ANNÉE 2012

THÈSE / UNIVERSITÉ DE RENNES 1
sous le sceau de l'Université Européenne de Bretagne

pour le grade de

DOCTEUR DE L'UNIVERSITÉ DE RENNES 1

Mention : Biologie

Ecole doctorale Vie-Agro-Santé

présentée par

Caroline Clouard

Préparée à l'UMR1348 PEGASE et à l'UR1341 ADNC
De l'Institut National de la Recherche Agronomique
Université Rennes 1

Mécanismes comportementaux et neurobiologiques de l'établissement des préférences et aversions alimentaires chez le porc : Applications en nutrition et santé animale et humaine

Thèse soutenue à Rennes
le 23 novembre 2012

devant le jury composé de :

Sylvie ISSANCHOU
DR1, INRA Dijon / *rapporteur*
Anne-Marie MOULY
CR1, CNRS, Université de Lyon / *rapporteur*
Hugo COUSILLAS
Pr, CNRS, Université Rennes 1 / e*xaminateur*
Ludovic DICKEL
Pr, Université de Caen / e*xaminateur*
Christine LETERRIER
DR2, INRA Tours / *examinateur*
Marie-Christine MEUNIER-SALAÜN
IR1, INRA Rennes / *directeur de thèse*
David VAL-LAILLET
CR1, INRA Rennes / *co-directeur de thèse*

REMERCIEMENTS

Ce travail de thèse a été réalisé à l'Institut National de la Recherche Agronomique (INRA), au sein de l'UMR1079 « Systèmes d'Élevage, Nutrition Animale et Humaine », qui s'est par la suite scindé en deux unités que sont l'UMR1341 « Physiologie, Environnement, Génétique pour l'Animal et les Systèmes d'Élevage », et l'UR1348 « Alimentation & Adaptations Digestives, Nerveuses et Comportementales ». Je remercie Jean Noblet, Jaap van Milgen et Charles-Henri Malbert, respectivement directeurs de l'UMR 1079 SENAH, de l'UMR1341 PEGASE et de l'UR1348 ADNC de m'avoir accueillie au sein de leurs unités à Saint-Gilles et de m'avoir donné les moyens de réaliser ce travail dans les meilleures conditions possibles.

Ce travail de thèse a été financé par la Région de Bretagne et l'INRA. Je remercie donc ces institutions pour m'avoir fourni, pendant ces trois années de recherche, les moyens financiers nécessaires à la réalisation de ces travaux. Je remercie également les laboratoires Phodé pour avoir financé une partie des expériences tout en me donnant l'opportunité de découvrir la recherche privée. Merci à Virginie Noirot, Pierre Étienne et Maxime Champagnac pour leur collaboration scientifique, ainsi qu'à Daniel Éclache, président, de nous avoir accueillis au sein de son entreprise.

Je tiens à exprimer ma reconnaissance et à remercier l'ensemble des membres du jury qui ont accepté d'évaluer ce travail de thèse : Sylvie Issanchou et Anne-Marie Mouly, en qualité de rapporteurs, ainsi que Hugo Cousillas, Ludovic Dickel et Christine Leterrier, en qualité d'examinateurs.

Je remercie vivement les membres de mon comité de thèse qui, à chaque rencontre, m'ont permis de prendre du recul sur ma thèse et de recadrer mon travail : Dominique Bligny, Catherine Blois-Heulin, Guillaume Ferreira, Cécile Ginane, Nathalie Quiniou et Jean-Pierre Royet. Merci pour vos critiques et suggestions, à la fois pertinentes et constructives. Nos échanges, toujours dans la bonne humeur, ont grandement contribué à la cohésion finale de cette thèse. Merci également à Catherine Blois-Heulin pour avoir accepté d'endosser au pied levé le rôle de tutrice.

J'ai également une pensée particulière, maintenant que la fin de cette aventure universitaire approche, pour les personnes que j'ai croisées au cours de ma vie étudiante et qui ont, sans le savoir, motivé mon choix de faire un doctorat. Je pense notamment aux professeurs qui m'ont fait découvrir et aimer l'éthologie, et en particulier Raymond Chichery, Ludovic Dickel, Marie-Annick Richard, Catherine Blois-Heulin... Je pense aussi, bien sûr, à Nicolas Devillers qui, avec sa formidable équipe de Sherbrooke, m'a fourni une première expérience professionnelle (et humaine) qui m'a faite « capoter ben raide » ! Et m'a convaincue que cette voie était la bonne. Merci à eux.

Je tiens à présent à adresser mes sincères remerciements ainsi que toute ma reconnaissance aux deux personnes sans qui ce travail m'aurait été bien difficile à réaliser, mes directeurs de thèse, Marie-Christine Meunier-Salaün et David Val-Laillet. La réussite d'une thèse dépend en grande partie de la qualité de l'encadrement et de la bonne entente entre les parties. Je ne sais pas si le résultat le reflètera (je l'espère !), mais vos qualités d'encadrants d'une part, et votre gentillesse et vos qualités humaines d'autre part, m'auront offert les meilleures conditions possibles pour réaliser ce travail de thèse avec plaisir et sérénité. Merci de m'avoir accordé votre confiance et d'avoir toujours su souligner les qualités du travail et pas seulement ses imperfections, cette pédagogie m'a fait beaucoup avancer durant ces trois années. Merci aussi pour votre investissement et votre grande disponibilité malgré des agendas bien remplis. Marie-Christine, je te remercie pour tes conseils aussi bien sur la thèse que sur l'après-thèse, merci pour m'avoir fait bénéficier de ton expérience, et de m'avoir appris à prendre du recul sur mon travail et à penser à l'après. David, merci pour ton investissement conséquent et quotidien dans cette thèse, aussi bien sur le terrain que sur le papier. Merci d'avoir su adapter ton encadrement progressivement pour me laisser le temps de m'approprier le sujet à mon rythme et de me sentir plus autonome. Un grand merci, enfin, pour ton implication concernant mon avenir post-thèse.

Ce travail était aussi un travail d'équipe, et j'aimerais remercier tous les acteurs qui y ont participé. Un énorme merci à Sylvie Guérin pour l'aide considérable (aussi bien professionnelle que personnelle !) qu'elle m'a apporté durant ces trois années. Merci pour ta participation active à l'ensemble des expériences, pour tes bonnes idées et ta réactivité (et tes drôles d'expressions !). Merci d'avoir toujours été disponible aussi bien pour les expériences que pour les discussions autour d'un petit thé ! Un grand merci aussi à Isabelle Nogret pour son aide et pour sa bonne humeur lorqu'il a fallu me donner un p'tit coup de main ! Merci aussi à Gwénaëlle Randuineau pour m'avoir initiée aux techniques d'immunohistochimie. Je remercie également Paul Meurice et Charles-Henri Malbert pour l'aide précieuse apportée sur l'ensemble des expériences d'imagerie, et pour le temps investi à trouver la solution lorsque le traitement et l'analyse des images se sont avérés plus compliqués que prévus.

Ce travail n'aurait pas pu aboutir sans l'aide de l'ensemble du personnel d'appui à la recherche et animaliers, qui ont toujours fait preuve d'une grande disponibilité, de sympathie et de bonne volonté pour s'adapter aux exigences expérimentales. Merci plus particulièrement à Alain Chauvin, Julien Georges, Régis Janvier, Francis Legouevec, Vincent Piedvache pour leur implication lors des chirurgies et des séances d'imagerie, et pour les soins aux animaux dans l'ensemble des expériences. Un grand merci également à Patrick Touanel (et son stock de blagues) et Fabien Guérin pour m'avoir si bien accueillie au bâtiment 11, pour leur disponibilité et leur constante bonne humeur. Merci à Hervé Demay et à Bernard Carrissant pour leur gestion efficace des animaux nécessaires aux expériences. Merci aussi à Michel Lefèvre, pour les installations vidéos, à Georges Guillemois et Jean-François Rouault du moulin, ainsi qu'à Armel Delalande et Jean-Claude Lemoine pour l'adaptation des cages de tests. Enfin, un grand merci à Henri Flageul pour les belles illustrations qui jalonnent ce manuscrit.

Mais une thèse, ce n'est pas que du travail, et encore du travail, c'est aussi une belle aventure humaine qui vous fait évoluer sur le plan personnel autant que professionnel. Pour l'ensemble des bons moments détente passés ensemble, pour les nombreux fous rires, pour leur bonne humeur et leur gentillesse, je tiens à remercier toutes les personnes des équipes qui m'ont si bien accueillie et que j'ai cotoyé quotidiennement pendant ces trois années : Carole Guérin, Sylvie Guérin, Marie-Christine Meunier-Salaün, Paul Meurice, Isabelle Nogret, Gwénaëlle Randuineau, Céline Tallet, David Val-Laillet...

Ma sympathie à l'ensemble du personnel de la station que j'ai côtoyé au quotidien autour d'un café, au restaurant, au détour d'un couloir, au cochon grillé ou dans un congrès, et particulièrement à l'ensemble des étudiants croisés pendant ma thèse. Je tiens également à adresser un grand merci à l'ensemble des stagiaires avec qui j'ai eu le plaisir de travailler. Mélanie Jouhanneau, Matthieu Chataignier, Barbara Réthoré, Florence Loison. Merci à vous pour avoir contribué activement à cette thèse. J'adresse bien sûr un clin d'œil particulier à Mélanie qui m'a accompagnée de sa bonne humeur et de sa gentillesse lors de mes tous premiers pas de thésarde.

Enfin, les derniers remerciements, et pas des moindres, iront à mes amis et à ma famille qui, sans le savoir, m'ont apporté un soutien irremplaçable pendant ces trois années. À mes meilleurs amis, Christo, Gill's, Lizou, Marie, Maud, Ophélie, Samy, un énorme merci pour me faire tellement rire depuis toutes ces années et pour m'avoir fait oublier le travail à chaque retrouvaille. À mes amis de Rennes : Alberto, Carlos, Caro, Enrique, Julien, Kike, Melissa, Marie, Nina, Paulo et Vinciane, merci pour tous les bons moments passés ensemble. Merci aussi à Florent (qui, lui, a eu son CLES...), c'est grâce à nos séances de révisions de M2 plus ou moins studieuses mais *a priori* efficaces qu'on en est là ! Le dernier merci ira à ma famille, mon frère et mes parents, pour avoir toujours cru en moi, avoir été d'un soutien sans faille, m'avoir accordé leur confiance depuis mes premiers pas à l'université et m'avoir toujours laissée maître de mes décisions. Enfin, je dédie cette thèse à Fabio. Ma plus grande fierté aurait été d'avoir la chance d'échanger avec toi sur cette belle expérience...

PUBLICATIONS SCIENTIFIQUES

Article n°1

Clouard, C., Meunier-Salaün, M.C., Val-Laillet, D. 2012. Food preferences and aversions in human health and nutrition: How can pigs help the biomedical research? *Animal* 6(1): 118-136.

Article n°2

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Article n°3

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COMMUNICATIONS ORALES AVEC COMITÉ DE LECTURE

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SOMMAIRE

Index des abréviations

Par convention, et pour plus d'homogénéité entre les articles de la thèse, écrits en anglais, et le corps du manuscrit écrit en français, l'ensemble des abréviations correspond aux dénominations anglaises.

Abbréviation	Dénomination anglaise	Dénomination française
5-HT	Serotonine	Sérotonine
^{18}F-FDG	Fluorodeoxyglucose (^{18}F)	Fluorodésoxyglucose (^{18}F)
^{99m}TC	Technetium 99m	Technétium 99m
(A/P)CC	(Anterior/postérior) cingular cortex	Cortex cingulaire (antérieur/postérieur)
AMY	Amygdala	Amygdale
APFC	Anterior prefrontal cortex	Cortex préfrontal antérieur
CAU	Caudate nucleus	Noyau caudé
CR	Conditioned response	Réponse conditionnée
CS	Conditioned stimulus	Stimulus conditionnel
DA	Dopamine	Dopamine
DLPFC	Dorsolateral prefrontal cortex	Cortex préfrontal dorsolatéral
GABA	Gamma-aminobutyric acid	Acide gamma-aminobutyrique
GP	Globus pallidus	Globus pallidus
IC	Insular cortex	Cortex insulaire
ITG	Inferior temporal gyrus	Gyrus temporal inférieur
LiCl	Lithium chloride	Chlorure de lithium
NAcc	Nucleus accumbens	Noyau accumbens
OFC	Orbitofrontal cortex	Cortex orbitofrontal
PeC	Perirhinal cortex	Cortex périrhinal
PET	Positrons emission tomography	Tomographie par émission de positrons
PHC	Parahippocampal cortex	Cortex parahippocampique
PiC	Piriform cortex	Cortex piriforme
PR	Progressive ratio	Ratio progressif
PUT	Putamen	Putamen
PreC	Prepiriform cortex	Cortex prépiriforme
SPECT	Single photon emission computed tomography	Tomographie par émission monophotonique
US	Unconditioned stimulus	Stimulus inconditionnel
VTA	Ventral tegmental area	Aire tegmentale ventrale

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INTRODUCTION GÉNÉRALE

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Le comportement alimentaire est un comportement complexe faisant intervenir deux voies principales de régulation : la voie homéostatique et la voie hédonique. Alors que la voie homéostatique se réfère d'avantage à la régulation interne du comportement alimentaire (signaux peptidiques, hormonaux et nerveux transmis par le système digestif au circuit central), la voie de régulation hédonique prend en compte l'influence de facteurs externes au processus digestif, tels que l'expérience de l'individu (mémoire, apprentissage) ou encore le contexte environnemental et social de la prise alimentaire, sur la perception affective des propriétés organoleptiques de l'aliment. D'un point de vue comportemental et chez de nombreuses espèces, la régulation de la prise alimentaire repose en grande partie sur la capacité des organismes à associer les caractéristiques sensorielles d'un aliment (*e.g.* goût, odeur, texture) avec les conséquences de sa mise en bouche (plaisir, dégoût) et/ou de son ingestion (*e.g.* satiété, malaise gastrique). Ces processus d'association aboutissent à l'établissement de préférences ou d'aversions alimentaires qui permettent aux individus de sélectionner les aliments riches en calories et/ou palatables, et d'éviter les aliments potentiellement toxiques dans leur milieu naturel. Les expériences passées d'un individu avec les aliments permettent donc à celui-ci de moduler son comportement alimentaire *via* des processus de sélection alimentaire qui sont eux-mêmes sous l'influence de mécanismes centraux complexes. Chez l'Homme et chez le rat, l'implication de nombreuses structures cérébrales dans la régulation de la prise alimentaire et plus précisément dans les volets hédonique (caractérisation hédonique des informations sensorielles), cognitif (mémoire, apprentissage) et/ou motivationnel (motivation et récompense alimentaire) de cette régulation a été mise en évidence.

L'étude des mécanismes comportementaux et neurobiologiques qui sous-tendent la modulation hédonique des comportements alimentaires chez le porc, *via* notamment l'établissement des préférences et aversions alimentaires, représente d'importants enjeux en termes d'application aussi bien dans le domaine de la production porcine que dans le domaine de la recherche biomédicale. En production porcine, comprendre les facteurs susceptibles de moduler le comportement et les préférences alimentaires chez l'animal pourrait permettre d'améliorer son niveau d'ingestion durant les phases sensibles de transition alimentaire. D'autre part, le porc, espèce omnivore qui possède d'importantes capacités cognitives (mémoire et d'apprentissage) et partage de nombreuses caractéristiques communes avec l'Homme en termes d'anatomie digestive et cérébrale, s'avère un modèle intéressant en re-

cherche biomédicale, et plus particulièrement dans les études en nutrition et neurosciences. Comprendre, chez le porc, sur quelles bases une préférence ou une aversion alimentaire se met en place, et comment se forme l'image sensorielle de l'aliment au niveau central en fonction de sa valeur hédonique peut notamment aider à comprendre l'origine de certains troubles du comportement alimentaire chez l'Homme, comme la mise en place d'aversions (e.g. patient cancéreux sous chimiothérapie) ou de préférences exacerbées (addictions alimentaires, obésité).

L'objectif principal de cette thèse sera donc de décrire les mécanismes comportementaux et neurobiologiques impliqués dans la régulation hédonique de la prise alimentaire, et plus particulièrement dans l'établissement des préférences et aversions alimentaires. Différentes questions de recherche émergent de cette thématique. D'un point de vue comportemental, s'il est clair que l'expérience passée avec un aliment influence les comportements de consommation, des doutes subsistent encore quant à la voie d'action (viscérale *vs* sensorielle) la plus déterminante dans l'établissement de préférences ou d'aversions alimentaires. En l'absence de renforcement gustatif, l'association entre un aliment et la perception d'un signal viscéral positif ou négatif est-elle suffisante à elle seule pour modifier les réponses vis-à-vis de cet aliment ? Si non, l'association entre renforcement gustatif et viscéral est-elle absolument nécessaire pour modifier durablement les choix alimentaires ? La démarche visera donc à comparer l'efficacité de différents composés agissant soit sur la sphère viscérale (apport calorique, malaise gastrique), soit sur la sphère gustative (goût plaisant), à modifier les choix alimentaires. Du point de vue de l'intégration centrale, nous tenterons d'évaluer si la perception de stimuli olfactifs et/ou gustatifs aux valeurs hédoniques contrastées entraîne l'activation différentielle de structures cérébrales reconnues pour être impliquées, chez l'humain, dans la régulation du comportement alimentaire.

Grâce à une approche pluridisciplinaire innovante, nous allons donc tenter de répondre aux différentes questions scientifiques exposées plus haut *via* la mise en œuvre de méthodes éthologiques (conditionnement pavlovien et opérant, tests de choix) et *via* l'utilisation de techniques d'imagerie cérébrale fonctionnelle (tomographie d'émission positonique, tomographie d'émission monophotonique).

SYNTHÈSE BIBLIOGRAPHIQUE

SYNTHÈSE BIBLIOGRAPHIQUE

Cette synthèse bibliographique va permettre de définir le contexte de la thèse et de décrire l'état de l'art sur les mécanismes comportementaux et neurobiologiques impliqués dans la modulation hédonique du comportement alimentaire. Dans une première partie, nous aborderons les processus comportementaux relatifs à la régulation de la prise alimentaire, avec notamment la description des processus impliqués dans l'établissement des préférences et aversions alimentaires conditionnées. Nous développerons ensuite l'utilisation du modèle porcin dans le cadre de la recherche biomédicale en santé et en nutrition en mettant en lumière les similitudes comportementales, physiologiques et neurobiologiques entre le modèle porcin et l'Homme (article n°1). Dans une seconde partie, nous nous intéresserons à l'approche neurobiologique en précisant les réseaux centraux connus pour être impliqués dans la régulation hédonique du comportement alimentaire chez le rat ainsi que chez les primates humains et non-humains.

Partie 1. Étude des préférences et aversions alimentaires chez le porc

Article n°1

Food preferences and aversions in human health and nutrition: How can pigs help the biomedical research?

C. Clouard, M. C. Meunier-Salaün and D. Val-Laillet, 2012

Animal, 6(1): 118-136

(Received 2 November 2010; Accepted 27 May 2011)

Abstract. The establishment of food preferences and aversions determines the modulation of eating behaviour and the optimization of food intake. These phenomena rely on the learning and memory abilities of the organism and depend on different psychobiological mechanisms such as associative conditionings and sociocultural influences. After summarizing the various behavioural and environmental determinants of the establishment of food preferences and aversions, this paper describes several issues encountered in human nutrition when preferences and aversions become detrimental to health: development of eating disorders and obesity, aversions and anorexia in chemotherapy-treated or elderly patients and poor palatability of medical substances and drugs. Most of the relevant biomedical research has been performed in rodent models, although this approach has severe limitations, especially in the nutritional field. Consequently, the final aim of this paper is to discuss the use of the pig model to investigate the behavioural and neurophysiological mechanisms underlying the establishment of food preferences and aversions by reviewing the literature supporting analogies at multiple levels (general physiology and anatomy, sensory sensitivity, digestive function, cognitive abilities, brain features) between pigs and humans.

Keywords: pig, conditioned learning, eating behaviour, animal model, biomedical applications

Acknowledgement. The authors wish to thank Cambridge University Press for granting permission to include the final pdf version of the article within the PhD thesis.

Animal (2012), 6:1, pp 118–136 & The Animal Consortium 2011
doi:10.1017/S1751731111001315

animal

Food preferences and aversions in human health and nutrition: how can pigs help the biomedical research?

C. Clouard, M. C. Meunier-Salaün and D. Val-Laillet[-]

INRA, UMR1079 SENAH, Domaine de la Prise, 35590 Saint Gilles, France

(Received 2 November 2010; Accepted 27 May 2011; First published online 19 August 2011)

The establishment of food preferences and aversions determines the modulation of eating behaviour and the optimization of food intake. These phenomena rely on the learning and memory abilities of the organism and depend on different psychobiological mechanisms such as associative conditionings and sociocultural influences. After summarizing the various behavioural and environmental determinants of the establishment of food preferences and aversions, this paper describes several issues encountered in human nutrition when preferences and aversions become detrimental to health: development of eating disorders and obesity, aversions and anorexia in chemotherapy-treated or elderly patients and poor palatability of medical substances and drugs. Most of the relevant biomedical research has been performed in rodent models, although this approach has severe limitations, especially in the nutritional field. Consequently, the final aim of this paper is to discuss the use of the pig model to investigate the behavioural and neurophysiological mechanisms underlying the establishment of food preferences and aversions by reviewing the literature supporting analogies at multiple levels (general physiology and anatomy, sensory sensitivity, digestive function, cognitive abilities, brain features) between pigs and humans.

Keywords: pig, conditioned learning, eating behaviour, animal model, biomedical applications

Implications

Investigation of the behavioural and neurophysiological mechanisms of the establishment of food preferences and aversions can lead to important developments in the context of human nutrition and health. Because the rodent models are not always adequate in this field, there is a need to develop alternative experimental models. Pigs have numerous similarities with humans in terms of the physiology, anatomy, sensory sensitivity, cognitive abilities and brain functions. The aim of this paper is to promote the use of pigs for biomedical research in human nutrition.

Introduction

Feeding is a complex behaviour, which can be described as 'the research and consumption of food and drink to maintain vital functions' (Bellisle, 1999) and to 'fulfil the metabolic needs of the organism' (Ferreira, 2004). Today, it is also well acknowledged that a high proportion of human food consumption in developed countries appears to be driven by pleasure (for a review, see Lowe and Butryn, 2007) and sociocultural influences. Food consumption is also involved in fundamental metabolic homeostasis regulation, as it controls the supply of energy and nutrients in the organism (Bellisle, 1999). According to Ferreira (2004), feeding behaviour implies that animals learn to consume high-energy foods and to avoid toxic foods. Establishment of food selection implies that, during its first experience with food, the organism memorizes the sensorial characteristics of the food (e.g. taste, odour, texture and visual cues) and the post-ingestive consequences of its ingestion, and associates these food characteristics with these consequences (Garcia *et al.*, 1974; Sclafani, 2001; Ferreira, 2004). This regulation of food choices requires learning and memory capacities (Bernstein, 1999; Houpt, 2000; Welzl *et al.*, 2001), which enable the animal to adapt its feeding behaviour towards a novel food. Such food selection leads to the constitution of a feeding repertoire, which is dependent on the particular feeding situation and on the needs of the organism (Bellisle, 1999). The feeding repertoire and food selection constantly evolve throughout life governed by several factors, such as genetic and environmental, and according to sensorial, physiological and psychological states (Bellisle, 2006). In numerous animal species, including humans, development of food preferences and aversions makes a major contribution towards the establishment of the feeding repertoire.

[-] E-mail: david.val-laillet@rennes.inra.fr

The aim of this review is threefold. In the first part, the characteristics and development of aversions and preferences, two phenomena involved in the establishment of eating behaviour and feeding repertoire, will be described in the light of recent literature. The second part of the review will summarize the current socio-economic and medical context related to preferences and aversions in human nutrition and will aim to justify the current needs for research in this topic. The last part of the review will focus on the methods and animal models currently used to address questions in this field, and put forth some arguments in favour of the use of pigs as a preferred model for studying the development of food preferences and aversions in humans.

Characteristics and development of preferences and aversions

Food preferences and aversions: a classical conditioning

Food preference or aversion learning is a form of classical conditioning first described by Pavlov (1960). A conditioned stimulus (CS) is associated with an unconditioned stimulus (US). In the case of conditioned food preference and aversion, animals come to consume or avoid a food (CS) that produces positive or negative post-ingestive symptoms (US), respectively (Pavlov, 1960; Garcia *et al.*, 1974).

When food intake generates unpleasant gustatory perception (e.g. bitter taste) or is followed by a visceral malaise (nausea, diarrhoea, etc.), the organism learns to avoid the consumption of that food or other food that presents the same sensory characteristics (Ferreira, 2004). This is known as conditioned food aversion. This ability to learn to avoid potentially toxic foods has been demonstrated in numerous animal species, from invertebrate to humans (for a review, see Bernstein, 1999; Paradis and Cabanac, 2004). Indeed, food aversion has been described in a variety of mammals, in addition to humans (Garcia *et al.*, 1974; Bellisle, 1999; Ravasco, 2005; Bellisle, 2006) or rats (Yasoshima *et al.*, 2000; Ferreira, 2004), and in livestock species such as horses, sheep and cattle (Houpt *et al.*, 1990; Burritt and Provenza, 1996; Halaweish *et al.*, 2002; Ginane and Dumont, 2006; Pfister *et al.*, 2007), and also in birds (Skelhorn and Rowe, 2006; Halpin *et al.*, 2008; Skelhorn *et al.*, 2008) and reptile species (Terrick *et al.*, 1995; Paradis and Cabanac, 2004). Experimentally induced food aversions are frequently conducted by an intragastric or an intraperitoneal injection of lithium chloride, an emetic substance known to induce visceral malaise. As a result, animals come to avoid the food that has been paired with this treatment (Pavlov, 1960; Garcia *et al.*, 1974).

Post-ingestive consequences can also lead to the establishment of food preferences. When food intake generates positive appetitive or post-ingestive consequences (e.g. abundant supply of energy), the organism learns to preferentially consume this particular food, which is known as a conditioned food preference. Two main categories of preferential conditioning are reported: the flavour–flavour and the flavour–nutrient conditionings. The first category consists of the association between the flavour of an unfamiliar food and one that is familiar and/or already has a high hedonic value. This kind of association has been widely studied in rats (Sclafani and Ackroff, 1994; Warwick and Weingarten, 1994 and 1996) and humans (Mobini *et al.*, 2007; Brunstrom and Fletcher, 2008). In contrast, flavour–nutrient conditioning is induced by pairing the flavour of an unfamiliar food with an energy supply, thats is, positive post-ingestive consequences (Myers and Sclafani, 2006). Flavour–nutrient learning has been studied in humans (Brunstrom and Mitchell, 2007; Mobini *et al.*, 2007; Zeinstra *et al.*, 2009) and rats (Sclafani and Ackroff, 1994; Warwick and Weingarten, 1994; Lucas *et al.*, 1997; Lucas and Sclafani, 1998; Sclafani, 2001). Although they are often combined, the ingestion of highly palatable food (e.g. sweet food) is often paired with an energy (caloric) supply (Myers and Sclafani, 2006). The two types of independently operating conditioned learning have been experimentally induced, especially in rats (Ackroff *et al.*, 2001; Gilbert *et al.*, 2003; Touzani and Sclafani, 2005; Myers, 2007; Touzani and Sclafani, 2007; Touzani *et al.*, 2009a and 2009b). Flavour–flavour association was achieved by adding an appetent taste or flavour in the test solution (e.g. non-caloric sweet taste) to induce an oral-hedonic reinforcement, whereas flavour–nutrient association was achieved by pairing food ingestion (CS) with an intragastric or an intraperitoneal injection (US) of energy (e.g. glucose or fructose) to induce positive post-ingestive consequences.

Considered as forms of classical conditioning, food preference and aversion learning have certain features in common: they are extremely robust and can be acquired in a single learning trial for a novel food, that is, after only one pairing of CS to US (Garcia *et al.*, 1974; Bellisle, 2006; Myers, 2007). Significant aversions also develop to the CS despite long delays between exposure to the CS and US (Garcia *et al.*, 1966). However, one should keep in mind that, for practical purposes, preferences are often stronger to acquire than aversions and their acquisition often requires more than one association to achieve a strong and long-lasting effect, although some studies in rats showed that the acquisition of a preference can be rapid (Myers, 2007; Ackroff *et al.*, 2009).

The development of food preferences and aversions is governed by sociocultural and familial influences

Even if conditioned food preferences and aversions broadly depend on factors related to learning and memory, food selection also depends on subtle factors that are genetic, hedonic, ontogenic and sociocultural. According to Birch (1999), genetic predispositions include the ability to express 'innate' preferences, the capacity to reject novel food (neophobia) and the ability to learn preferences. The development of food choices implies that environmental factors combine with these genetic predispositions (for a review, see Wardle and Cooke, 2008) and this complex association of factors leads to the formation of 'innate' and learned preferences and aversions.

In humans and several animal species, reflex responses to taste and smell are present in the neonate before any spontaneous feeding experience (Birch, 1999), suggesting

that the development of food preferences and aversions does not depend only on learning processes. The study of facial expressions induced by taste stimulations showed that neonates prefer foods that are sweet (sugar) and reject sour or bitter food (Steiner, 1979). Moreover, preference for salt develops in human infants approximately 4 months postnatally (Beauchamp *et al.*, 1994). However, it is necessary to be cautious with the use of the term 'innate preferences'. As the foetus can perceive some sensorial stimuli even during the last weeks of pregnancy (i.e. has functional olfactory receptors or taste papillae; Mennella and Beauchamp, 1996; Bellisle, 1999; Doty and Shah, 2008), these newborn preferences are likely to have been influenced by several pre- and postnatal stimulations.

The environment and especially mother–child interactions also play an important role in shaping children's preferences (Birch, 1999). What the mother eats during pregnancy and lactation can have an impact on children's food choice, as volatile compounds of the mother's diet (e.g. vanilla, garlic, anis, alcohol) are transferred from the maternal circulatory system to the amniotic fluid (Doty and Shah, 2008) and milk (Mennella and Beauchamp, 1993 and 1996). Mennella and Beauchamp (1993) showed an effect of prior experience with garlic in mother's milk on the breast-feeding behaviour of their infants: children with mothers who had consumed garlic during pregnancy showed a weaker aversion to garlic odour compared with non-exposed children. Similarly, the mother's consumption of vanilla altered the behaviour of her infant during breast-feeding: human infants whose mothers had consumed vanilla during gestation showed greater acceptance of vanilla flavour than non-exposed infants (Mennella and Beauchamp, 1996). Similar results on the impact of mother–young interactions were found in pigs (Campbell, 1976). Langendijk *et al.* (2007) showed that pre- and postnatal exposure to flavours (garlic or anis) increases postweaning feed intake in pigs. Exposure to flavours through the sow's diet during gestation and lactation increases acceptance by piglets (Oostindjer *et al.*, 2010). Saint-Dizier *et al.* (2007) also found that the development of food preference in lambs depends on observation of the mother that provided visual and behavioural cues to eat or avoid the food.

In addition to the maternal influences, these food choices are also strongly modulated throughout life via feeding experiences in association with sociocultural influences (Bellisle, 1999 and 2006; Birch, 1999) including the family circle, the social group or the cultural environment of children. For instance, exposure to a variety of flavours in the familial environment enhances food acceptance in children (Gerrish and Mennella, 2001), whereas children who rarely have the opportunity to try new food, perhaps because of rigid control by parents of the food environment of their infant, are more likely to be neophobic in the future (Hursti and Sjödén, 1997).

Overall, these findings suggest that social environment is important and that genetic factors may play a minimal role in the phenomenon of food preferences. The association between these two factors may explain the considerable inter-individual variability between children and between adults in their food preferences (Bellisle, 2006; Wardle and Cooke, 2008). In summary, food preferences and aversions are complex phenomena and their development does not only rely on classical learning processes but also on numerous factors, genetic or socio-cultural.

Study of feeding behaviour and the current socio-economic context

Investigation on the development of feeding preferences and aversions and their inherent mechanisms (behavioural and neurobiological) may fulfil the current needs of research and development in human nutrition and health. The relevance of studying feeding behaviour for human health applications is addressed in this chapter of the review by drawing up a non-exhaustive list of possible applications. The first section will introduce the problems of appetite and feeding disorders, especially obesity, a condition that is reaching epidemic proportions in wealthy countries. In the second section, the applications in biomedical and pharmacological research will be investigated.

Obesity and eating disorders

The establishment mechanisms of food selection described above have a strong adaptive value and so does the organisms' capacity to store energy. These mechanisms present an unquestionable advantage in an environment where resources are scarce. However, with the recent development of fundamental, unprecedented increases in food availability in modern human societies (i.e. plethoric and appetent food), these same mechanisms can lead to detrimental conditions, such as obesity and eating disorders (Lowe and Butryn, 2007).

Indeed, obesity has become a worldwide phenomenon and a major health issue (Popkin and Doak, 1998; Spurlock and Gabler, 2008). In 2005, the World Health Organization stated that approximately 400 million adults were obese (Singh-Manoux *et al.*, 2009). Obesity is characterized by an unbalanced hunger/satiety ratio and by an overaccumulation of fat in adipocytes. It is a multifactorial disease that can cause or arise as a consequence of eating disorders, although the relationship between obesity and eating disorders is very complex. Obesity may result from several influences, including genetic, metabolic, nutritional, hormonal, behavioural, environmental (e.g. stress) or iatrogenic (i.e. due to medical treatment) factors (Bellisle, 1999; Stein and Colditz, 2004). Regarding environmental influences, it seems that activity changes (e.g. urbanization, structure of work, more passive leisure-time and sedentary activities) are responsible for decreased physical activity and energy consumption of excess empty calories (for a review, see Popkin and Doak, 1998). Although low levels of physical activity contribute towards increased obesity rates, the onset of an excessive and unbalanced diet is also a major contributor to overweight and obesity (Blundell and Finlayson, 2004; Lowe and Levine, 2005; Lowe and Butryn, 2007). This so-called 'western diet' is

characterized by a high proportion of palatable foods, such as high-carbohydrate and high-fat foods, that are responsible for food binges with exaggerated preferences (Yanovski, 2003). Fat consumption is considered to be pleasurable because fat increases the palatability of foods, enhancing food sensorial characteristics, such as flavour, odour and texture (Drewnowski, 1997; Yanovski, 2003; Mizushige *et al.*, 2007). The tendency to prefer high-fat and high-carbohydrate foods is enhanced by the lower cost of those diets compared with the cost of healthy diets including fruit and vegetables (Bernstein *et al.*, 2010). Low-income consumers are particularly concerned about the cost of food rather than its nutritive and health benefits and prefer low-cost foods rather than healthy foods (Hampson *et al.*, 2009). The modern food transition and the widespread availability of highly palatable and low-cost food providing an 'obesogenic environment' have stimulated food intake, leading to energy intake beyond that required to balance energy expenditure (Wardle, 2007). Thus, although socio-economical factors play a predominant role in the emergence of feeding disorders, the sensorial characteristics of foods are also likely to be involved.

Being overweight or obese has various negative consequences and can cause several chronic health diseases. The disorders that develop are associated with increased mortality and risks for coronary heart diseases, type-2 diabetes, hypertension and some types of cancer (for a review, see Sturm, 2002; Stein and Colditz, 2004). Cole *et al.* (2010) reported that overeating and consumption of high-fat/high-caloric diets increase the risk of age-related brain diseases later in life, such as Alzheimer's disease, Parkinson's disease or frontal temporal dementia. As obesity is strongly associated with clinical diseases, it also reduces health-related quality of life and increases health-care and medication costs (Sturm, 2002).

Considering the epidemic of obesity and its detrimental consequences on health, there is an urgent need for optimization of methods to prevent and treat obesity. As the sensorial characteristics of food may be involved in the development of eating disorders, such as binge eating and food addictions, a better understanding of food preferences and aversions may lead to improved methods for the prevention and treatment of obesity and eating disorders (Yanovski, 2003). Study of food preferences and aversions could thus lead to the development of new, more efficient strategies to promote the establishment of good eating habits and diversified food repertoires in children, through acceptance of novel healthy food, from a young age. The development of such preventive methods is crucial as the prevalence of nutritional pathologies and diseases such as obesity can only be reduced by means of a close association between preventive and palliative methods. Children often exhibit some spontaneous neophobic responses and/or aversions towards novel food, and especially healthy food (e.g. vegetables), which are known to have a 'low reinforcement value' (Zeinstra *et al.*, 2009). Some behavioural techniques are already being used to facilitate the acceptance of novel and healthy foods by children. For instance, mixing vegetables with other more palatable ingredients may encourage the intake of vegetables later in life (Zeinstra *et al.*, 2009). Preference for a food can also be acquired in children by regular and repeated exposure to it (Wardle and Cooke, 2008). Using food as a reward may also be an effective strategy to increase food acceptance by children, although this strategy is slightly controversial, as the reward strategy may be strongly related to the child's perception of the context (Wardle and Cooke, 2008). Therefore, it is necessary to develop our knowledge of the behavioural and neurophysiological mechanisms underlying the development of such learning. This may lead to recommendations in terms of feeding learning and diversification in children and adults.

Biomedical applications

Chemotherapy and radiotherapy. In cancer patients, chemotherapy and radiotherapy treatments often have detrimental or harmful side effects (e.g. nausea and vomiting) that may lead to the establishment of food aversions and ultimately to clinical anorexia and cachexia (Bernstein, 1978). Cancer patients under chemotherapy often show avoidance or aversion for a meal taken before the administration of treatment, because the meal is associated with therapy-induced malaise (Bernstein, 1978), which acts like a CS. Moreover, patients often complain about these symptoms before the infusion. The environmental context of drug administration (e.g. entry of the nurse or the doctor, sight of the syringe and of the infusion apparatus, hospital odours) can be associated with the symptoms and acts like a CS in itself. Thereby, after some pairings of CS) and US, some anticipatory symptoms may occur before the onset of the infusion, which clearly indicates conditioning (Stockhorst *et al.*, 1998; Stockhorst *et al.*, 2007). Holmes (1993) reported that 82% of patients under chemotherapy developed food avoidance, whereas, according to Mattes *et al.* (1987), over 50% of patients developed a food aversion after chemotherapy. Moreover, a reduction in taste sensitivity (hypogeusia), an absence of taste sensation (ageusia) or a change in taste sensitivity (dysgeusia) often occurs in patients receiving radiotherapy against cancers (Ripamonti *et al.*, 1998; Berteretche *et al.*, 2004). These taste alterations, which decrease the hedonic value of food, are another cause of nausea or vomiting in these patients (Lévy *et al.*, 2006; Bernhardson *et al.*, 2007)

The conditioned aversions to food and beverages developed after chemotherapy or radiotherapy might explain the loss of appetite and the decreased energy intake recorded in some cancer patients (Bernstein, 1978). The detrimental consequences of this malnutrition are diverse: poor prognosis, morbidity, decreased quality of life and clinical management of patients, but also anorexia (Bernstein, 1978; Andreyev *et al.*, 1998; Berteretche *et al.*, 2004). Taste changes, which are among the most common chemotherapy-associated side effects (Ravasco, 2005), are not only distressing for patients and impact on their quality of life (Epstein *et al.*, 1999 and 2002; Ohrn *et al.*, 2001), but also lead to food aversions and reduced food intake (Ravasco, 2005).

Although absent in rodents, the emetic reflex exists in several mammalian species, including humans, monkeys, dogs, cats and ferrets. As a result, the ferret has been used as an alternative model to rodents for chemotherapy-induced emesis (Andrews and Horn, 2006). Those pharmacological studies enabled the identification of efficient antiemetic agents such as serotonin type 3 receptor antagonists (anti-5HT3) or neurokinin type 1 receptor antagonists (anti-NK1) that are frequently used during chemotherapy treatments to inhibit nausea and vomiting in cancer patients (Durand *et al.*, 2009). As nausea and vomiting appear to be responsible for significant decreases of food intake in cancer patients, treatments based on these antiemetic drugs may result in an increase of food intake. However, despite modern antiemetic treatment, approximately 25% to 30% of chemotherapy patients still exhibit anticipatory nausea or vomiting immediately after re-exposure to the stimuli that usually signal the drugs' infusion (Stockhorst *et al.*, 2007). According to Schwartz *et al.* (1996), it seems that the presence of nausea following chemotherapy administration is correlated with a decrease in hedonic rating towards food but not with a decrease in consumption. Mattes *et al.* (1987) also suggest that nausea and vomiting may not be essential stimuli for the acquisition of conditioned food aversions. Antiemetic medications during chemotherapy may also be ineffective in preventing the development of aversion to foods, and thereby ineffective in increasing food intake (Schwartz *et al.*, 1996).

As a result of these issues, the study of the development of food aversions is clearly needed to develop new treatments and strategies to increase food intake in these patients. One of the interesting strategies developed as a result of the study of food aversions in humans is the 'scapegoat' technique (Broberg and Bernstein, 1987; Mattes *et al.*, 1987; Stockhorst *et al.*, 1998). This technique is based on the overshadowing principle underlying the principles of the classical conditioning technique (Pavlov, 1960). It consists of the presentation of a compound of two stimuli as a potential CS, which is paired with the US. The more salient of the stimuli is assumed to override the effects of the less salient one and the conditioned response elicited by the less salient element is weaker than if it alone had been paired with the US (Miller *et al.*, 1990; Stockhorst *et al.*, 1998). Broberg and Bernstein (1987) found that using strongly flavoured candies as scapegoats reduces food aversions during chemotherapy and, thereby, increases food consumption among paediatric patients. Furthermore, Mattes (1994) showed that patients exposed to a particular sensory stimulus demonstrate a statistically significant 30% reduction in the development of food aversion compared with the non-exposed patients.

The elderly and undernutrition. In addition to application to cancer patients under chemotherapy, the study of food aversions and preferences has other interesting biomedical applications, particularly in the hospitalized elderly. During the past century, the proportion of older individuals in developed countries has increased to a considerable extent and continues to grow rapidly. A decline in appetite is often observed in this population and is logically associated with a decreased food intake (for a review, see MacIntosh *et al.*, 2000; Beckoff *et al.*, 2001; Kagansky *et al.*, 2005; Fetissov *et al.*, 2009). This phenomenon is known as 'physiological anorexia of ageing'. Consequently, malnutrition is frequent in elderly populations, even in the developed countries, and even among the hospitalized elderly, nutritional status can be poor (MacIntosh *et al.*, 2000; Kagansky *et al.*, 2005). As for cancer patients, malnutrition is found to negatively influence the quality of life of older adults in nursing homes (Crogan and Pasvogel, 2003). Moreover, poor nutritional status has been implicated in the development and progression of chronic diseases commonly affecting the elderly and leading to complications during hospitalization, poorer clinical outcome and increased mortality (Kagansky *et al.*, 2005). Malnutrition is a predictor of long hospital stay and high mortality in geriatric and cancer patients (Chima *et al.*, 1997; Kagansky *et al.*, 2005).

St-Arnaud-McKenzie *et al.* (2004) suggest that the development of nutritional interventions to maintain hunger and reduce aversion may be necessary to ensure optimal food intake among hospitalized people (cancer patients, geriatric patients, etc.). For instance, Beckoff *et al.* (2001) showed that the use of glucose or other carbohydrate supplements in the diet can increase the total energy intake of older subjects and thus prevent weight loss in the elderly. Improving the pleasurable qualities of food, that is, taste and smell, may stimulate an increase in appetite and food intake in the elderly (MacIntosh *et al.*, 2000). As the sense of taste decreases with ageing (Bellisle, 1999; MacIntosh *et al.*, 2000), and given that taste and smell (i.e. flavour) are important features for the motivation to eat, an increased understanding of the sensorial characteristics of food that induce a deterioration in food intake in terms of quality and quantity in the elderly seems necessary. This should facilitate the development of appropriate preventive and treatment strategies to improve the health of older individuals.

Optimization of pharmaceutical medicines. The study of the perceived and preferred sensorial characteristics of food may also lead to an improved tolerance of oral medications, through enhancement of their palatability. Indeed, several medicines and active pharmaceutical ingredients may be difficult to ingest or may not very palatable due to their propensity to irritate the mouth or throat and their unpleasant taste (e.g. too bitter). This is particularly true for paediatric patients. These patients may have many of the same diseases and are often treated with the same drugs as those used to treat adults, although they are often more sensitive to gustatory cues (Mennella and Beauchamp, 2008). For instance, this is the case with oral contrast agents (Arya *et al.*, 2009) used before computed tomography examinations; especially large volumes must be ingested for investigations of intra-abdominal pathology (Weyant *et al.*, 2000). Paediatric patients' care is often disrupted because they have difficulty in tolerating the oral contrast solution, which has low palatability. Arya *et al.* (2009) have demonstrated that

oral contrast is more palatable when mixed with flavoured commercial drink mixes compared with the standard contrast mixed with water. Similarly, in their review, Mennella and Beauchamp (2008) argued that children's acceptance of many medicines may be increased by improving their palatability. For example, addition of sugars or salt substances may be effective in suppressing the bitter taste of some medications. Altogether, these results prove that a better understanding of the sensorial characteristics of food and beverages that are preferred or disliked may be very useful to improve biomedical treatments in hospital.

As reviewed above, in patients suffering from malnutrition, such as the elderly or cancer patients, stimulation of appetite by appetitive factors or by the addition of aroma to food might be a useful method to maintain weight and food intake. Further investigations are needed to identify the more pertinent food characteristics that could be manipulated to promote food intake and fight aversion in a clinical context.

The development of new strategies and innovative techniques may have a significant impact on the outcome of therapy and on the patients' quality of life. This biomedical research requires the use of animal models, depending on the experimental design and research paradigm to be investigated. It is obvious that the choice of an animal model has to be well considered, according to their biological characteristics and the research topic addressed. Most of the biomedical research is performed in rodent models, although this approach has severe limitations, especially in the nutrition field. An alternative model to rodents or non-human primates is the pig, which has several similarities to humans in terms of the digestive physiology, feeding behaviour, sensory sensitivity and brain organization and functioning. Pigs also have high cognitive capacities that allow them to integrate very complex conditioned learning, especially when this learning is coupled with socio-environmental determinants. The last part of the review focuses on the features that make the pig an ideal model to study preferences and aversions in human nutrition and health research.

The pig: a preferential animal model in human nutrition research?

Numerous studies on the development of food aversions and preferences have been carried out in rodent models (e.g. Touzani and Sclafani, 2005 and 2007; Touzani *et al.*, 2009aand 2009b) and have led to many significant and useful findings on the behavioural and neurobiological mechanisms underlying these feeding processes (for a review, see Ferreira, 2004). However, due to the huge phylogenic difference between rodents and humans, rodents are not suitable models to study such processes in humans. The considerable metabolic and physiological differences between humans and rodents have complicated the translation of research findings into applications in human biomedical and nutrition research (Table 1; Spurlock and Gabler, 2008). Moreover, due to ethical and practical reasons, some research on feeding behaviour cannot be conducted in humans or in non-human primates, especially since a recent European directive (EU Directive E4131, 2008) limits the use of non-human primates as animal models. The need for new animal models for applications in the domain of human health and nutrition emerged during the last decades (Vodicka *et al.*, 2005), with alternative and complementary models allowing the translation of science into biomedical methods for prevention and intervention, especially in the case of obesity (Spurlock and Gabler, 2008). In this context, the pig has been used extensively in human nutrition research. In addition to having a longer lifespan than mice, greater cost savings in housing under controlled conditions than for non-human primates (Vodicka *et al.*, 2005) and lesser risk of zoonoses (diseases spread by animals), pigs have several similarities with humans.

Anatomo-physiological similarities

Pigs and humans have several anatomical and physiological features in common. Pigs are monogastric omnivores, such as humans, with proportionally similar organ sizes and very comparable gastrointestinal tract anatomy, morphology and physiology (Spurlock and Gabler, 2008), despite some slight anatomical differences in their digestive systems. The total length of the gastrointestinal tract of a growing pig weighing approximately 30 to 40 kg is similar to that of an adult human. Moreover, the relative diameters of human and pig gastrointestinal tracts are very comparable. Pigs and humans also have approximately the same dietary requirements in terms of nutrients, although the quantitative requirements for each nutrient differ between the two species (Gandarillas and Bas, 2009). As a consequence of their similar digestive physiology, pigs have been extensively used as a model for assessing nutrient absorption in humans (Gandarillas and Bas, 2009).

The similarities between the two species extend to numerous other physiological functions (Vodicka *et al.*, 2005). For example, pigs and humans also have very similar cardiovascular systems (Xi *et al.*, 2004; Sahni *et al.*, 2008; Spurlock and Gabler, 2008), making pigs an excellent model for cardiovascular studies and for the development of new surgical procedures. Moreover, due to the similar size and physiological capacity of the organs, pigs may be the most suitable donors for animal-to-human xenotransplantation (Vodicka *et al.*, 2005; Sahni *et al.*, 2008). For the same reasons, pigs have also been used as a general surgical model for most organs and systems, particularly to assess the feasibility of surgical techniques or to evaluate their postoperative metabolic consequences (for a review, see Gandarillas and Bas, 2009). Another interesting factor is that pigs can develop some of the same disease as humans, such as obesity, diabetes, or cardiovascular diseases such as atherosclerosis (for a review, see Jokinen *et al.*, 1985). For instance, miniature Ossabaw pigs have a 'thrifty genotype' that confer them with a naturally increased predisposition to the development of obesity or insulin resistance in response to high-fat/high-carbohydrate diets (Dyson *et al.*, 2006; Clark *et al.*, 2011). Gôttingen minipigs also have a high propensity

Table 1 *A non-exhaustive comparison of features and strains in pigs and rodents, highlighting the similarities or discrepancies of these models with humans, and their advantages and limitations for studies on human nutrition and eating disorders*

	Pigs	Rodents
General features		
Phylogeny	Close to humans	Huge difference from humans
Lifespan	Long (12 to15 years), enabling long-term studies	Short (2 to 3 years)
Availability	Numerous breeds, including conventional and miniature pigs	Some rodent models (e.g. obesity) derived from closely bred strains - homogeneous genetic data altering the translation of knowledge to humans with high genetic heterogeneity (Augustine and Rossi, 1999)
Genome	Sequenced (Archibald *et al.*, 2010)	Sequenced, easily modified by genetic engineering, but extreme cost of maintaining the offspring at a sufficient scale (Speakman *et al.*, 2008)
Housing recommendations (EU Directive 8869/10)	5- to 50-kg pig or minipig: compartment . 2 m^2, surface per animal of 0.20 to 0.70 m^2 in the case of group housing 50- to 100-kg pig: compartment . 3 m^2, surface per animal of 0.80 to 1 m^2 in the case of group housing	20- to 30-g mouse: 330 cm^2 in laboratory 200- to 600-g rat: 800 cm^2 in laboratory . 600g-rat: 1500 cm^2
Behavioural features		
Sweet craving, phagomania	Reported both in obese pigs (Val-Laillet *et al.*, 2010c) and in humans (Yanovski, 2003)	Reported in obesity-prone compared with obesity-resistant rats (Pickering *et al.*, 2009)
Learning and cognitive abilities	Efficient learning abilities during behavioural tests (e.g. the open field or the novel object tests; Lind *et al.*, 2007; Kornum and Knudsen, 2011)	Rats less efficient compared with pigs during some cognitive tasks (e.g. progressive ratio; Ferguson *et al.*, 2009) or social recognition tests (Held *et al.*, 2005)
Anatomo-physiological features		
General anatomy	Organ sizes proportionally similar to humans	Small size of the organs with a different overall organization
(GIT)	Very comparable to humans, (e.g. similar length and diameter of the GIT of a growing pig and that of a human; Spurlock and Gabler, 2008)	Same overall organization of the GIT as in humans, but few differences (e.g. relative lengths of the small intestine; DeSesso and Jacobson, 2001). Different anatomical and functional development of the GIT (Ménard, 2004)
Digestive physiology	Similar dietary requirements, digestive physiology and nutrient absorption processes as in humans (Gandarillas and Bas, 2009)	Differences in the relative absorptive surface areas of the GIT (e.g. faster nutrient absorption in humans than in rats; DeSesso and Jacobson, 2001)
Ability to develop human diseases	Obesity (Val-Laillet *et al.*, 2010a, 2010b and 2010c; Clark *et al.*, 2011), diabetes (Bellinger *et al.*, 2006; Liu *et al.*, 2007), atherosclerosis (Xi *et al.*, 2004; Miyoshi *et al.*, 2010).	Obesity, metabolic syndrome (Li *et al.*, 2008; Aleixandre de Artiñano and Miguel Castro, 2009)
Adipokines and obesity	Same adipokines linked to obesity in pigs and in humans (e.g. adiponectin and leptin; Spurlock and Gabler, 2008)	Conflicting results compared with humans (e.g. lower adipsin rates in obese than in lean mice *v.* higher rates in obese than in lean humans, TNF-a released into the circulation in obese animals but not in obese humans; Arner, 2005)
Taste receptors	Intestinal taste receptor subunits (T1R2 1 T1R3, associated with the gustatory G-protein (gustducin) involved in sweet taste recognition characterized in pigs (Moran *et al.*, 2010b) humans (Li *et al.*, 2002) and rats (Mace *et al.*, 2007)	
Sweet perception	Perception of the sweet taste of some compounds known to be sweet to humans by pigs (Hellekant and Danilova, 1996 and 1999) and rats (Frank and Blizard, 1999)	
Neurobiological features		
Brain anatomy	Gyrencephalic brain of approximately 180 g (1300 g in humans; Sauleau *et al.*, 2009)	Lissencephalic brain of approximately 10 g (Sauleau *et al.*, 2009)
Brain structures	Brain similar to that of humans in terms of structure, vascularization, anatomy, growth and development (Vodicka *et al.*, 2005; Lind *et al.*, 2007)	Many differences in the organization of some brain structures and in neuronal density compared with humans (e.g. amygdala; Pitkänen and Kemppainen, 2002)

Table 1 Continued

	Pigs	Rodents
Imaging techniques	Large brain that enables the identification of cortical and subcortical structures by neurosurgery or conventional imaging techniques in living animals (MRI, CT, SPECT, PET; Sauleau *et al.*, 2009)	Small brain compatible for micro-imaging techniques (micro-PET, micro-MRI, micro-CT; e.g. Tai *et al.*, 2005; Wu *et al.*, 2008), but with higher radiation exposure to obtain the same resolution as in humans - potential tissue damage (Ritman, 2007)
Neurotransmitters	Similar neurotransmitters involved in feeding behaviour (serotonin, dopamine, opioid systems), e.g. developing 5-HT system in human infants and piglets (Niblock *et al.*, 2005)	Similar neurotransmitters involved in feeding behaviour (e.g. the dopamine system related to the food reward perception; Barbano and Cador, 2007), serotonin system involved in hedonic processing during food intake (Berridge, 2000)
Brain and obesity	Deactivation of some brain structures (e.g. prefrontal cortex) in obese compared with lean subjects (Val-Laillet *et al.*, 2011), as in humans (Le *et al.*, 2006)	Deactivation of the frontal cortex and activation of the superior colliculus in obese compared with lean rats (Thanos *et al.*, 2008)
Examples of the strains currently used as models for human obesity and/or eating disorders		
Induced models of obesity		
Genetic models	Knockout models of pigs (e.g. Casu *et al.*, 2010), but not dedicated to the study of feeding behaviour or nutritional diseases	Numerous knockout models to study eating pathologies in humans (e.g. the *axl* mouse ; action on the tyrosine kinase receptor; progressive obesity without hyperphagic behaviour but with an increase of TNF-a)
Dietary models	High propensity of Gôttingen minipigs (Val-Laillet *et al.*, 2010a, 2010b and 2010c) and microminipigs (Miyoshi *et al.*, 2010) to develop obesity in response to diets enriched with carbohydrates and lipids in only 15 weeks	Diet-induced obesity rodents with increase of body weight, adiposity, circulating leptin and insulin levels and decrease of insulin sensitivity. But, discrepancies in gene-expression alterations between diet-induced obese rats and obese humans (Li *et al.*, 2008)
Spontaneous models of obesity		
Genetic models	Thrifty genotype of Ossabaw minipigs with a natural predisposition to the development of obesity in response to high-fat/high-carbohydrates diets and even in absence of high-fat diets (Dyson *et al.*, 2006; Spurlock and Gabler, 2008; Clark *et al.*, 2011)	Ten spontaneous single-gene mutations leading to obesity (Augustine and Rossi, 1999; Speakman *et al.*, 2008; e.g. *ob/ob* mice; mutations in the leptin gene), *db/db* mice and Zucker (*fa/fa*) obese rats (mutations in the leptin receptor gene) - spontaneous obesity with increased weight gain and hyperphagia
Spontaneous or induced models of anorexia nervosa		
	The wasting pig syndrome, infectious disease caused by porcine circovirus 2 (Chae 2004) used as a model of anorexia nervosa with decreased appetite, great weight loss and acute motor activity (Casper *et al.*, 2008; Treasure and Owen, 1997)	'Activity-stress' or 'activity-based anorexia' model in mice and rats with restricted food intake in the presence of hunger, weight loss, excessive activity (Casper *et al.*, 2008) Anorexic (*anx/anx*) mouse, spontaneous mouse mutation with decreased food intake leading to death)
Induced models of binge eating		
	Sweet craving induced thanks to dietary model of obesity in minipigs, with exacerbated preference of obese minipigs for high-carbohydrate diets paired with high food intake (Val-Laillet *et al.*, 2010c)	Genetic models: link between high sensitivity to stress and binge eating disorders - genetic mouse model of stress sensitivity used to induce binge eating to high-fat or high-carbohydrate diets (e.g. CRFR2-deficient mice; Teegarden and Bale, 2008)

GIT 5 gastrointestinal tract; MRI 5 magnetic resonance imaging; CT 5 computed tomography; SPECT 5 single photon emission computed tomography; PET 5 positron emission tomography; 5-HT 5 the medullary serotoninergic system; TNF 5 tumour necrosis factor.

to develop obesity (i.e. weight gain, overeating) in only 15 weeks and in response to diets enriched in carbohydrates and lipids (e.g. Val-Laillet *et al.*, 2010a, 2010b and 2010c). Thus, conventional pigs and minipigs are often used as models of high-fat and/or high-carbohydrate diet-induced obesity (Val-Laillet *et al.*, 2010a, 2010b, 2010c and 2011, Clark *et al.*, 2011), diabetes (Bellinger *et al.*, 2006; Liu *et al.*, 2007) or atherosclerosis (Xi *et al.*, 2004).

With regard to hormonal regulation of feeding behaviour, pigs and humans share some taste receptors and hormones that are involved in appetite/satiety regulation. Pigs' intestines have numerous sugar transporters similar to those in humans (for a review, see Wood and Trayhurn, 2003), such as GLUT5, a Na^1-independent fructose transporter, or the Na^1/glucose co-transporter 1 (SGLT1) that transports glucose and galactose from the lumen of the intestine into enterocytes (Moran *et al.*, 2010b; Shirazi-Beechey *et al.*, 2011). Moran *et al.* (2010a) reported that the supplementation of the diet of weaning piglets with artificial sweeteners (i.e. Sucram, a combination of saccharin and neohesperidin dihydrochalcone) led to an enhancement of the expression of SGLT1 and of the subsequent intestinal glucose transport function by acting on the intestinal and lingual sweet taste receptor T1R2 1 T1R3, subunits that are associated with the gustatory G-protein gustducin (for a review, see Shirazi-Beechey *et al.*, 2011). These intestinal taste receptor subunits and their involvement in sweet taste recognition have been characterized in pigs (Moran *et al.*, 2010b), humans (Li *et al.*, 2002) and rats (Mace *et al.*, 2007; Sclafani, 2007). Food intake also induces the release of several gut hormones from the endocrine cells of the small and large intestines, such as glucagon-like peptide 1 (GLP-1) or 2 (GLP-2) or the leptin, a hormone that is particularly expressed in adipocytes and acts as a satiety signal. These hormones and their involvement in the induction of satiety and regulation of feeding behaviour have been identified both in humans (Ahima and Antwi, 2008; Steinert *et al.*, 2011) and in pigs (Schlatter *et al.*, 2007; Liu *et al.*, 2011).

Neurobiological similarities

The use of pigs in neurosciences has increased widely in the past decade due to interesting neurobiological similarities between pigs and humans (for a review, see Lind *et al.*, 2007; Sauleau *et al.*, 2009). Pigs and humans have most of their cerebral structures in common and their brains appear to be comparable in terms of structure, vascularization, anatomy, growth and development (for a review, see Vodicka *et al.*, 2005; Lind *et al.*, 2007).

In terms of gross neuroanatomy, pigs have a convoluted or a gyrencephalic cortical surface, superficially resembling the human brain (Figure 1; Hofman, 1985), whereas rodents have a small lissencephalic brain. The pig brain, which has human-like vascularization characteristics, is large enough to enable the identification of cortical and subcortical structures by neurosurgery and conventional imaging techniques in living animals (Lind *et al.*, 2007; Sauleau *et al.*, 2009). The pig's brain, being relatively large, is suitable for imaging techniques and machines used for humans, for instance, magnetic resonance imaging, computed tomography, single photon emission computed tomography (SPECT) or positron emission tomography (PET; Figure 1). Thus, pigs have been used as a model for human research in a wide range of imaging studies, such as in traumatic brain injury (Grate *et al.*, 2003), Parkinson's disease (Mikkelsen *et al.*, 1999; Cumming *et al.*, 2003) or stroke (Sakoh *et al.*, 2000; Røhl *et al.*, 2002). Anatomical brain imaging studies on pigs have allowed the identification of swine cerebral structures and the conception of stereotaxic atlases of the pig brain (e.g. Felix *et al.*, 1999; Watanabe *et al.*, 2001; Saikali *et al.*, 2010). Thanks to these atlases, numerous anatomical brain analogies between pigs and humans have been highlighted.

Despite these anatomical similarities and the huge number of neurobiological studies, few studies have focused on the characterization of structures that are specifically involved in feeding behaviour and especially in the establishment of food preferences and aversions (Figure 2; Biraben *et al.*, 2008; Val-Laillet *et al.*, 2010d). Brain structures involved in the establishment of conditioned food preference or aversion and structures of the 'brain reward system' involved in the hedonic perception of food have been widely described in the rat model (for a review, see Ferreira, 2004; Berridge, 2009). This functional brain network consists of structures such as the amygdala (Gilbert *et al.*, 2003), the insular cortex (Desgranges *et al.*, 2009; Roman *et al.*, 2009) or the parabrachial nucleus (Reilly, 1999; Reilly and Trifunovic, 2000), which are involved in the establishment of a feeding preference or aversion, depending on the sensorial stimuli involved. Literature data also report 'hedonic hotspots' distributed in different brain structures such as the nucleus accumbens (Baldo and Kelley, 2007; Barbano and Cador, 2007; Pritchett *et al.*, 2010), the ventral pallidum (Berridge, 2009) or the subthalamic nucleus (Baunez *et al.*, 2002). The ventral striatum (i.e. nucleus accumbens) is also involved in feeding behaviour (Kelley *et al.*, 2002; Will *et al.*, 2006). These hedonic hotspots play a role in the perception of the hedonic features of food intake and in the characterization of food palatability, that is, mediate pleasure associated with the gustatory signals.

In contrast, few functional studies have been carried out in pigs on the brain structures specifically involved in feeding behaviour and especially in the establishment of food preferences and aversions. In the past decades, some neurobiological studies used pigs to investigate human brain anomalies and feeding behaviour disorders (Sauleau *et al.*, 2009). The changes in the metabolism of some brain structures in obese pigs, used as a model of obese humans, were studied using a SPECT imaging technique (Val-Laillet *et al.*, 2011). This study suggests that, as in obese humans, compared with lean subjects, obese minipigs (Figure 3) had relatively less activation in specific brain structures, including the prefrontal cortex, the nucleus accumbens and the ventral tegmental area. Moreover, it has been demonstrated that chronic vagus nerve stimulation, which was originally used as a treatment for refractory epilepsy in humans, also affected food intake and weight gain in humans and obese

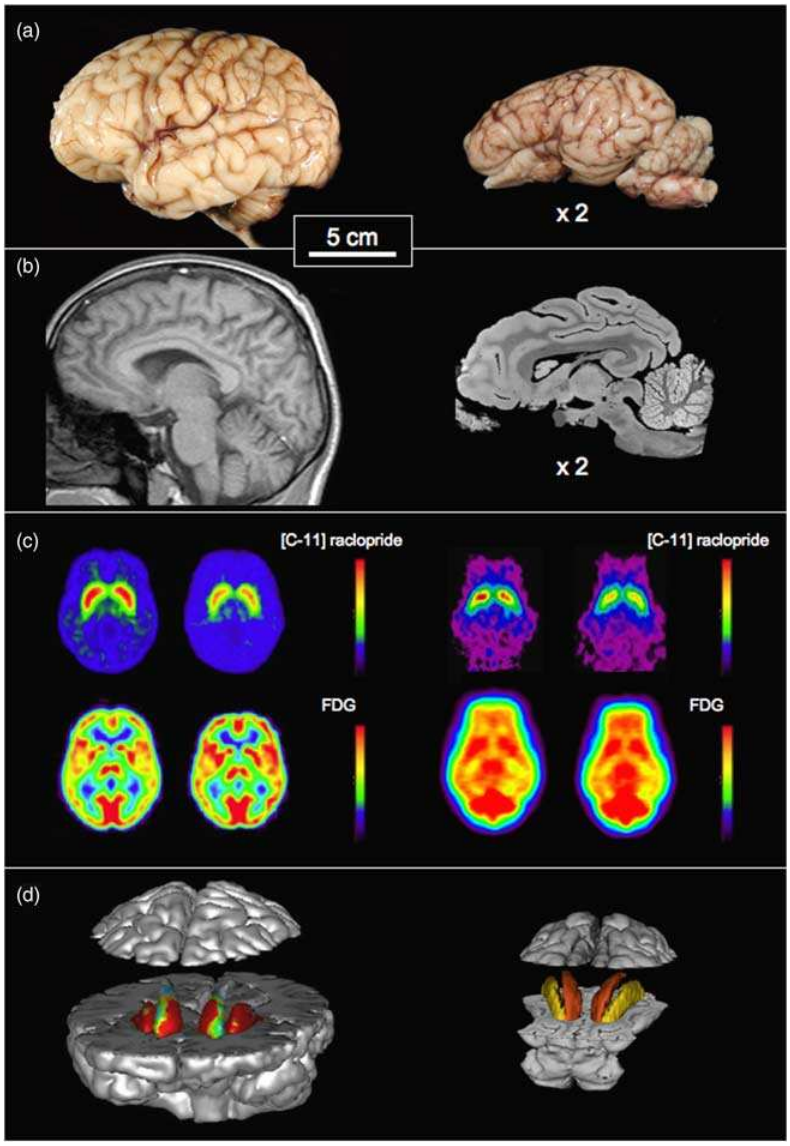

Figure 1 Comparison of human (left) and pig (right) brain images. (a) *Ex vivo* anatomical brain and (b) magnetic resonance brain images. The image of the extracted human brain was used with the permission of J. C. Fournet (University Hospital Sainte-Justine, Montreal, Canada, http://www.humpath.com). The other images are from our institution. (c) ^{11}C-Raclopride positron emission tomography (PET) and ^{18}fluorodeoxyglucose (^{18}FDG) PET brain images. PET images of humans were obtained with the permission of Gene-Jack Wang (Brookhaven National Laboratory, Upton, New York, USA) and Elsevier (Wang *et al.*, 2001), illustrating the metabolic differences between a lean and an obese patients, respectively. ^{11}C-Raclopride PET images of pigs were obtained with the permission of P. Cumming (Pet Center, Århus University Hospitals, Aarhus, Denmark; http://www.cfin.au.dk/index.php?menu 5 262). ^{18}FDG PET images of pigs are from our institution. (d) A three-dimensional (3D) view of the dopaminergic nuclei in both species. The human model was obtained from the website http://www.brainvisa.info/museum.html. The pig model was obtained from a stereotactic 3D atlas realized in our institution (Saikali *et al.*, 2010).

minipigs (Biraben *et al.*, 2008; Val-Laillet *et al.*, 2010c). Indeed, vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs (Val-Laillet *et al.*, 2010c). Numerous studies support the idea that this potential therapy against obesity would be as effective in humans as in animal models such as pigs. Interestingly, Biraben *et al.* (2008) studied the activation of cerebral structures during chronic vagus nerve stimulation using the SPECT imaging technique. They reported that chronic vagus nerve stimulation activated some cerebral structures known to be involved in feeding behaviour and the reward system (e.g. nucleus tractus solitarius and dorsal motor nucleus of the vagus, the olfactory bulb, the globus pallidus, the hippocampus and the cerebellum).

More recently, a study investigated for the first time the brain structures specifically involved in the establishment of food preferences and aversions in pigs (Gaultier *et al.*, 2011). The paradigm was based on the use of flavours positively or

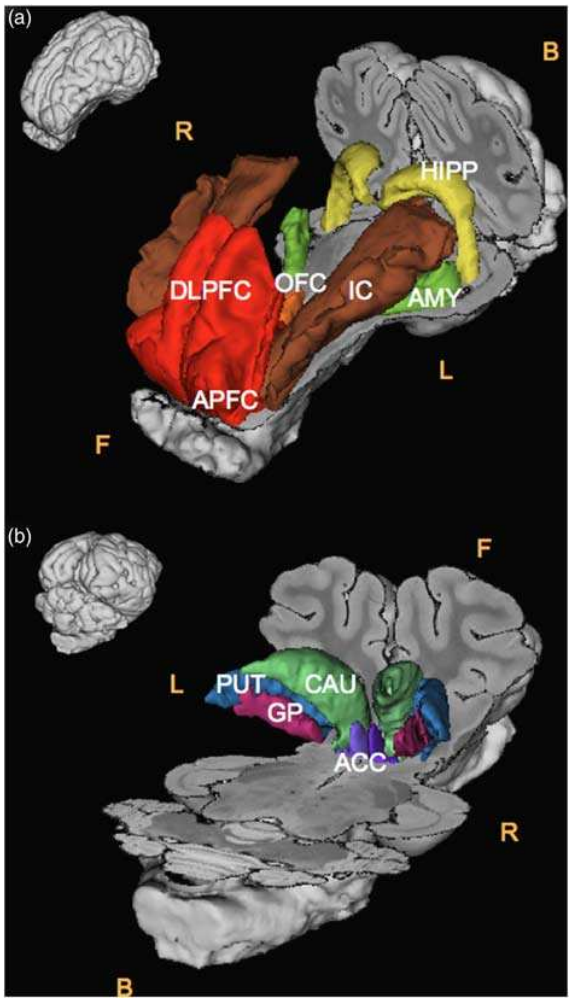

Figure 2 Localization of some brain structures involved in the establishment of food preferences and aversions, in reward expectation and/or in the characterization of food palatability. (a) Skinned front view of the pig brain with three-dimensional (3D)representations of the amygdala (AMY), the insular cortex (IC), the hippocampus (HIPP) and some structures of the frontal and prefrontal cortices, including the anterior prefrontal cortex (APFC), the dorsolateral prefrontal cortex (DLPFC) and the orbitofrontal cortex (OFC). (b) Skinned back view of the pig brain with 3D-representations of several structures of the 'brain reward system' including the nucleus accumbens (ACC), the globus pallidus (GP), the putamen (PUT) and the caudate nucleus (CAU). All these images were obtained from a stereotactic 3D atlas realized in our institution (Saikali *et al.*, 2010). In the top left corner of each part of the figure (a and b), complete 3D models of the pig brain in the same orientation as the skinned representations are shown (F 5 front; b 5 back; R 5 right; L 5 left).

negatively conditioned through the ingestion of a flavoured meal coupled with an intraduodenal injection of NaCl (sham treatment) or lithium chloride, respectively. The brain activations were then explored via SPECT during olfacto-gustatory stimulations with the conditioned flavours. The results showed contrasting brain activation patterns in response to the different flavours. Positively and negatively associated flavours notably induced different metabolic responses in the brain structures involved in food recognition, memorization and

Figure 3 The minipig is a good model for studying human diseases and pathologies in biomedical research. (a) Lean Gôttingen minipig; (b) obesity induced in a Gôttingen minipig after a high-fat and high-carbohydrate diet ('Western diet').

reward. These results are quite promising and could be coupled with the strong parallels highlighted in brain metabolism between pigs and humans. Such investigations represent interesting biomedical findings for the comprehension of the neurobiological mechanisms underlying the establishment of feeding behaviour in humans, with interesting opportunities for applications, notably for the treatment of eating disorders and obesity.

To extend the comparison between the brain metabolism of pigs and humans, it would be interesting to compare the neurotransmitter systems associated with the brain structures involved in feeding behaviour. It is well acknowledged today that the dopamine and opioid systems play an important role in the modulation of feeding behaviour, although they are involved in different steps of this process. These two systems, which are important for the 'reward circuit' and play a major role in food pleasure and selection, have been relatively well characterized in humans and rats (Berridge, 2000; Kelley *et al.*, 2002; Barbano and Cador, 2007; Barbano *et al.*, 2009; Wassum *et al.*, 2009) but not yet in pigs.

Literature data in rodents report that dopamine release could be related to the perception of the stimulus that predicts the reward (e.g. food reward; Barbano and Cador, 2007). The dopamine system is thus rather related to the appetitive phase of feeding behaviour, that is, the phase that precedes the consumption itself. Regarding the opioid system, it seems to

be involved in the modulation of the food hedonic perception and in the characterization of food palatability (Barbano and Cador, 2007; Barbano *et al.*, 2009). To summarize, although the opioid system seems to be involved in the modulation of the perception of the hedonic features of food, dopamine plays more of a role in the anticipatory aspect of feeding. It is obvious that other neurotransmitter systems are involved in the modulation of feeding behaviour. In his review, Berridge (2000) mentioned that the serotonin system may be involved in hedonic processing during food intake, suggesting that serotonin causes a specific negative shift in palatability.

Thanks to molecular imaging techniques, the distribution of the dopamine and serotonin neurotransmitters has been well characterized in pigs' brains. In their review, Niblock *et al.* (2005) carried out a comparison between the medullary serotoninergic (5-HT) system development and the anatomy of human infants and piglets. They concluded that the developing 5-HT systems of human infants and piglets are very close, although some structural and developmental differences exist. Despite these slight differences, some serotonin receptors (e.g. $5HT_{1B}$) are very similar to those of humans (Lind *et al.*, 2007). As impairments in the serotoninergic system (5-HT) are known to be involved in several brain diseases in humans (e.g. depression, schizophrenia, Alzheimer's disease), some authors developed and validated pig models for serotonin depletion. Cumming *et al.* (2007) reported that the vulnerability of serotonin transporters in pigs to 3,4-methylendioxymethamphetamine treatment and the distributions of serotonin transporters and $5HT_{1A}$ receptors in the brain of Göttingen minipigs are similar to those reported in humans. Ettrup *et al.* (2011) also investigated the distribution of 5-HT_{1A} and 5-HT_{2A} receptors in the pig brain. Their results showed that the binding of 5-HT_{1A} and 5-HT_{2A} receptors was not affected by serotonin depletion achieved by a parachlorophenylalanine treatment, whereas this treatment increased 5-HT_4 receptor binding, especially in the nucleus accumbens. They also showed that, overall, the distributions of 5-HT_{1A} and 5-HT_{2A} receptors were concordant with those of humans. Interestingly, according to Prelusky's study (1993), serotoninergic activity is negatively correlated to food intake, given that a decrease in food intake after the administration of a toxic substance (mycotoxin : deoxynivalenol) is associated with a decrease in brain serotonin turnover. Although the dopamine system has received less attention, the distribution of mesencephalic neurons is similar in pig and human brains (Minuzzi *et al.*, 2006; Lind *et al.*, 2007). The availability of D_2 dopamine receptors for binding of radioligands (e.g. ^{11}C-raclopride) is influenced by competition from endogenous dopamine. In their PET study, Lind *et al.* (2005) reported some similarities in the decreased availability of ^{11}C-raclopride-binding sites for D_2 receptors in the striatum caused by amphetamine treatment between pigs and humans. In their autoradiography study using [^{3}H]raclopride and [^{3}H]SCH 23390, respectively, Minuzzi *et al.* (2006) showed that the distribution and the density of dopamine $D_{2/3}$ and D_1 receptor-binding sites of Göttingen minipigs are very similar to those of humans, with a high abundance of these receptors. The use of dopamine receptor ligands such as ^{11}C-raclopride may indeed represent an interesting tool to understand the normal and pathological molecular mechanisms underlying feeding behaviour. Some studies identified efficient radioligands and isotopes currently used to explore the dopamine transporter (DAT) because this molecular target is involved in numerous neurological diseases such as Parkinson's disease in humans. In their study, Wang *et al.* (2007) used the ^{18}F-FP-CIT, a radiotracer that binds specifically to DAT, whereas Chalon *et al.* (2006) used the ^{11}C-LBT-999 to investigate the DAT variations in baboons. Minuzzi *et al.* (2006), however, reported that several usual radioligands failed to bind to DAT in the pig brain, although they revealed the presence of DAT in rat, ferret, monkey and human brains. However, according to previous studies using a ^{11}C-raclopride paradigm, pigs possess functional DAT (Rosa-Neto *et al.*, 2004). These discrepancies may be due to the aberrant binding properties of DAT in pigs compared with those in other species. Altogether, these results emphasize the limitation of using pigs for some dopamine studies. These radioligands have not yet been used to investigate the involvement of these neurotransmitters during feeding behaviour in pigs. The establishment of eating disorders in humans is strongly influenced by the perception of food sensorial characteristics and palatability, and interestingly, some studies showed that obesity and/or food addiction, for example, are associated with brain metabolic disorders including the low availability of D_2-receptors (Wang *et al.*, 2001; Volkow *et al.*, 2008). The existing similarities of some neurotransmitter systems involved in the perception and characterization of food in pigs and humans represent a huge opportunity to gain a better understanding of these diseases. It would be interesting to use these radioligands to quantify the involvement of neurotransmitter receptors and transporters in the acquisition of food preferences or aversions.

Behavioural similarities

Neophobic responses towards food. The knowledge generated about pig behaviour in livestock production enables to draw an interesting parallel between the pig and human feeding behaviour, for example, in the development of food preferences and aversions, or the emergence of neophobic responses towards novel food. In livestock production, pigs may face stressful periods during which their feeding activity is strongly disrupted due to unfamiliar feeding and environmental conditions (Meunier-Salaün and Picard, 1996). For instance, at weaning, piglets have to face a huge and abrupt modification of their diet associated with important changes in their physical and social environment. Weaned piglets are separated from their mother (disruption of the mother–young bond) and are classically mixed in pens with unfamiliar congeners. Their diet changes drastically, with the disappearance of the mother's milk and the supply of a concentrate diet mainly formulated with cereals. During the growth period, diets are formulated to satisfy the animal's nutrients and energy requirements and depend on the available dietary sources. When exposed to novel food during the food transition, a period of slow growth is often

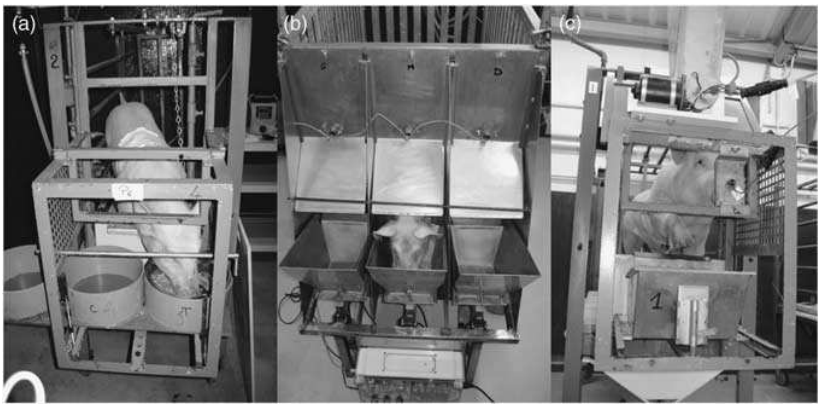

Figure 4 Examples of the experimental cages and operating devices used to investigate the feeding behaviour and motivation in pigs. From left to right: (a) simple two-choice feeding test, in which the animal has to choose between two different diets, (b) three-choice feeding test, in which the animal has to choose between three different diets held in three different troughs equipped with mechanical trap doors controlled by three different buttons accessible to the animal and (c) operant conditioning test, in which the animal has to push a button to activate the food dispenser, the number of pushes necessary to obtain a small ration of food being variable. The operating devices and testing parameters are controlled by a computer and all troughs can be connected to strain gauges, allowing for a precise calculation of the quantity consumed and the ingestion speed. All these images are from our institution. The operating devices and analysis software solutions were designed by C. H. Malbert and E. Bobillier.

reported in pigs, until such time as they accept their novel feeding and environmental conditions fully (Campbell, 1976; Dong and Pluske, 2007). Humans, and especially children, also exhibit this 'neophobic response' towards novel foods and this phenomenon is reinforced by an associated novel environment (Hursti and Sjödén, 1997). This response is caused by the fear of novelty and is responsible for transiently decreased food consumption.

The development of feeding behaviour and its environmental determinants. In addition to this neophobic response towards food, pigs and humans share some development characteristics of their feeding behaviour and especially for the acquisition of food preferences and aversions. As in humans (Mennella and Beauchamp, 1993; Beauchamp *et al*., 1994), the food choices in weaned piglets can be modulated by the mother's diet or early experience (King, 1979). Indeed, piglets weaned from sows fed with a flavoured diet and then fed with a post-weaning diet of a similar flavour ate significantly more food and grew significantly faster during the immediate post-weaning period than pigs that were not familiar with the flavour (Campbell, 1976; Langendijk *et al*., 2007; Oostindjer *et al*., 2010).

The impact of conspecifics is also an important social factor that influences food choices and intake among pigs (Forbes, 1995; Meunier-Salaün and Picard, 1996; Meunier-Salaün *et al*., 1997; Meunier-Salaün and Bergeron, 2005). In the study of Meunier-Salaün *et al*. (1997), piglets aversively conditioned towards a diet with concanavalin A (an emetic substance) added and re-exposed to the aversive diet showed diet refusals, indicating that they remembered the conditioning. However, when re-exposed to the aversive diet in the presence of a naïve congener, conditioned pigs resumed eating. This social facilitation phenomenon is also encountered in humans: observing people eating may influence children's food preferences, thanks to the tendency of children to imitate their peers' behaviour, especially in the home environment (Hursti and Sjödén, 1997; Wardle and Cooke, 2008). Moreover, food diversity allows for better food intake in humans (Gerrish and Mennella, 2001). When a choice of diets is offered to pigs during growth, pig performance (i.e. food intake, daily weight gain) is highly improved compared with a situation where pigs have no food choice (Lawlor *et al*., 2003).

Cognitive abilities during behavioural tests aiming to assess feeding behaviour. As feeding behaviour requires learning and memory capacities, the animal model chosen for studying feeding behaviour in humans must have significant cognitive capacities. Numerous studies have investigated and attested to the learning and memory abilities of pigs during behavioural tests (e.g. the open field or the novel object tests; for a review, see Lind *et al*., 2007; Kornum and Knudsen, 2011).

The numerous tests developed to assess feeding preferences are based on the hypothesis that preferred food (i.e. the most palatable food) would be consumed the most (for a review, see Meunier-Salaün and Picard, 1996; Meunier-Salaün and Bergeron, 2005). Two main types of methods emerge: choice tests and operant conditioning (Figure 4). Two methodologies exist in the feed choice tests: a one-way test in which various diets are alternatively presented, and the 'multiple-way choice test' in which two or more diets are presented simultaneously in a free-choice situation (e.g. Schöne *et al*., 2006; Guillemet *et al*., 2007; Sola-Oriol *et al*., 2009). In the free-choice situation, the result does not predict the behaviour in a practical situation in which a unique food is usually supplied, whereas the one-way test allows the analysis of feeding preference, but at the same time limits the influence of alternate food resources

available in the livestock environment, such as straw indoors or various herbaceous and invertebrate resources outdoors (Meunier-Salaün and Picard, 1996). In the case of the operant conditioning methodology, pigs must work (e.g. push a button) to obtain a resource, food (e.g. Bergeron *et al.*, 2000; Robert *et al.*, 2002), space or a social stimulus. Operant conditioned tests are used to assess the feeding motivation and feeding preferences, based on the assumption that the quantity of work provided would be higher for food and for preferred food.

From the perspective of animal production, these methods have been used extensively to understand the feeding problems (under- or overconsumption) encountered in livestock production and to improve the rate of weight gain (e.g. growth of growing pigs: Campbell, 1976; King, 1979; Lawlor *et al.*, 2003; Edge *et al.*, 2005; Schöne *et al.*, 2006; Langendijk *et al.*, 2007; Sola-Oriol *et al.*, 2009; or reproductive sows: Bergeron *et al.*, 2000; Robert *et al.*, 2002; Guillemet *et al.*, 2006 and 2007). Just like weaning pigs, reproductive sows have to cope with changes in their physical and social environment and modifications in their diet throughout their breeding cycle. Pregnant sows are subjected to a food restriction to prevent overeating and subsequent excessive weight gain. After farrowing, lactating sows receive *ad libitum* feeding and a novel food, which is adapted to the very high energy requirements of milk production (Forbes, 1995). During this transition phase, usually, the spontaneous food consumption of the animal is low, especially in primiparous sows (Forbes, 1995). Insufficient food intake generally induces lower productivity (decreased milk production and/or fertility) and decreased animal welfare (weight loss, weakened state; Dourmad *et al.*, 1994).

As high-fibre diets may have beneficial effects on sows' welfare during both gestation and lactation (Philippe *et al.*, 2008), several studies have investigated the use of such diets to regulate food consumption of reproductive sows. The use of fibrous diets is a promising method to prevent overeating in gestating sows because such diets seem to reduce hunger and maintain satiety for a longer period of time after feeding in restricted-fed sows (Meunier-Salaün *et al.* 2001; Robert *et al.*, 2002). In their study, Bergeron *et al.* (2000) also showed that a high-fibre diet efficiently increased satiety in gestating sows, but they failed to demonstrate that this diet reduced food motivation in operant tests. The discrepancy between this study and previous ones may be due to protocol differences, such as their use of relatively old sows when other studies used gilts. Providing a high-fibre diet during gestation may also be beneficial for lactating sows because it prepared the sows for an *ad libitum* food supply after farrowing and increased food consumption, especially in primiparous young sows and during the first week of lactation (Guillemet *et al.*, 2006). However, when subjected to two-way choice tests, gestating sows preferred standard gestation and lactation diets to a high-fibre diet, consistent with its lower palatability (Guillemet *et al.*, 2007 and 2010). These results showed the positive impact of a fibrous diet in improving animal welfare during pregnancy when the diet was supplied without any alternative choice, and also highlighted the necessity to ameliorate its organoleptic properties, so as to prevent its avoidance under circumstances of multiple food choices.

Even more than sows, adult humans are subjected to a plethora of physiological (e.g. pregnancy, ageing) and social changes throughout life and have to adapt their feeding behaviour according to these changes. Studying the modulation of feeding behaviour in sows may enable a better understanding of the mechanisms underlying modulations of human feeding behaviour. For instance, interesting parallels can be drawn between the effects of dietary fibre supplementation in reproductive sows and in humans. Lindström *et al.* (2006) showed that an increased fibre intake coupled with a low-fat diet induced a long-term weight reduction in overweight adult humans, suggesting that fibres may also be used to prevent overweight in humans.

Gustatory responses to food. As the flavour of food plays a major role in the establishment of preferences and aversions, a good animal model must have well-developed sensorial capacities and share some characteristics with humans in terms of taste and odour responses. Using behavioural feeding choice tests, Glaser *et al.* (2000) highlighted several similarities in gustatory responses towards some carbohydrates (mono- and oligosaccharides), polyols and natural or artificial compounds used as sweeteners in humans. Pigs showed gustatory preference for all the 15 carbohydrates (e.g. sucrose, fructose, glucose) tested over water, as for all the seven polyols (e.g. xylitol). Moreover, for 12 out of the 15 carbohydrates tested in pigs (like sucrose or fructose), detection and recognition thresholds on a molar basis were relatively close to the thresholds found in humans. In terms of natural or artificial sweeteners (e.g. sucralose, saccharin), 5 out of the 12 sweeteners tested elicited gustatory responses in pigs, but of a weaker intensity than that in humans. Tinti *et al.* (2000) carried out similar experiments to compare pigs' and humans' gustatory responses to glycine and 28 amino acids. Out of 17 amino acids, which are sweet to humans, 12 were preferred by pigs over water during two-bottle tests. Altogether, these results confirm the existence of a general positive correlation between pigs' and humans' preferences towards sweet compounds. However, Nofre *et al.* (2002) also tested gustatory responses of pigs towards 60 compounds perceived as sweet by humans using the two-bottle preference test method. According to their results, only 35 out of the 60 compounds tested elicited preference responses in pigs, among these most notably lugduname and carrelame (i.e. two of the most potent artificial sweeteners known in humans). These results emphasize that it is essential to make no hasty conclusions and to take into account the fundamental differences between pigs' and humans' preferences towards sweet compounds, especially because data refer to a different method of evaluation (Tinti *et al.*, 2000; Nofre *et al.*, 2002). In humans, evaluation of sweetness is based on a subjective assessment of the intensity of a compound's sweet taste. In pigs, sweetness

evaluation refers to solutions' palatability, which is assessed by the mean of preference tests, based on consumption rates and feeding behaviour.

Studies based on electrophysiological recordings allow better comparison between the gustatory responses of pigs and humans. Responses to taste stimulations in pigs were recorded at the level of the chorda tympani nerve (CT) and the glossopharyngeal nerve (Hellekant and Danilova, 1999). The information of taste is assessed through nerve fibres classified according to their response to salt, sour, sweet and bitter compounds (e.g. the fibres are designated as sweet if sucrose elicits the maximum responses; Hellekant and Danilova, 1996). In electrophysiological studies on pigs, recordings of the spontaneous nerve impulses after various taste stimulations (i.e. rinsing the tongue with different solutions of interest) have been used to classify the fibres' responses in terms of quality and intensity. In Hellekant and Danilova's study (1996), 13 compounds known to be sweet to humans have been tested in pigs. Out of the 13 compounds, three (sucrose, glucose and fructose) elicited responses of the CT fibres, thus demonstrating that pigs perceived the sweet taste of these compounds. Conversely, 7 out of these 13 compounds, including alitame, aspartame, super-aspartame and saccharine, did not elicit or elicited little nerve response, although these compounds are perceived as sweet-tasting by humans. Similarly, among 30 compounds tested that are sweet to humans, only glycine, xylitol, sucrose, fructose and glucose elicited nerve activity (Hellekant and Danilova, 1999). These electrophysiological data showed that it is of fundamental importance to exercise caution in assuming the cross-species identity of taste preference because some porcine gustatory responses are different from those of humans.

Conclusions

The extensive physiological similarities between the pig model and humans in the major mechanisms involved in the regulation of the feeding behaviour emphasize the research perspectives using a pig model to investigate the behavioural and neurophysiological mechanisms underlying the establishment of food preference and aversions, in relation to human nutrition issues. However, the use of pigs is not free from limitations. Owing to the high weight of adult standard pigs, imaging studies are carried on juveniles and the translation of research findings into applications in adult human biomedical research must be carried out carefully. The emergence of minipig models represents an interesting alternative to the use of standard pigs in biomedical research. Various strains of minipig promise to enable longitudinal studies and/or studies on adult stages, providing an accurate translation into human applications.

Acknowledgements

Caroline Clouard was supported by a grant from the Brittany Region (Région Bretagne) and INRA (Department of Human Alimentation; Department of Animal Physiology and Livestock Systems). The authors are grateful to Dr Charles-Henri Malbert, who is the initiator and head of the PRISM imagery platform (INRA, St Gilles, France) and the INRA MINIPIG program. The authors thank Dr Céline Tallet for helpful comments on the manuscript. They also thank the two anonymous reviewers for their interesting and valuable suggestions.

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Comme nous venons de le décrire dans la première partie, le porc apparaît comme un modèle prometteur pour l'étude du comportement alimentaire et de sa régulation. Ses similitudes avec l'Homme, en termes de comportement ou d'anatomie digestive et cérébrale, offrent des opportunités intéressantes d'application en santé et nutrition humaine. De telles applications en recherche biomédicale supposent que, outre des similitudes sur l'anatomie des structures cérébrales, le porc et l'Homme partagent aussi des caractéristiques cérébrales fonctionnelles communes, notamment concernant les circuits neuronaux impliqués dans la régulation hédonique du comportement alimentaire. Dans un premier temps, il est donc nécessaire de caractériser et de définir précisement l'organisation fonctionnelle de ces réseaux chez l'Homme, afin d'obtenir une base solide qui permettra une comparaison pertinente avec les systèmes qui seront caractérisés chez le porc au cours de cette thèse. Ainsi, dans cette deuxième partie de l'introduction, nous allons nous attacher à décrire les mécanismes centraux du traitement hédonique des informations sensorielles, et plus particulièrement des informations olfacto-gustatives, à partir de données recueillies principalement chez les rongeurs, l'Homme et les primates non-humains.

Partie 2. Mécanismes centraux du traitement sensoriel et hédonique des informations olfacto-gustatives

La prise alimentaire est régulée par deux boucles principales : la voie homéostatique et la voie hédonique (pour revue, Berthoud et Morrison, 2008). La voie homéostatique, dont l'hypothalamus fait office de pierre angulaire au niveau central, régule la prise énergétique et la balance faim/satiété en fonction de différents signaux périphériques nerveux, hormonaux ou peptidiques (*e.g.* insuline, leptine, ghréline) provenant du tractus gastro-intestinal et d'organes annexes de la digestion comme le pancréas, le tissu adipeux ou le foie (pour revue, Badman et Flier, 2005). La voie hédonique, quant à elle, intègre les valeurs motivationnelle, émotionnelle et cognitive de la prise alimentaire qui peuvent être modulées par de nombreux facteurs externes au processus digestif, comme l'expérience du sujet (apprentissage, mémoire), le contexte environnemental et social de la prise alimentaire, ou encore la différence entre la prévision et l'obtention effective d'une récompense.

Dans cette synthèse bibliographique, nous nous intéresserons exclusivement à la boucle de régulation hédonique du comportement alimentaire, et nous concentrerons donc uniquement sur les structures cérébrales extra-hypothalamiques et extra-médullaires qui sont impliquées dans la régulation de la prise alimentaire. Après avoir introduit brièvement les

voies de traitement périphérique et central primaire de l'identité sensorielle des informations olfacto-gustatives (lors de l'approche ou de la mise en bouche de l'aliment par exemple), nous décrirons plus en détail les processus sous-tendant le traitement central hédonique des informations olfacto-gustatives en détaillant les structures cérébrales et les systèmes de neurotransmetteurs impliqués.

1. Traitement périphérique et central de l'identité sensorielle des informations olfacto-gustatives

Les voies de traitement périphérique et central de l'identité sensorielle des informations olfacto-gustatives, ainsi que leurs connexions avec les structures impliquées dans le traitement hédonique de ces informations, sont illustrées dans la **Figure 1**.

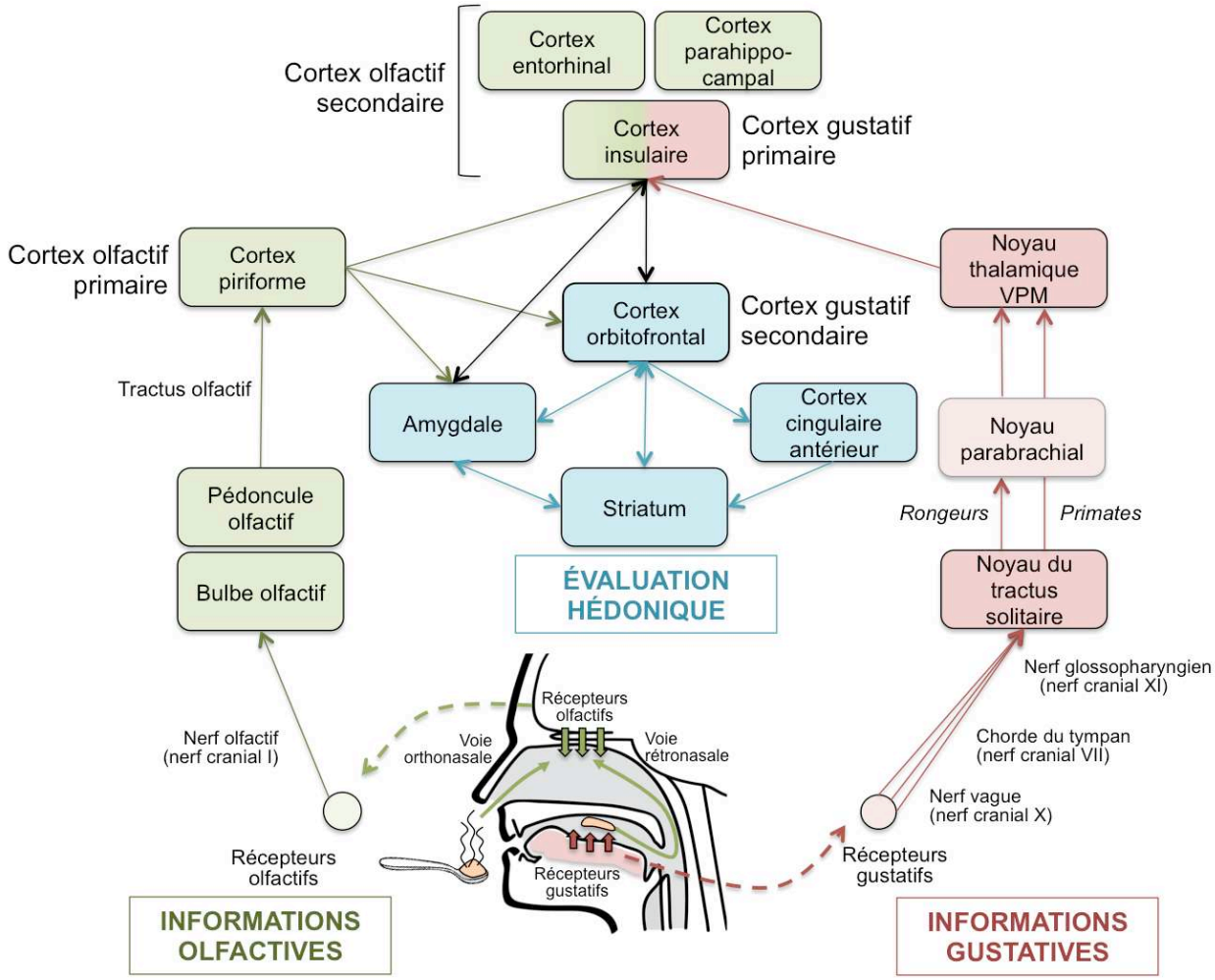

Figure 1. Voies de traitement périphérique et central des informations olfactives et gustatives, et connexions avec le système du traitement hédonique des informations sensorielles. VPM : ventro-postéro-médian (adapté d'après Rolls et al. 2004a)

1.1. Traitement sensoriel des informations olfactives

D'un point de vue anatomique, le traitement sensoriel des informations olfactives a été relativement bien étudié chez les modèles mammifères (rongeurs et primates non-humains), mais peu chez l'Homme (pour revues, Gottfried, 2010 ; Soudry *et al.*, 2011). Les informations olfactives perçues d'abord par la voie orthonasale, *i.e. via* la cavité nasale lors d'inhalation de molécules olfactives, puis par la voie rétronasale, *i.e. via* l'épithélium olfactif lors de la mise en bouche d'un aliment, convergent *via* le nerf olfactif (nerf cranial I) vers le bulbe olfactif qui représente le premier relais central du système olfactif. Les informations traversent ensuite successivement le pédoncule olfactif puis le tractus olfactif pour rejoindre le cortex olfactif primaire qui est composé principalement du cortex piriforme (PiC), bien que les frontières du cortex olfactif primaire soient encore mal connues chez l'Homme (Gottfried, 2010). D'un point de vue fonctionnel, le PiC est notamment impliqué dans l'évaluation de l'intensité des odeurs (Rolls *et al.*, 2003). Le cortex olfactif primaire est lui-même connecté à différentes structures cérébrales, parmi lesquelles le cortex insulaire (IC) et le cortex entorhinal attaché au cortex parahippocampique (PHC), qui constituent le cortex olfactif secondaire. En outre, le cortex olfactif primaire émet des projections vers d'autres structures cérébrales telles que le cortex orbitofrontal (OFC), le cortex cingulaire antérieur (ACC), les noyaux de la base ou encore l'amygdale (AMY) (Gottfried, 2010 ; Soudry *et al.*, 2011).

1.2. Traitement sensoriel des informations gustatives

Chez les mammifères, lors de la mise en bouche d'un aliment, les informations gustatives perçues par les papilles gustatives, situées sur la langue, sont transportées par différents types de fibres nerveuses : le nerf cranial X (nerf vague), le nerf de la chorde du tympan (*chorda tympani*, nerf cranial VII), qui part des papilles fongiformes, et le nerf glossopharyngien (nerf cranial XI) qui prend son origine dans les papilles foliées et circumvallées – ou caliciformes (Hellekant et Danilova, 1999). Les informations gustatives sont alors transportées *via* ces nerfs vers le noyau du tractus solitaire, première structure cérébrale impliquée dans la perception du goût. Chez les primates, ces informations gustatives convergent ensuite directement vers le noyau thalamique postério-médio-ventral alors que, chez les rongeurs, elles transitent par le noyau parabrachial (Yamamoto, 2006). Enfin, ces informations sont transmises à l'aire gustative du IC, qui est à ce titre appelé cortex gustatif primaire. Ce dernier est impliqué dans le traitement qualitatif des informations gustatives ainsi que dans l'évaluation de l'intensité d'un stimulus gustatif (pour revue, Small, 2010). Cependant, nous verrons plus tard que l'IC

n'est pas uniquement impliqué dans ces processus du traitement primaire des informations gustatives, mais joue également un rôle dans le traitement affectif des goûts. De ce cortex gustatif primaire partent des fibres efférentes vers différentes structures cérébrales et notamment vers le cortex gustatif secondaire principalement situé dans l'OFC (Baylis *et al.*, 1994 ; Rolls, 2004b).

1.3. Convergence entre informations gustatives et olfactives : la reconnaissance des flaveurs

Au niveau central, la formation et la reconnaissance d'une flaveur résultent de l'intégration d'informations sensorielles multiples, et notamment d'informations olfactives, gustatives mais aussi somatosensorielles (*e.g.* perception de la texture dans la bouche ; Small et Prescott, 2005). Certaines structures cérébrales répondent à la fois à des stimuli olfactifs et gustatifs et sembleraient donc impliquées dans la représentation centrale des flaveurs (de Araujo *et al.*, 2003 ; Small *et al.*, 2004), et notamment l'OFC qui est une des régions principales impliquées dans la formation et la reconnaissance des flaveurs. Cependant, cette structure fonctionne en liaison avec de nombreuses autres structures corticales issues du cortex préfrontal, mais aussi de l'ACC et de l'IC, ainsi qu'avec des structures subcorticales comme l'AMY (pour revue, Verhagen et Engelen, 2006).

1.3.1. Le cortex orbitofrontal

Comme présenté précédemment, l'OFC, en tant que cortex gustatif secondaire, reçoit des fibres provenant du cortex gustatif primaire situé dans le IC (Baylis *et al.*, 1994 ; Rolls, 2004a, b). L'OFC comprend également une aire olfactive (Carmichael *et al.*, 1994) qui reçoit des afférences du cortex olfactif primaire, le PiC, et semblerait être impliqué dans la reconnaissance et la perception consciente des odeurs, indépendamment de leur valence affective, ainsi que dans la mémoire olfactive (Zald et Pardo, 1997). Ainsi, l'OFC, est une des premières structures cérébrales dans laquelle convergent les informations gustatives et olfactives, auxquelles viennent s'ajouter des informations somatosensorielles, auditives et visuelles (Rolls, 2004a, b). Il semble donc être impliqué dans la perception et la formation des flaveurs au niveau central (Öngür et Price, 2000 ; Rolls, 2004a, b).

1.3.2. Le cortex insulaire

Au même titre que l'OFC, l'IC est une structure vers laquelle convergent à la fois des informations gustatives et olfactives. Chez les primates humains et non-humains, l'IC, en tant que cortex gustatif primaire, est impliqué dans la perception primaire du goût en termes de qualité ou d'intensité, ainsi que, comme nous le verrons plus tard, dans son évaluation affective (pour revue, Small, 2010). Cependant, il semblerait également que la perception de stimuli olfactifs entraîne des réponses dans l'IC, au même titre que la perception de stimuli gustatifs (de Araujo *et al.*, 2003 ; Small *et al.*, 2004). Ceci suggère que cette structure est, elle aussi, impliquée dans la formation des flaveurs.

1.3.3. L'amygdale et le cortex cingulaire antérieur

Bien que l'activation de l'AMY (structure limbique du lobe temporal) suite à la perception de stimuli gustatifs ait été largement rapportée dans la littérature (*e.g.* O'Doherty *et al.*, 2001), de Araujo *et al.* (2003) sont les premiers à avoir mis en évidence, chez l'Homme, une activation de l'AMY à la fois par des stimuli gustatifs et olfactifs. De même, l'ACC répond à la fois à la perception de stimuli olfactifs (Rolls *et al.*, 2003) et gustatifs (de Araujo *et al.*, 2003 ; Small *et al.*, 2004). De Araujo *et al.* (2003) proposent que cette activation bimodale serait due en partie à des afférences provenant de l'IC et de l'OFC. Cependant, il semblerait que ces deux structures soient davantage impliquées dans la caractérisation hédonique de la flaveur plutôt que dans son identification sensorielle. C'est pourquoi leurs caractéristiques fonctionnelles seront abordées plus tard dans ce manuscrit.

2. Traitement central de l'identité affective des informations olfacto-gustatives

De nombreuses structures cérébrales sont connues pour jouer un rôle majeur dans le contrôle de la prise alimentaire, que ce soit sur le volet motivationnel, affectif (plaisir) et/ou cognitif (apprentissage, mémoire). Ces structures peuvent être soit corticales, avec notamment la participation du cortex préfrontal, du ACC ou encore du IC, soit subcorticales, avec en particulier les noyaux de la base (noyau caudé (CAU), putamen (PUT), noyau accumbens (NAcc), globus pallidus (GP)), l'AMY ou l'aire tegmentale ventrale (VTA). Après avoir fait un tour d'horizon de ces différentes structures, de leurs connexions respectives et de leur implication spécifique dans la régulation hédonique de la prise alimentaire, nous décrirons brièvement les principaux systèmes de neurostransmetteurs qui entrent en jeu au sein de ces structures pour réguler le comportement alimentaire.

2.1. Structures cérébrales impliquées dans le traitement affectif des informations olfacto-gustatives

2.1.1. Structures corticales

L'ensemble des structures corticales impliquées dans le traitement affectif/hédonique des informations olfacto-gustatives est illustré dans la **Figure 2**.

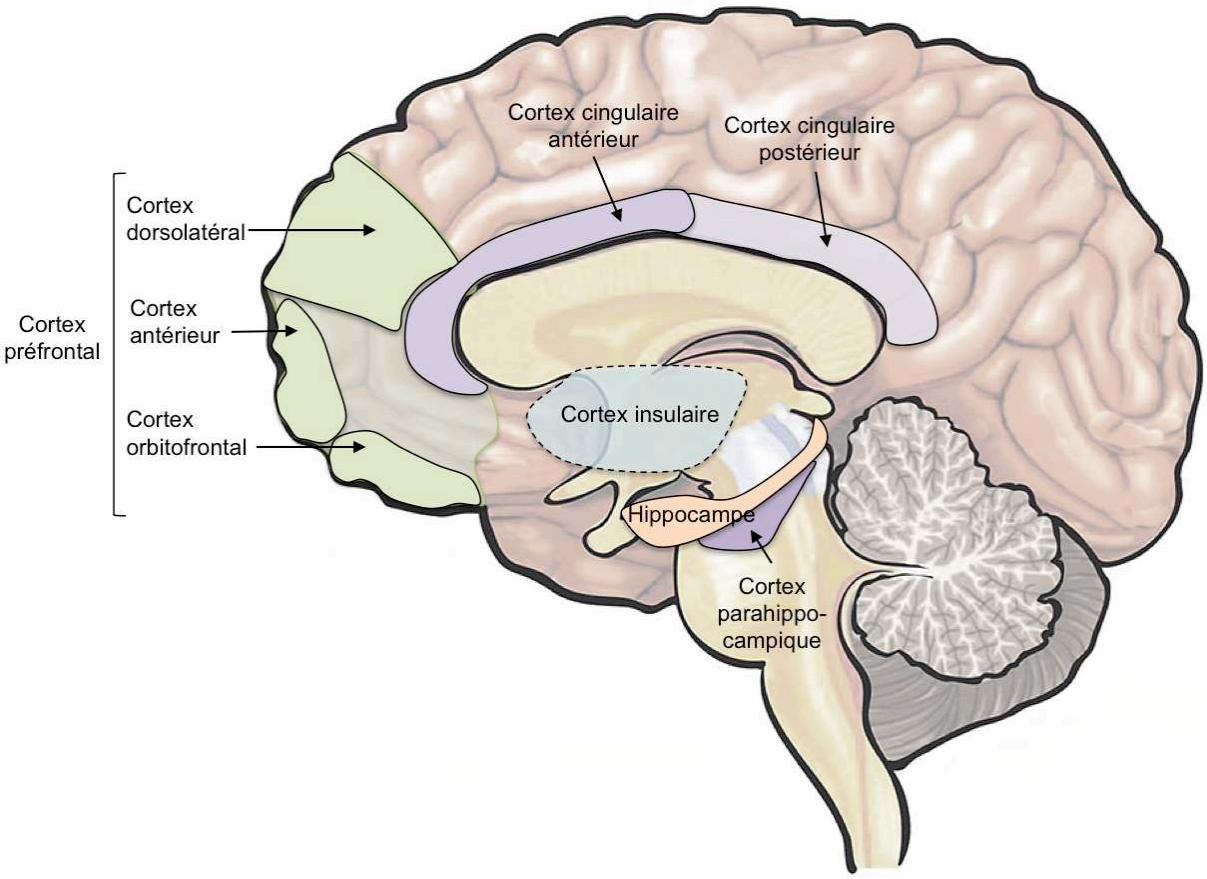

Figure 2. Structures corticales impliquées dans le traitement affectif/hédonique des informations sensorielles, et plus particulièrement des informations olfacto-gustatives chez l'Homme. Pour une meilleure lisibilité de l'illustration, les structures sont représentées de manière schématique.

Le cortex orbitofrontal : entre traitements sensoriel et hédonique. L'OFC se retrouve au carrefour de nombreuses voies de signalisation nerveuses et reçoit des informations en provenance de structures corticales et subcorticales (pour revues, Rolls, 2004a ; Rolls, 2004b). Outre ses connexions avec les structures impliquées dans le traitement primaire des informations olfacto-gustatives (PiC et IC), il reçoit des projections directes ou indirectes importantes

de structures subcorticales comme l'AMY. Indirectement, l'OFC reçoit également des afférences du gyrus temporal inférieur (ITG) et du cortex prépyriforme (PreC, cortex olfactif) et émet des projections vers le ITG, le cortex entorhinal, et le cortex cingulaire (CC). Il envoie également des projections vers des structures profondes comme la VTA et le CAU.

Outre son rôle dans l'identification des flaveurs (*cf.* plus haut), l'OFC semble avoir un rôle important dans le traitement hédonique des informations olfacto-gustatives (pour revues, Rolls, 2004a ; Rolls, 2004b). Il en est de même pour les phénomènes de récompense alimentaire (Haber et Knutson, 2010 ; Schultz, 2000), comme en témoignent ses nombreuses connexions avec des structures du circuit de la récompense, notamment les noyaux gris centraux et l'ACC. Ce double traitement des informations olfacto-gustatives fait de cette structure une des seules structures où sont codées à la fois l'identité des stimuli sensoriels ainsi que leurs propriétés hédoniques. Bien que tous les auteurs s'accordent à dire que l'OFC est impliqué dans le traitement hédonique des goûts, odeurs et flaveurs, les avis divergent quant à la direction de sa réponse (*i.e.* activation *vs* désactivation) lors de la perception de stimuli à valeur hédonique positive ou négative. Alors que certaines études chez l'Homme et le porc affirment que les stimuli positifs sont plus déterminants que les stimuli négatifs pour l'activation du OFC (Gaultier *et al.*, 2011 ; Rolls *et al.*, 2003) et que son activation est corrélée avec les jugements hédoniques de différents stimuli sensoriels (de Araujo *et al.*, 2005 ; Savic, 2005), d'autres démontrent que l'OFC est activé à la fois par la perception de stimuli plaisants et aversifs (O'Doherty *et al.*, 2001 ; Zald et Pardo, 1997 ; Zald *et al.*, 1998). Plus précisément, les odeurs négatives seraient préférentiellement associées à une activation dans l'hémisphère gauche alors que les odeurs plaisantes seraient associées à une activation à droite.

Autres structures du cortex préfrontal. Chez les rats et les primates, le cortex préfrontal est connecté, directement ou indirectement, à de nombreuses structures du circuit de la récompense, telles que l'AMY, la VTA, l'ACC, l'IC, le striatum (CAU, PUT, NAcc) ou encore le pallidum ventral, *i.e.* le GP (pour revues, Öngür et Price, 2000 ; Yamamoto, 2006), ces connexions passant notamment par le biais d'innervations dopaminergiques.

Du fait de ces connexions, le cortex préfrontal est une des interfaces majeures entre les voies de traitement de l'information gustative et le système de la récompense, au même titre que d'autres structures corticales et subcorticales comme le striatum, l'OFC, l'ACC ou encore l'AMY. En particulier, le cortex préfrontal dorsolatéral (DLPFC) est une partie intégrante du système de la récompense puisqu'il joue un rôle dans l'anticipation de la récom-

pense et la prise de décision en fonction du but (*i.e.* goal-oriented decision making). Le DLPFC ainsi que d'autres structures corticales comme le cortex temporal médial, qui participe à la détection et à la prédiction d'une récompense, ou l'OFC qui participe à l'évaluation de la valeur plaisante d'une récompense, envoient des informations sur le stimulus récompense au striatum (pour revue, Schultz, 2000).

Outre ce rôle dans l'anticipation de la récompense, le cortex préfrontal a un rôle dans la mémoire, la motivation et le contrôle cognitif de la prise de décision (Kouneiher *et al.*, 2009). Il est également activé dans les stimulations relatives à l'aliment, comme c'est le cas du cortex préfrontal antérieur (APFC) (Ramnani et Owen, 2004) et du DLPFC (Kringelbach *et al.*, 2004). Plus précisément, certaines études montrent que le DLPFC, et plus particulièrement l'hémisphère gauche, est activé durant la perception d'un goût plaisant (Gautier *et al.*, 1999) et est impliqué dans le traitement des signaux alimentaires et donc dans la régulation de la prise alimentaire (*e.g.* études sur l'obésité, Del Parigi *et al.*, 2002 ; Gautier *et al.*, 2000 ; Le *et al.*, 2006). Selon ces auteurs, l'activation du cortex préfrontal, et plus particulièrement du DLPFC, participerait aux processus centraux impliqués dans la prise de décision pour l'arrêt de la prise alimentaire. Le DLPFC exercerait en fait une action inhibitrice sur les réseaux centraux orexigéniques (*i.e.* stimulant l'appétit), composés notamment de l'hypothalamus et de certaines structures limbiques, ce qui entrainerait alors la suppression de la faim et l'arrêt de la prise alimentaire.

Le cortex cingulaire antérieur et le cortex parahippocampique Le lobe limbique comprend des structures subcorticales, telles que l'AMY et l'hippocampe, et des structures corticales, telles que le PHC et l'ACC. Le lobe limbique étant impliqué dans la régulation des émotions et dans les processus de mémoire, ces structures jouent logiquement un rôle important dans le traitement émotionnel des informations olfacto-gustatives.

Outre son implication dans la reconnaissance d'odeurs alimentaires (Cerf-Ducastel et Murphy, 2001) et de goûts (Verhagen et Engelen, 2006), l'ACC, au même titre que le PHC, est impliqué dans l'évaluation émotionnelle et hédonique des stimuli sensoriels (Small *et al.*, 2001), dans les volets motivationnel, hédonique ou cognitif (Kringelbach et Berridge, 2010). L'ACC est d'ailleurs connecté à de nombreuses structures impliquées dans ces fonctions, comme l'IC, l'OFC et l'AMY. L'ACC est également fortement connecté avec les noyaux de la base, où se situent les « hotspots » hédoniques, points chauds sous la dépendance de régulateurs opioïdes. Il est donc considéré comme une partie intégrante du circuit de la récom-

pense (pour revues, Haber et Knutson, 2010 ; Kringelbach et Berridge, 2010 ; Schultz, 2000). Cependant, d'autres études montrent que l'ACC est plutôt activé pendant la perception de stimuli déplaisants et très aversifs (Zald et Pardo, 1997), alors que le PHC, quant à lui, serait plutôt activé par les stimuli gustatifs plaisants (Gautier *et al.*, 1999), suggérant un effet opposé de ces dernières structures.

Le cortex insulaire. Comme indiqué précédemment, l'IC, représentant le cortex gustatif primaire, joue un rôle majeur dans l'évaluation qualitative des informations gustatives et est interconnecté à différentes structures impliquées dans le traitement des informations olfacto-gustatives comme le cortex préfrontal, l'OFC, l'AMY, la VTA ou encore le striatum. Cependant, chez l'Homme, l'IC n'a pas un rôle seulement dans la perception primaire des goûts et des informations relatives à l'aliment (Small, 2010 ; Wang *et al.*, 2004) mais semblerait également impliqué dans l'évaluation de l'intensité des informations gustatives, et dans le traitement de leur valeur hédonique (pour revue, Kringelbach et Berridge, 2010). Plus précisément, il semblerait que la perception d'une stimulation sensorielle plaisante entraîne l'activation de cette structure (Gaultier *et al.*, 2011 ; Rolls *et al.*, 2003), bien que certaines études affirment que l'IC serait activé à la fois par des stimuli plaisants et aversifs (Gottfried *et al.*, 2002 ; O'Doherty *et al.*, 2001).

Outre son implication évidente dans le traitement hédonique des informations alimentaires, des études lésionnelles chez le rat ont permis de démontrer que l'IC n'était pas nécessaire à l'acquisition de préférence alimentaire conditionnée (Touzani et Sclafani, 2007) alors qu'il apparaît essentiel au développement d'aversion gustative conditionnée (Desgranges *et al.*, 2009 ; Roman *et al.*, 2009). Ainsi, cette structure, au même titre que l'AMY, l'OFC et l'ACC (pour revue, Veldhuizen *et al.*, 2010) semblerait jouer un rôle important dans les processus d'apprentissage, et donc dans le volet cognitif du contrôle de la prise alimentaire.

2.1.2. Structures subcorticales

L'ensemble des structures subcorticales impliquées dans le traitement affectif/hédonique des informations olfacto-gustatives est illustré dans la **Figure 3**.

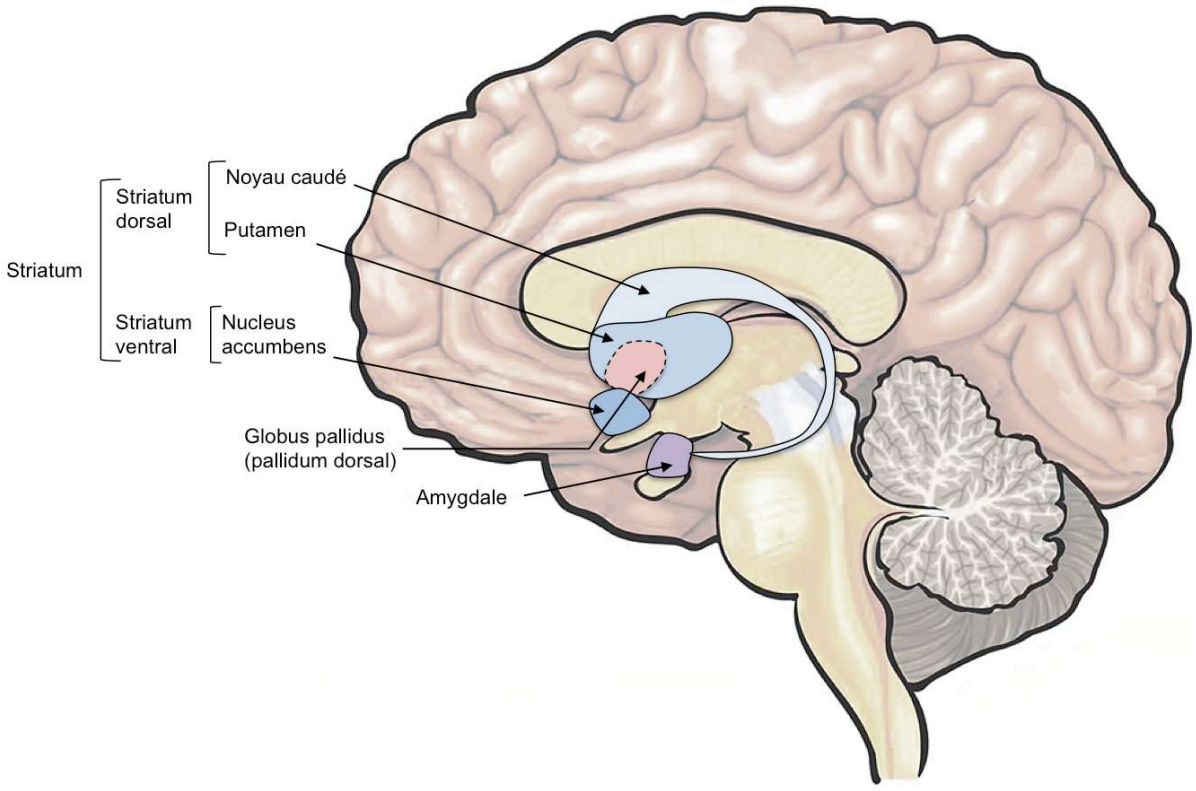

Figure 3. Structures subcorticales impliquées dans le traitement affectif/hédonique des informations sensorielles, et plus particulièrement des informations olfacto-gustatives chez l'Homme. Ces structures correspondent à l'ensemble des noyaux gris de la base et à l'amygdale.

Les noyaux gris de la base. L'ensemble des noyaux gris de la base, encore appelés noyaux gris centraux ou noyaux de la base, est constitué de différentes structures centrales connectées entre elles : le striatum dorsal (CAU et PUT), auquel s'ajoute le NAcc pour former le striatum ventral, et le pallidum (formé du pallidum dorsal ou GP, auquel s'ajoute une zone de la substance grise pour former le pallidum ventral). Outre des connexions réciproques, ces noyaux sont connectés à de nombreuses structures corticales ou subcorticales impliquées dans la régulation de la prise alimentaire. Par exemple, en plus de connexions avec le NAcc, le pallidum ventral est connecté avec des structures jouant un rôle dans le traitement de la récompense comme l'AMY, le noyau parabrachial ainsi que certaines régions du cortex préfrontal comme l'OFC (pour revue, Peciña *et al.*, 2006). Le NAcc, quant à lui, reçoit des afférences des aires corticales du goût, c'est-à-dire de l'IC et du cortex périrhinal (PeC). Outre ses interconnexions avec le pallidum ventral, le striatum ventral reçoit des afférences de zones subcorticales comme l'AMY et la VTA, mais également de l'OFC, du DLPFC ou encore de l'ACC (pour revue, Haber et Knutson, 2010). Comme en témoignent les nombreuses

connexions existant entre les noyaux de la base et les structures de la régulation de la prise alimentaire, ces noyaux gris centraux, acteurs majeurs du circuit de la récompense, sont impliqués dans différents volets de la prise alimentaire et notamment dans l'évaluation de la dimension hédonique des aliments et dans la composante motivationnelle de la prise alimentaire.

Le striatum, qui englobe le CAU, le PUT et le NAcc, participe activement à la modulation de la consommation alimentaire en jouant notamment un rôle dans les comportements d'apprentissage, et plus particulièrement dans les apprentissages émotionnels ou associés à une récompense (pour revues, Fattore *et al.*, 2010 ; Schultz, 2000). Chez l'Homme et les primates non-humains, le striatum est impliqué dans le traitement des récompenses, et plus particulièrement pendant l'anticipation ou la prévision d'une récompense alimentaire (pour revues, Haber et Knutson, 2010 ; O'Doherty *et al.*, 2002). Chez le porc, Gaultier *et al.* (2011) ont mis en évidence une activation du striatum dorsal suite à la perception d'une flaveur plaisante comparé à la perception d'une flaveur aversive. Des résultats similaires ont été mis en évidence chez l'Homme, puisque la perception d'odeurs alimentaires plaisantes induit une activation dans le striatum – ainsi que dans le CC et l'IC (Bragulat *et al.*, 2010), et la diminution de la valeur hédonique d'une récompense entraîne une désactivation du striatum dorsal (Small *et al.*, 2001).

Outre son implication dans l'anticipation de la récompense (O'Doherty *et al.*, 2002), Gottfried *et al.* (2002) ont démontré l'activation du striatum ventral, et plus particulièrement du NAcc, suite à un conditionnement alimentaire préférentiel, suggérant une implication de ce noyau dans la reconnaissance des aliments très palatables chez l'Homme. Ce rôle dans le traitement des préférences alimentaires est tenu *via* l'action de nombreux systèmes de neurotransmetteurs que nous détaillerons à la fin de cette introduction, comme les systèmes opioïde et GABA (Kelley *et al.*, 2002 ; Woolley *et al.*, 2006 ; Woolley *et al.*, 2007), ou encore le système dopaminergique (Barbano et Cador, 2007 ; Bassareo *et al.*, 2002). En effet, l'injection d'antagonistes des récepteurs opioïdes (naltrexone) du NAcc provoque une diminution de la consommation d'une flaveur préférée (Barbano et Cador, 2007), alors qu'à l'inverse, l'injection d'un agoniste des récepteurs opioïdes (DAMGO) augmente la consommation d'aliments palatables et d'une flaveur préférée (Woolley *et al.*, 2006). Concernant le système GABA, l'injection d'un agoniste des récepteurs $GABA_A$ (muscimol) dans le NAcc a égale-

ment pour effet une augmentation de la prise alimentaire, mais indépendamment de la valeur hédonique de l'aliment (Woolley *et al.*, 2006).

Ensemble, le NAcc et le pallidum ventral jouent un rôle important dans la régulation de la prise alimentaire notamment *via* l'échange d'informations *via* le système opioïde (Taha *et al.*, 2009). Cet échange direct ou indirect d'informations pourrait être requis pour le traitement de la valeur hédonique des aliments, suggérant l'implication du pallidum dans ces processus (pour revues, Peciña *et al.*, 2006 ; Smith et Berridge, 2005, 2007). En effet des « hotspots » hédoniques ont été identifiés dans le pallidum ventral et dans le striatum ventral, suggérant l'implication du GP et du NAcc dans le plaisir et la motivation pour les récompenses alimentaires. Gaultier *et al.* (2011) ont d'ailleurs démontré que le pallidum dorsal (GP) était activé par la perception de stimuli olfacto-gustatifs alimentaires plaisants.

L'amygdale. Du point de vue anatomique, l'AMY est une structure limbique subcorticale située dans le lobe temporal et qui reçoit des projections olfactives du PiC (*i.e.* le cortex olfactif primaire), et joue donc, à ce titre, un rôle important dans le traitement quantitatif (intensité) des informations olfactives (*e.g.* Anderson *et al.*, 2003). L'AMY reçoit également des projections de nombreuses structures impliquées dans la reconnaissance du goût comme l'IC ou l'OFC (Carmichael *et al.*, 1994). Les projections de l'AMY partent vers l'IC, le cortex préfrontal, et particulièrement vers l'OFC (Morris et Dolan, 2001), ainsi que vers le NAcc et l'hippocampe, ce qui lui donne un rôle prépondérant dans les processus mnésiques (Soudry *et al.*, 2011). Chez le singe, Cho et Fudge (2010) ont démontré que l'AMY recevait également d'importantes connexions dopaminergiques en provenance de la VTA.

En tant que structure limbique, l'AMY est impliquée dans la régulation des émotions et dans les processus mnésiques, et joue donc un rôle important dans le traitement émotionnel des informations olfacto-gustatives lors de la prise alimentaire. En effet, outre son rôle dans la reconnaissance primaire de l'aliment, l'AMY, en connexion étroite avec l'OFC, a un rôle dans les volets motivationnel et cognitif (mémoire, apprentissage) du contrôle de la prise alimentaire (Morris et Dolan, 2001), ainsi que dans le traitement des récompenses (Schultz, 2000). Chez l'Homme, bien que la réponse de l'AMY ne soit pas corrélée avec les évaluations subjectives de la valeur hédonique de stimulations gustatives et/olfactives (de Araujo *et al.*, 2003 ; Kringelbach *et al.*, 2003), l'activation de l'AMY semble tout de même étroitement liée à la présentation de stimuli à forte valence émotionnelle, notamment des stimuli olfactifs et gustatifs (voir la méta-analyse de Costafreda *et al.*, 2008). De nombreuses études souli-

gnent en effet l'implication de l'AMY dans le traitement affectif des informations sensorielles, avec une activation préférentielle suite à la présentation de stimuli aversifs ou déplaisants, bien que certaines études révèlent également une activation suite à la présentation d'un stimulus positif (e.g. O'Doherty *et al.*, 2001 ; Zald et Pardo, 1997 ; Zald *et al.*, 1998).

Cependant, il semblerait que l'AMY ne joue pas un rôle dans la représentation du caractère plaisant *per se* des stimuli alimentaires. Certains auteurs ont suggéré que l'AMY, tout comme l'IC et en opposition à l'OFC ou à l'ACC, était davantage impliquée dans l'évaluation de l'intensité des stimuli (Small *et al.*, 2003 ; Winston *et al.*, 2005). Veldhuizen *et al.* (2010) vont plus loin et proposent que l'intensité et la qualité des stimuli sont indépendamment traitées dans l'AMY et l'IC, puisque ces informations convergent vers l'OFC où elles formeraient alors une information hédonique unique du stimulus. D'autres auteurs suggèrent encore que l'AMY code plutôt pour la valeur motivationnelle des stimuli, qui peut par exemple évoluer en fonction de l'état de satiété de l'individu (Morris et Dolan, 2001).

Outre son implication dans la caractérisation affective des stimuli sensoriels, il apparaît que cette structure est aussi impliquée dans les processus d'expérience et d'apprentissage alimentaires. Des études lésionnelles ont permis de mettre en évidence le rôle de l'AMY dans le développement de préférences (Dwyer et Iordanova, 2010 ; Touzani et Sclafani, 2005 ; Touzani *et al.*, 2009b) ou d'aversions alimentaires conditionnées chez le rat (*e.g.* Rollins *et al.*, 2001), suggérant que cette structure est indispensable à la formation de telles associations.

2.2. Neurotransmetteurs impliqués dans le traitement affectif des informations olfacto-gustatives

La régulation hédonique de la prise alimentaire se fait *via* l'action de neurotransmetteurs spécifiques distribués dans les structures cérébrales décrites plus haut. Ces neurotransmetteurs peuvent être impliqués dans différents volets de la prise alimentaire, que ce soit dans la composante motivationnelle ou dans la composante hédonique de la prise alimentaire. Il nous semble donc nécessaire de décrire brièvement les différents systèmes de neurotransmetteurs connus à ce jour comme partie intégrante du circuit de régulation de la prise alimentaire.

2.2.1. Évaluation conjointe du plaisir (« liking ») et de la motivation (« wanting ») alimentaire

Le système opioïde. Les opioïdes (*e.g.* enképhaline, endorphine), qui comprennent une multitude de familles de peptides, se retrouvent dans de nombreux circuits cérébraux, mais plus particulièrement dans les régions impliquées dans la régulation émotionnelle, et dans la prise alimentaire comme le NAcc, le IC, l'hippocampe, le pallidum ventral ou encore l'AMY (Barbano et Cador, 2007 ; Colantuoni *et al.*, 2001 ; Kelley *et al.*, 2002 ; Taha *et al.*, 2009 ; Wassum *et al.*, 2009). En effet, un réseau de « hotspots » (ou points chauds) hédoniques utilise la neurotransmission des opioïdes pour augmenter à la fois la motivation (« wanting ») et le plaisir (« liking ») pour des récompenses alimentaires (pour revues, Peciña *et al.*, 2006 ; Smith et Berridge, 2005, 2007). Ces « hotspots » hédoniques ont été identifié notamment dans le NAcc (striatum ventral), le GP (pallidum ventral) ainsi que dans le noyau parabrachial ; d'autres pourraient exister dans l'AMY ou dans des régions corticales comme l'OFC ou le CC.

Alors que les opioïdes ne semblent pas avoir d'impact sur la perception ou la reconnaissance du goût *per se* (Arbisi *et al.*, 1999 ; O'Hare *et al.*, 1997), ces neurotransmetteurs sembleraient réguler le plaisir provoqué par la perception de certains goûts (pour revues, Berridge, 2000 ; Kelley *et al.*, 2002). Les opioïdes régulent ainsi l'évaluation de la palatabilité et de l'hédonicité des aliments, et plus particulièrement des aliments riches en lipides ou en sucres, *i.e.* les aliments hautement palatables (Drewnowski *et al.*, 1992). En plus d'avoir un rôle dans l'évaluation de la palatabilité des aliments ou dans la caractérisation hédonique d'une récompense (Levine et Billington, 2004 ; Taha *et al.*, 2006), les opioïdes participent à la régulation de la motivation alimentaire. En effet, chez les rats, la détérioration du système opioïde par l'utilisation de naloxone diminue la motivation à obtenir un aliment, probablement par le biais d'une diminution de la palatabilité de la récompense alimentaire (Barbano *et al.*, 2009 ; Glass *et al.*, 1999). À l'inverse, la stimulation des opioïdes du NAcc, *via* l'injection de DAMGO, un agoniste des récepteurs opiacés, provoque une augmentation de la prise alimentaire pour des aliments très palatables (riches en lipides), avec une augmentation de la palatabilité des aliments et des préférences alimentaires (Zhang *et al.*, 1998). Le système opioïde va donc avoir un effet sur la prise alimentaire et la motivation alimentaire *via* une modulation de l'évaluation des propriétés plaisantes ou hédoniques des aliments (pour revue, Barbano et Cador, 2007).

L'évaluation de la valeur motivationnelle et de la palatabilité d'un(e) aliment/récompense dépend donc en partie de l'activation des récepteurs opioïdes. Il est intéressant de noter que l'implication de ces récepteurs se différencie sur les plans fonctionnel et anatomique : chez les rats, Wassum *et al.* (2009) ont observé une activation des opioïdes du NAcc et du pallidum ventral lors de l'évaluation de la palatabilité d'une récompense, et des opioïdes de l'AMY basolatérale pour encoder la valeur motivationnelle utilisée pour les actions dirigées vers un but.

Le système benzodiazépines/GABA. Au même titre que le système opioïde, le système benzodiazépines/GABA est impliqué dans la régulation du comportement alimentaire. Les récepteurs GABA, sous le contrôle des neurones cholinergiques et dopaminergiques du striatum ventral (NAcc), sembleraient notamment réguler la consommation de nourriture palatable et/ou préférée (Kelley *et al.*, 2005 ; Woolley *et al.*, 2006 ; Woolley *et al.*, 2007). Cependant, il semblerait que, contrairement au système opioïde, les récepteurs GABA dans le pallidum ventral répondent indépendamment de la valeur hédonique des stimulations (Peciña *et al.*, 2006).

L'administration de benzodiazépines augmente la prise alimentaire chez les animaux (pour revue, Cooper, 2005) et chez l'Homme (Haney *et al.*, 1997). Il semblerait que les benzodiazépines agissent sur la prise alimentaire *via* une augmentation de la palatabilité et de la perception hédonique de l'aliment chez les rats (Dwyer, 2009 ; O'Hare *et al.*, 2006), bien que cette action sur la palatabilité n'ait pas été démontrée chez l'Homme ou chez d'autres espèces. Cependant, alors que les benzodiazépines semblent influencer la perception hédonique des aliments, Dwyer (2009) a démontré que l'infusion d'agonistes des benzodiazépines comme le midazolam n'avait pas d'effet majeur sur l'expression de préférences alimentaires conditionnées *via* un stimulus gustatif (fructose), ou *via* un stimulus calorique (maltodextrine).

Le système sérotoninergique. Chez l'Homme, comme chez de nombreuses espèces telles que le rat la souris, le lapin, le mouton ou les primates non humains (Niblock *et al.*, 2005), la sérotonine (5-HT) est majoritairement synthétisée dans l'ensemble des noyaux du raphé, eux-mêmes distribués dans le bulbe rachidien (Hornung, 2003). Chez l'Homme et les primates non humains, le circuit sérotoninergique étend ensuite ces ramifications neuronales vers de nombreuses structures cérébrales, notamment vers les centres hypothalamiques, mais aussi

vers l'ensemble des ganglions de la base, comme le PUT, le CAU, le NAcc et le GP (Ikemoto *et al.*, 1996 ; Parent *et al.*, 2011), et vers l'AMY (Lam *et al.*, 2010).

De nombreuses études ont démontré le rôle déterminant du circuit sérotoninergique dans la régulation et le contrôle de la prise alimentaire ainsi que dans la prise de poids (pour revue, Lam *et al.*, 2010). Cependant, la majorité des études suggère que le circuit sérotoninergique est davantage impliqué dans la régulation homéostatique de la prise alimentaire. En effet, des interactions importantes ont été mises en évidence entre le circuit sérotoninergique et la libération de certains neuropeptides impliqués dans le contrôle de la prise alimentaire et situés dans les centres hypothalamiques (*e.g.* oxytocine, orexines, neuropeptides Y, etc.). Cependant, et au regard de ces nombreuses innervations vers les noyaux de la base, il est probable que le système sérotoninergique soit également impliqué dans le traitement hédonique des informations sensorielles, et dans les aspects motivationnels et de récompense de la prise alimentaire, bien que les conclusions des différentes études divergent. Certaines études ont démontré que l'administration d'un agent responsable d'une augmentation des taux de 5-HT, la fenfluramine, provoquait une diminution de la palatabilité perçue (Gray et Cooper, 1996) ainsi qu'une diminution de la prise alimentaire (Asin *et al.*, 1992). Barnfield *et al.* (1994) ont également démontré que la fenfluramine, faute de modifier les réponses hédoniques positives induites par la présentation de sucrose, augmentait les réponses aversives induites par la présentation de quinine. Enfin, la 5-HT semblerait impliquée dans les phénomènes d'addiction, avec notamment l'augmentation des propriétés de récompense de l'abus de drogues qui suit l'exposition antérieure à ces drogues (pour revue, Rothman *et al.*, 2008).

2.2.2. Cas particulier du système dopaminergique : évaluation de la motivation (« wanting ») alimentaire

La dopamine (DA) est synthétisée dans la VTA et dans la substance noire pour être ensuite distribuée dans diverses structures cérébrales comme le cortex préfrontal (Bassareo *et al.*, 2002), le striatum dorsal et le NAcc, l'AMY ou encore l'hippocampe (**Figure 4**). Ce neurotransmetteur joue un rôle fondamental dans les mécanismes d'addiction et de récompense, alimentaire ou autre. Plus particulièrement, la DA est impliquée dans différents comportements liés à la récompense, comme l'apprentissage, le renforcement et l'addiction. Au cours des dernières décennies, le rôle de la DA dans le traitement de la récompense a fait l'objet de nombreuses théories (pour revue, Barbano et Cador, 2007 ; Berridge et Robinson, 1998).

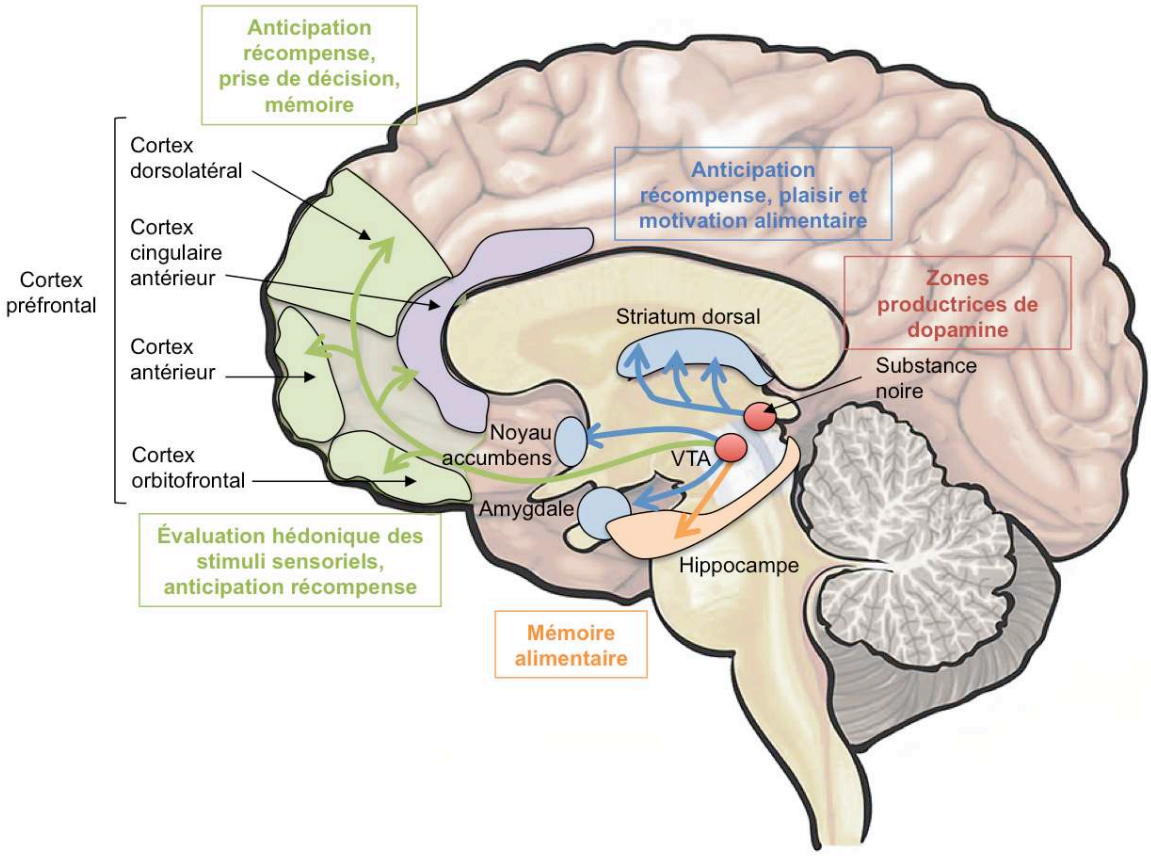

Figure 4. Schéma du circuit dopaminergique chez l'Homme. Des connexions nerveuses partent des zones productrices de dopamine (en rouge) pour rejoindre différentes structures subcorticales (en bleu), corticales (en vert) ou hippocampiques (en orange). Ces structures sont toutes impliquées dans la régulation hédonique du comportement alimentaire et dans le traitement affectif des informations olfacto-gustatives plus particulièrement.

« The anhedonia hypothesis », proposé par Wise (Wise, 1982) repose sur le fait que la DA régule le plaisir produit par la nourriture et les récompenses en général, et que, par conséquent, l'administration d'antagonistes de la dopamine provoque une disparition du plaisir associé à un stimulus. Cependant, cette hypothèse a été largement remise en cause et, bien qu'autrefois considérée comme un neurotransmetteur « hédonique », il est à présent généralement admis que la dopamine ne modifie pas la perception affective du goût mais joue plutôt un rôle dans d'autres aspects du traitement de la récompense alimentaire ou de la motivation à manger. Ce constat a favorisé l'émergence de nouvelles théories, comme notamment la théorie de la « prédiction de la récompense » proposée par Schultz (Schultz, 2002) qui propose que la DA soit un signal codant pour la différence entre la récompense vraiment reçue et la récompense attendue. La théorie de la « saillance/pertinence motivationnelle » (*i.e.* « the incentive salience theory »), théorie la plus communément admise, postule quant à elle que la DA code pour la valeur motivationnelle perçue d'un stimulus plutôt que pour sa valeur hédo-

nique (pour revues, Barbano et Cador, 2007 ; Berridge et Robinson, 1998 ; Peciña *et al.*, 2006).

Selon la théorie de la « salience motivationnelle », bien que le système dopaminergique contribue au traitement de la récompense, il ne joue pas de rôle direct dans l'impact hédonique des goûts *per se* et dans l'évaluation de la palatabilité mais plutôt dans d'autres aspects de la prise alimentaire, comme notamment dans la régulation de la motivation alimentaire et dans les phases anticipatoires de la prise alimentaire – en opposition à la phase consommatoire de la prise alimentaire pendant laquelle l'évaluation hédonique est maximale (pour revues, Barbano et Cador, 2007 ; Kelley, 2004). Comme reporté par Barbano et Cador (2007), la DA semble notamment jouer un rôle dans la transcription de la motivation alimentaire en des comportements adaptés pour obtenir la nourriture, ou encore dans l'évaluation entre les coûts/bénéfices d'une action destinée à obtenir l'accès à un stimulus (Barbano *et al.*, 2009). Selon cette théorie, le système dopaminergique, notamment dans le NAcc, aurait plutôt un rôle dans l'anticipation et le calcul coût/bénéfice de la récompense, avec des modulations de la production de DA au niveau de l'VTA, que dans la composante affective de la prise alimentaire. En effet, des modifications de l'activité dopaminergique par l'utilisation d'antagonistes dopaminergiques provoquent une diminution de la motivation alimentaire, avec notamment une diminution de la prise alimentaire (Barbano *et al.*, 2009), mais ne parvient pas à modifier les réponses hédoniques vis-à-vis des stimuli sensoriels, notamment lors de tests de réactivité gustative (Treit et Berridge, 1990) (pour revue, Berridge, 2000). De même, Peciña *et al.* (2003) ont montré que, chez des souris mutantes, un taux chronique élevé de DA entraîne une plus grande motivation à obtenir une récompense sucrée et une augmentation des quantités consommées, mais n'augmente pas les réactions de plaisir provoquées par la perception de ces stimuli palatables lors de tests de réactivité gustative (expressions faciales positives).

Conclusions

En conclusion de cette synthèse, les études chez l'Homme, mais également chez les rongeurs et les primates non-humains, ont permis de définir de manière précise et complète les mécanismes comportementaux et cérébraux impliqués dans la régulation hédonique de la prise alimentaire. Cette régulation complexe repose en partie sur les expériences passées des individus avec les aliments et se caractérise par des modifications constantes des choix alimentaires *via* notamment l'établissement de préférences et d'aversions alimentaires. Les struc-

tures cérébrales impliquées dans la régulation hédonique de la prise alimentaire, ainsi que dans la reconnaissance et la caractérisation hédonique des stimuli olfacto-gustatifs ont également été bien caractérisées chez ces espèces. Ces structures, qui agissent sur le volet cognitif, motivationnel ou affectif du contrôle de la prise alimentaire, sont elles-mêmes sous la dépendance de mécanismes moléculaires complexes portés par des systèmes de neurotransmetteurs qui agissent en synergie au sein de ces structures.

À ce jour, cependant, peu d'études se sont intéressées aux facteurs comportementaux et cérébraux liés à la régulation hédonique du comportement alimentaire chez le porc. Pourtant, la première partie de l'introduction (**article n°1**) montre que de telles études chez cette espèce pourraient s'avérer très bénéfiques en termes d'applications biomédicales, mais également en termes de production animale.

Ces dernières années, des avancées scientifiques prometteuses ont néanmoins été réalisées dans ce domaine chez le porc. Pour commencer, l'anatomie du cerveau de porc est aujourd'hui relativement bien caractérisée (Felix *et al.*, 1999 ; Saikali *et al.*, 2010). Grâce à la réalisation d'un atlas du cerveau de porc, Saikali *et al*. (2010) ont mis en évidence de nombreuses similitudes anatomiques entre les cerveaux humain et porcin. Ils ont notamment identifié et localisé l'ensemble des structures cérébrales connues pour être impliquées chez l'Homme dans la régulation hédonique du comportement alimentaire, telles que l'ensemble des structures du cortex préfrontal, le CC, l'IC, les noyaux de la base, ou encore l'AMY. D'un point de vue fonctionnel, Gaultier *et al.* (2011) sont les premiers à avoir souligné des similitudes fonctionnelles prometteuses entre l'Homme et le porc, compte tenu des activations cérébrales successives à la perception de stimuli olfacto-gustatifs caractérisés par des valeurs hédoniques contrastées, dans ces mêmes structures. D'un point de vue comportemental enfin, ces mêmes auteurs ont mis en évidence le développement d'une aversion alimentaire robuste chez cette espèce *via* l'utilisation d'un paradigme de conditionnement pavlovien.

Ainsi, dans la suite de ce manuscrit, nous nous attacherons à approfondir les avancées majeures soulevées par ces travaux récents. Ainsi, après avoir rappelé la méthodologie générale de la thèse, nous chercherons dans les prochains chapitres à développer et à caractériser les différents volets impliqués dans la régulation de la prise alimentaire chez le porc, en nous focalisant tout particulièrement sur les mécanismes comportementaux et cérébraux sous-tendant l'établissement les préférences et aversions alimentaires.

MÉTHODOLOGIE GÉNÉRALE

MÉTHODOLOGIE GÉNÉRALE

1. Modèle animal

Le modèle animal utilisé dans cette thèse est le **porc,** plus particulièrement des **femelles juvéniles sevrées**. Outre les possibles champs d'applications des travaux présentés dans cette thèse pour l'amélioration de la prise alimentaire en élevage porcin, le porc est un modèle privilégié pour les recherches en nutrition humaine et neurosciences (*cf.* **Article n°1**).

Le choix de ne travailler que sur des **femelles** repose sur une volonté d'éliminer les biais interindividuels liés au sexe en termes de réponses comportementales et neurophysiologiques (e.g. Cornier *et al.*, 2010 ; Killgore et Yurgelun-Todd, 2010). De plus, l'utilisation de femelles permet de faciliter les interventions chirurgicales par laparotomie (pose de cathéters duodénaux) notamment grâce à l'absence d'étui pénien au niveau de la région ombilicale où est réalisée la laparotomie. Le choix de travailler sur des **animaux juvéniles sevrés** est, quant à lui, motivé par deux raisons principales. D'une part, les porcs juvéniles sevrés sont particulièrement concernés par les problématiques soulevées dans cette thèse : (1) en termes d'applications pratiques en élevage porcin, notamment pour l'amélioration de la prise alimentaire lors des phases sensibles de transition alimentaire chez le porc en croissance, et (2) en termes d'applications chez l'Homme, les étapes précoces de vie (enfance, adolescence) étant une période importante pour l'acquisition du répertoire alimentaire de l'individu. D'autre part, la petite taille des animaux juvéniles facilite l'hébergement en cages expérimentales et métaboliques et permet la réalisation d'imagerie fonctionnelle cérébrale *in vivo* par tomographie par émission de positons (PET) ou par tomographie d'émission monophotonique (SPECT). Un porc adulte est en effet trop lourd et imposant pour les machines d'imagerie de la plateforme expérimentale PRISM du site INRA de Saint Gilles, initialement conçues pour l'Homme et dédiées à l'imagerie médicale.

Ainsi, un total de 163 animaux a été utilisé pour réaliser l'ensemble des expériences présentées de la thèse (**Tableau I**). Parmi ces animaux, 139 étaient âgés d'environ 2 à 3 mois le premier jour des tests, avec un poids moyen de 25,38 ± 0,35 kg. Une des expériences du chapitre IV ayant nécessité l'utilisation de porcelets au sevrage, 24 de ces animaux étaient âgés de seulement un mois le premier jour des tests, avec un poids moyen de 8,86 ± 0,14 kg. À ces animaux, s'ajoutent 27 animaux supplémentaires utilisés pour les phases de mise au point des protocoles (*i.e.* expériences non présentées dans le manuscrit). Les expériences de

la thèse ont toutes été réalisées dans les installations expérimentales de l'INRA, UMR1079 SENAH (Systèmes d'Élevage, Nutrition Animale et Humaine) sur le site de Saint Gilles (scindée en UMR1348 PEGASE et UR1341 ADNC à partir du 1er janvier 2012), Ille-et-Vilaine (48° 09' 13'' N, 01° 49' 34'' O) dans deux zones distinctes : le plateau AniScans (imagerie multimodalité de l'animal moyen) de la plateforme d'imagerie PRISM et la zone « élevage », dans laquelle se trouve la majeure partie du troupeau expérimental. Trois lignées différentes, issues de croisements (♂ × ♀) entre trois races de porcs, ont été utilisées en fonction des disponibilités au sein du cheptel, avec principalement des animaux issus de la lignée Piétrain × (Large White × Landrace), puis de la lignée Large White × (Large White × Landrace), et enfin de la lignée Large White × Landrace.

Tableau I. Répartition des effectifs et des lignées de porcs utilisés dans les différentes expériences de la thèse. Les lignées de porcs étaient choisies en fonction des disponibilité du cheptel. Par convention, les croisements indiquent la race du mâle (race pure) × la race de la femelle (race pure ou elle-même issue d'un croisement).

Chapitre	Expérience	Effectif	Lignée (♂ × ♀)
Chapitre I			
	Expérience préliminaire	24	Piétrain x (Large White x Landrace)
	Article n°2	12	Piétrain x (Large White x Landrace)
Chapitre II			
	Article n°3	27	Piétrain x (Large White x Landrace)
Chapitre III			
	Article n°4	9	Piétrain x (Large White x Landrace)
	Article n°5	11	Large White x Landrace
Chapitre IV			
	Article n°6	12	Large White x (Large White x Landrace)
		12	Piétrain x (Large White x Landrace)
		24	Piétrain x (Large White x Landrace)
	Expérience complémentaire	32	Piétrain x (Large White x Landrace)

2. Conditions d'élevage

Deux types de logements expérimentaux ont été utilisés au cours de cette thèse : des **cages individuelles de type métabolique** et des **loges individuelles standard** d'élevage. Le choix de travailler sur des animaux maintenus en loges ou en cages individuelles repose sur des considérations pratiques. L'étude du comportement alimentaire sur des individus isolés physiquement permet une précision sur les mesures individuelles de consommation lors des expériences tout en supprimant la compétition alimentaire. Les phénomènes de facilitation so-

ciale engendrés par les interactions directes entre les animaux sont limités, bien que les cages, proches les unes des autres, permettent tout de même une communication visuelle et acoustique constante entre les animaux, maintenant ainsi une interaction sociale nécessaire au bien-être de cette espèce.

2.1. Logement en cages individuelles métaboliques

Les **cages métaboliques** se trouvaient dans un bâtiment du plateau AniScans. Ces cages sont des « unités de détention délimitées de tous côtés par des parois à claire-voie avec un sol perforé en vue de la collecte des excréments et de l'urine séparément, permettant l'obtention de données métaboliques qualitatives et quantitatives » (Directive Protection des animaux 2.06). L'utilisation de cages métaboliques limitant les mouvements de l'animal a été requise dans les expériences pour lesquelles les animaux étaient équipés de cathéters (duodénal et/ou jugulaire), afin de faciliter leur utilisation lors des expériences ou lors des soins. De plus, le logement en cages métaboliques était indispensable dans le cas d'animaux soumis à des séances d'imagerie cérébrale puisque seul ce type de logement permet de récupérer les urines et fèces radioactives afin de ne pas contaminer le circuit classique d'évacuation des déchets. Les animaux étaient alors hébergés dans des cages métaboliques individuelles (150 × 60 × 80 cm ; **Figure 5a**), dans une salle à température contrôlée (22-24°C) et avec un cycle jour/nuit 12h/12h. Les cages étaient équipées d'une chaîne suspendue de manière à ce que les animaux puissent exprimer leur propension naturelle pour les comportements d'investigation et de jeu.

2.2. Logement individuel en élevage

Lorsque les animaux n'étaient soumis ni à une chirurgie ni à des séances d'imagerie cérébrales, ils étaient hébergés dans des **loges individuelles standard d'élevage** (**Figure 5b**, 126 × 75 cm et **Figure 5c**, et 132 × 122 cm) dans un bâtiment de la zone « élevage » du site de Saint Gilles. Les salles étaient maintenues dans des conditions contrôlées avec une température constante de 24 ± 2°C, et un cycle jour/nuit naturel, la salle étant équipée de fenêtres donnant sur l'extérieur.

Il est à noter également que, pour la réalisation des tests comportements décrits ci-dessous, les deux types de logements étaient équipés, au besoin, d'auges bipartites amovibles ou d'abreuvoirs doubles reliés à des bidons amovibles afin de faciliter les mesures de consommation à l'issue de tests comportementaux.

Figure 5. Types de logements utilisés au cours des différentes expériences de la thèse. (a) Cage métabolique permettant la récupération des urines et fèces radioactives et/ou la réalisation sécurisée de prélèvements et d'infusions *via* les cathéters veineux et duodénaux, respectivement. **(b, c)** Loges individuelles standard (126 x 75 cm et 132 x 122 cm, respectivement) pour les expériences ne nécessitant que des mesures comportementales. Les loges sont équipées d'auges bipartites amovibles (a, c) ou d'abreuvoirs doubles (b) pour l'implémentation de tests de préférence alimentaire.

3. Chirurgie : pose de cathéters duodénaux et jugulaires

Lorsque les expériences nécessitaient de réaliser des **infusions intra-duodénales (i.d.)**, les animaux subissaient une opération chirurgicale sous conditions stériles pour la pose d'un cathéter duodénal sous laparotomie. Après un jeûne de 24 heures, les animaux étaient pré-anesthésiés grâce à une injection intra-musculaire de kétamine avant d'être mis sous isoflurane (3-5% v/v). Après intubation, un niveau moyen d'anesthésie était maintenu pendant l'ensemble de l'acte chirurgical grâce à l'inhalation d'isoflurane (2-3% v/v) alors que l'analgésie était réalisée par injection intra-veineuse (i.v.) d'un agent morphinique (Fentanyl 4 ml). Sous surveillance des constantes vitales (*e.g.* rythme cardiaque et respiratoire, oxymétrie sanguine, capnométrie, etc.), une laparotomie était réalisée puis un cathéter était inséré dans le duodénum proximal, tunnélisé sous la peau et extériorisé entre les épaules de l'animal pour faciliter l'accès et l'utilisation ultérieurs. Certaines expériences ont également nécessité la réalisation de **prélèvements sanguins** sur de longues périodes, ce qui était permis par la pose d'un cathéter jugulaire. Dans ce cas, sous les mêmes conditions d'anesthésie et

d'analgésie, une incision était réalisée au niveau du cou de l'animal afin d'atteindre une veine périphérique rejoignant la veine jugulaire. Le cathéter était inséré dans la veine, tunnélisé sous la peau et extériorisé au niveau de la nuque de l'animal. Les animaux disposaient ensuite d'une semaine de récupération, avec 4 jours de traitement antibiotique post-opératoire (Ampiciline 4 ml/jour) et alimentation contrôlée et progressive. Durant cette période, un suivi post-opératoire avec évaluation de la douleur et de l'état général de l'animal était également réalisé (*cf.* **Annexe 1**).

4. Tests comportementaux

Différents tests comportementaux ont été conduits dans les expériences de cette thèse afin de conditionner une préférence ou une aversion alimentaire pour une flaveur d'intérêt (conditionnement pavlovien), d'évaluer les préférences spontanées ou conditionnées (tests de choix), ainsi que la motivation alimentaire (tests de ratio progressif ou conditionnement opérant) des animaux pour les aliments ou les solutions liquides proposés. Il s'agit ici de décrire sommairement le principe de ces différents tests dont les détails de mise en œuvre sont rapportés plus précisément dans les différents articles constituant ce manuscrit.

4.1. Conditionnement pavlovien

Principe

Le **conditionnement pavlovien** (ou conditionnement classique) repose sur l'association entre un stimulus conditionnel (CS) et un stimulus inconditionnel (US). Dans le cas d'un conditionnement alimentaire préférentiel ou aversif, les animaux apprennent à consommer préférentiellement ou à éviter un aliment additionné d'une flaveur inconnue (CS) qui est associée à des renforcements gustatifs et/ou viscéraux provoquant des conséquences appétitives et/ou post-ingestives (US) positives (*e.g.* goût plaisant, satiété) ou négatives (*e.g.* nausées, vomissements), respectivement. Après plusieurs associations entre CS et US, une réponse conditionnée (CR) doit alors apparaître (*e.g.* augmentation de la consommation ou évitement de l'aliment). Ces techniques d'apprentissage comportemental ont été utilisées tout au long de la thèse afin de conditionner des préférences ou des aversions pour des aliments solides ou des eaux de boisson aromatisé(e)s en utilisant différents types de renforcements détaillés ci-après.

Paradigmes expérimentaux

Dans chaque expérience utilisant ce type d'apprentissage, le conditionnement était systématiquement précédé d'une période d'habituation destinée, d'une part à la récupération post-opératoire dans le cas d'animaux cathétérisés, et d'autre part, à la familiarisation aux dispositifs expérimentaux et aux cages expérimentales. Les différents paradigmes expérimentaux utilisés dans cette thèse lors des conditionnements préférentiels ou aversifs sont résumés dans le **Tableau II**. Les variables de ces conditionnements sont les suivantes :

- **le type de CS** : la flaveur cible du conditionnement était ajoutée soit dans un aliment solide, soit dans de l'eau de boisson. Les flaveurs utilisées (thym, orange, cannelle) étaient fournies sous forme d'huiles essentielles par les Laboratoires Phodé (Terssac, France). Elles étaient diluées dans l'eau de boisson (0,025 %.) ou ajoutées dans l'aliment (diluées dans un mélange d'huiles végétales à 0,4 % pour le thym, 0,15 % pour l'orange ou 0,1 % pour la cannelle). L'**Annexe 2** présente l'ensemble des aliments de base utilisés au cours de cette thèse. Le choix des concentrations d'huiles essentielles dans la boisson et dans l'aliment a été effectué lors de tests préliminaires et sur la base de l'absence de réponse néophobique trop marquée et de préférence spontanée pour l'un(e) ou l'autre des aliments/eau de boisson ainsi aromatisé(e)s (*cf. Chapitre I – Expérience préliminaire : choix des concentrations des flaveurs dans l'aliment*) ;

- **le type de US** : le renforcement utilisé pour développer une aversion alimentaire était le chlorure de lithium (LiCl) qui s'est rapidement avéré efficace. Différents renforcements jouant sur la sphère viscérale et/ou gustative ont été testés pour le développement d'une préférence alimentaire conditionnée et sont détaillés dans le **Tableau II** ;

- **la modalité d'administration du US** : le US était délivré soit *per os*, *i.e.* ajouté directement dans l'eau de boisson ou l'aliment, soit en injection i.d., notamment lorsque l'étude s'intéressait aux stimuli jouant sur la sphère viscérale (apport calorique, malaise intestinal) ;

- **le nombre de sessions de conditionnement**, *i.e.* le nombre d'associations nécessaires entre le US et le CS (*e.g.* 4 associations) ;

- **la durée de chaque session de conditionnement**, *i.e.* la durée d'association entre le CSet le US par jour (*e.g.* 1 heure *vs* 7 heures par jour).

Tableau II. Différents paradigmes expérimentaux utilisés dans le cadre de la thèse pour la réalisation de conditionnements aversifs ou préférentiels. Les aliments et les eaux de boisson ont été aromatisés par l'ajout d'huiles essentielles de thym, orange et/ou cannelle fournies par les laboratoires Phodé. Les taux de dilutions des huiles essentielles dans les aliments et eaux de boisson ont été déterminés au préalable de manière à ce que les animaux n'expriment pas de préférences spontanées au début des tests, *i.e.* de manière à obtenir des aliments et eaux de boisson aux palatabilités similaires.

Chapitre	Stimulus conditionnel	Stimulus inconditionnel	Modalité d'administration	Durée du conditionnement	Nombre de sessions
I	Aliment aromatisé	LiCl 8%	i.d	30 minutes	4 sessions
		Glucose 15%	i.d.	30 minutes	4 sessions
II-1A	Boisson aromatisée	Sucrose 1,125%	*Per os*	7 heures	3 sessions
II-1B	Boisson aromatisée	Sucrose 10%	*Per os*	7 heures	6 sessions
II-2	Boisson aromatisée	Maltodextrine 2,25%	*Per os*	7 heures	4 sessions
		Saccharine 0,37%	*Per os*	7 heures	4 sessions
III	Boisson aromatisée	Sucrose 16%	*Per os*	1 heure	4 sessions
		Sucrose 16%	i.d.	1 heure	4 sessions

i.d. : injection intra-duodénale

4.2. Tests de préférences

Afin d'évaluer les préférences alimentaires spontanées ou conditionnées des animaux, différents tests de préférences alimentaires ont été réalisés. Ces tests reposent sur l'hypothèse principale que l'aliment ou l'eau de boisson préféré(e) (*i.e.* le/la plus palatable) sera consommé(e) en plus grande quantité. Deux méthodologies d'évaluation des préférences alimentaires ont été utilisées au cours de cette thèse : le test de double choix et la présentation d'un aliment unique.

4.2.1. Tests de double choix alimentaires

Principe

Dans les **tests de choix alimentaires**, plusieurs aliments expérimentaux (ou d'eaux de boisson expérimentales) sont présenté(e)s simultanément aux animaux pendant une période de temps équivalente. Dans la thèse, des **tests de double choix** pendant lesquels deux aliments/eaux de boisson étaient présenté(e)s simultanément aux animaux ont été utilisés.

Paradigmes expérimentaux

Lors des tests de choix sur l'aliment solide, deux aliments étaient présentés aux animaux pendant 30 min dans des auges bipartites (**Figure 6a**). Lorsque les tests de choix étaient réalisés sur les eaux de boisson, les animaux avaient accès à deux distributeurs d'eau de boisson pendant 7 heures ou pendant 1 heure, selon l'expérience concernée. Les solutions étaient disponibles à partir de bidons amovibles situés en hauteur au-dessus de la loge, et reliés à des abreuvoirs-sucettes de part et d'autre de la loge (**Figure 6b**). Après les sessions de tests, les refus d'aliment ou d'eau étaient pesés et des compléments alimentaires à base d'aliment non expérimental étaient fournis pour que les animaux reçoivent tous la même quantité d'aliment. En dehors des périodes de tests, les animaux avaient accès à de l'eau courante accessible soit *via* le circuit d'eau classique (logement en élevage), soit *via* les bidons (cages métaboliques).

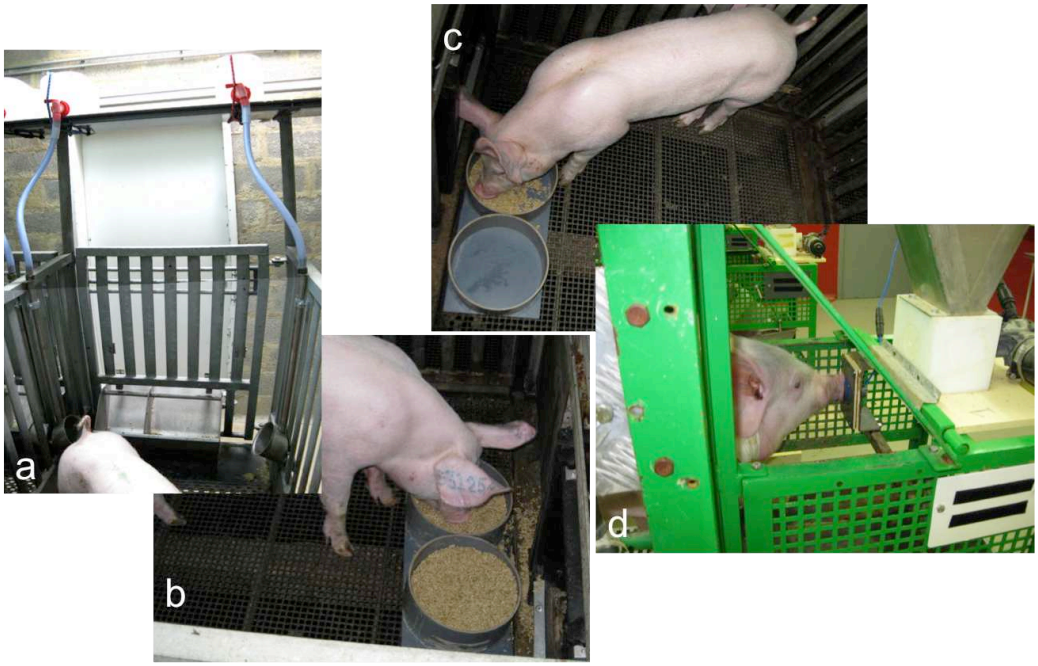

Figure 6. Types de dispositifs expérimentaux utilisés au cours de la thèse pour la mise en œuvre de tests comportementaux de mesure de la prise et des préférences alimentaires. (a) Tests de double choix réalisés sur des eaux de boisson. Les bidons amovibles contenant les boissons expérimentales sont reliés à des abreuvoirs-sucettes de part et d'autre de la loge. **(b)** Tests de double choix alimentaire réalisés dans une auge amovible. **(c)** Distribution d'un aliment unique. **(d)** Tests de conditionnement opérant (ou ratio progressif) durant lequel l'animal doit appuyer sur un bouton pour obtenir une récompense alimentaire, reflétant ainsi sa motivation à obtenir l'aliment.

4.2.2. Distribution d'un aliment unique

Principe

Dans la situation d'une **distribution d'aliment unique** qui se rapproche le plus des conditions d'élevage standard où les animaux sont généralement confrontés à un aliment unique plutôt qu'à une situation de choix alimentaire (Meunier-Salaün et Picard, 1996), les tests consistent à présenter successivement aux animaux différents aliments expérimentaux pendant une période de temps égale, et dans un ordre différent pour chaque animal. Ce type de test permet donc de déterminer de manière plus fiable les préférences alimentaires qui pourraient être observées dans une situation pratique d'élevage, contrairement aux tests de double choix alimentaire.

Paradigme expérimental

Dans notre cas, le paradigme expérimental consistait à présenter aux animaux un premier aliment pendant 15 min, puis l'aliment était retiré et un autre aliment était immédiatement présenté pendant 15 min supplémentaires avant d'être retiré à son tour (**Figure 6c**). Le choix de laisser l'accès à chaque aliment pendant seulement 15 min reposait sur la volonté d'avoir un temps total d'accès aux aliments équivalent à celui observé pendant les tests de double choix alimentaires, *i.e.* 30 min. L'ordre de présentation des aliments était alterné entre les animaux et les sessions de tests pour éviter tout biais expérimental. Les refus alimentaires pour chaque aliment étaient ensuite pesés et une ration complémentaire était fournie aux animaux afin que tous les animaux reçoivent une ration journalière équivalente.

4.3. Conditionnement opérant

Principe

Dans les tests de **conditionnement opérant**, aussi appelé tests de ratio progressif (PR), les animaux doivent travailler (*i.e*. appuyer sur un bouton) pour obtenir une récompense alimentaire représentée par une petite quantité d'aliment. Ces tests qui permettent d'évaluer la motivation alimentaire ainsi que les préférences alimentaires reposent sur l'idée simple que la quantité de travail fournie par l'animal serait d'autant plus grande que l'animal est motivé à manger, motivation qui elle-même est fonction de la palatabilité ou du caractère préféré de l'aliment.

Paradigme expérimental

Le dispositif expérimental était constitué d'un bouton poussoir disposé au-dessus de l'auge et connecté à un système automatisé de distribution des récompenses (5 g d'aliment granulé ; **Figure 6d**). Le dispositif était relié à un ordinateur (MacIntosh II vx, software 7.5.3, Apple Computer) équipé d'un logiciel (LabVIEW 3.1.1) contrôlant la distribution des récompenses et enregistrant le nombre de récompenses obtenues et le nombre d'appuis réalisés en fonction du temps. Les animaux étaient d'abord soumis à une semaine d'entraînement avec de l'aliment non expérimental. Puis, pendant la semaine suivante, après avoir reçu 200 g d'aliment non expérimental pour minimiser la motivation strictement due à la faim, les animaux étaient testés en suivant un programme de PR de 4 heures au cours duquel le nombre de fois où l'animal devait appuyer sur le bouton pour obtenir 1 récompense était augmenté toutes les 3 récompenses par incrément de 5 appuis (*i.e.* 1,1,1, 6,6,6, 11,11,11 ,16,16,16,… appuis pour 1 récompense). À l'issue des tests, un complément alimentaire aux caractéristiques oro-sensorielles différentes de celles des aliments présentés pendant les tests de PR était distribué.

4.4. Analyse comportementale

Pour l'ensemble des tests comportementaux, des relevés de consommation d'aliment et d'ingestion d'eau à la fin du chaque test ont été systématiquement réalisés. Pour les tests de PR, différentes variables supplémentaires ont été relevées comme le nombre total d'appuis, le nombre de récompenses obtenues, le taux d'appuis (appuis/min), le nombre d'appuis requis pour obtenir la dernière récompense (breakpoint), et la pause post-récompense (temps moyen entre l'obtention d'une récompense et l'appui suivant). Certaines expériences ont également nécessité la réalisation de relevés comportementaux. Pour ce faire, deux méthodes d'échantillonnage ont été utilisées : le **scan sampling** et le **focal sampling**.

Le **scan sampling** consiste à relever pour l'ensemble des animaux, et à intervalle de temps régulier, le comportement de chaque individu au moment précis de l'observation (*e.g.* observation pendant 30 min avec un point d'échantillonnage toutes les 30 sec). Ces mesures ont été réalisées en observation directe, *via* l'utilisation du logiciel Pocket Observer® (Noldus, Information Technology, Wageningen, Pays-Bas) installé sur un PC de poche (iPAQ 214, Hewlett-Packard, Palo Alto CA, USA). Les observations ont permis d'établir des budget-temps, détaillant la proportion de temps passé à réaliser différents items comportemen-

taux (*e.g.* boit, mange, explore l'auge, explore le sol, défèque, etc.), ainsi que la proportion de temps passé dans différentes postures (debout, assis, à genoux, couché). Le **focal sampling** consiste à observer un individu cible pendant un temps donné et à noter en continu les comportements d'intérêt de cet individu. Ces mesures ont été réalisées à partir des enregistrements vidéo et ont notamment permis de relever le temps total passé en contact avec chaque aliment/abreuvoir, la latence d'accès aux aliments/eaux de boisson ou le nombre d'alternance d'accès à chaque auge pendant les tests de choix alimentaires par exemple.

4.5. Analyse statistique des résultats comportementaux

L'ensemble des données comportementales est présenté sous forme de moyennes et d'erreurs-standard. Les analyses statistiques ont été réalisées avec le logiciel Statview 4.57 (Abacus Concepts Inc., Berkeley, USA) et le logiciel R (R.app for GUI 1.43, R Foundation for Statistical Computing, 2011). La normalité des résidus et l'égalité des variances ont été vérifiées pour les données continues (temps, consommation). Pour les données ne suivant pas de loi normale (*e.g.* variables non continues), des tests non-paramétriques ont été utilisés. Les variables, le plus souvent appariées, ont été analysées grâce au test de Friedman. Lorsque le test de Friedman se révélait significatif, les variables étaient comparées deux à deux grâce au test de Wilcoxon. Dans le cas de données non-appariées, un test de Kruskal-Wallis était réalisé, suivi d'un test de Mann-Whitney si le Kruskal-Wallis était significatif. Dans le cas de comparaisons multiples, une correction de Bonferroni était appliquée (α/nombre de comparaisons). Le cas échéant, une mention spéciale indique les résultats présentés avant la correction de Bonferroni. Lorsque les données suivaient une loi normale (*e.g.* données de consommation), des tests paramétriques ont été utilisés. Des analyses de variance de type ANOVA (ANalyse Of VAriance) à deux facteurs sur mesures répétées, suivies d'une analyse d'effets principaux (« simple main effet ») ont été utilisées pour évaluer les interactions session × traitement et l'effet de ces variables sur la consommation lors des conditionnements. Des tests *t* de Student pour données appariées ont permis de comparer les consommations deux à deux lors des tests de choix par exemple. Pour l'ensemble des données comportementales, le seuil de significativité a été fixé à $\alpha = 0,05$, les valeurs de *P* comprises entre 0,1 et 0,05 étant considérées comme des tendances.

5. Imagerie fonctionnelle couplée à une stimulation sensorielle

5.1. Anesthésie des animaux

Avant la stimulation, les animaux étaient anesthésiés d'une manière similaire à l'anesthésie obtenue lors des actes chirurgicaux. Après un jeûne de 24 heures, les animaux étaient pré-anesthésiés grâce à une injection intra-musculaire de kétamine avant d'être mis sous isoflurane (3-5% v/v). Après intubation, un niveau moyen d'anesthésie était maintenu pendant l'ensemble de la procédure couplant stimulation sensorielle et imagerie fonctionnelle grâce à l'inhalation d'isoflurane (2-3% v/v). Les constantes vitales (*e.g.* rythme cardiaque et respiratoire, oxymétrie sanguine, capnométrie, etc.) étaient surveillées tout au long de la procédure.

5.2. Stimulation sensorielle sur animal anesthésié

Principe

Le dispositif de stimulation (**Figure 7**) est composé de deux automates indépendants assistés par ordinateur permettant de réaliser une stimulation olfactive et/ou gustative combinées ou indépendantes. Le principe de la stimulation olfactive consiste à diffuser de l'air odorisé au débit souhaité (4 L/min) dans la narine de l'animal *via* un cathéter nasal relié à l'**olfactautomate**. L'olfactautomate est constitué d'une bouteille d'air médical et de deux flacons contenant de l'eau additionnée d'une flaveur qui, par bullage, permet la diffusion d'air odorisé dans le dispositif jusqu'à la narine de l'animal. La stimulation gustative consiste à diffuser au débit souhaité (24 mL/min) de la salive aromatisée ou additionnée d'une saveur d'intérêt (*e.g.* sucre) sur la langue de l'animal *via* un cathéter lingual relié au **gustautomate**. Les paramètres de la stimulation ainsi que la synchronisation étaient alors contrôlés par ordinateur.

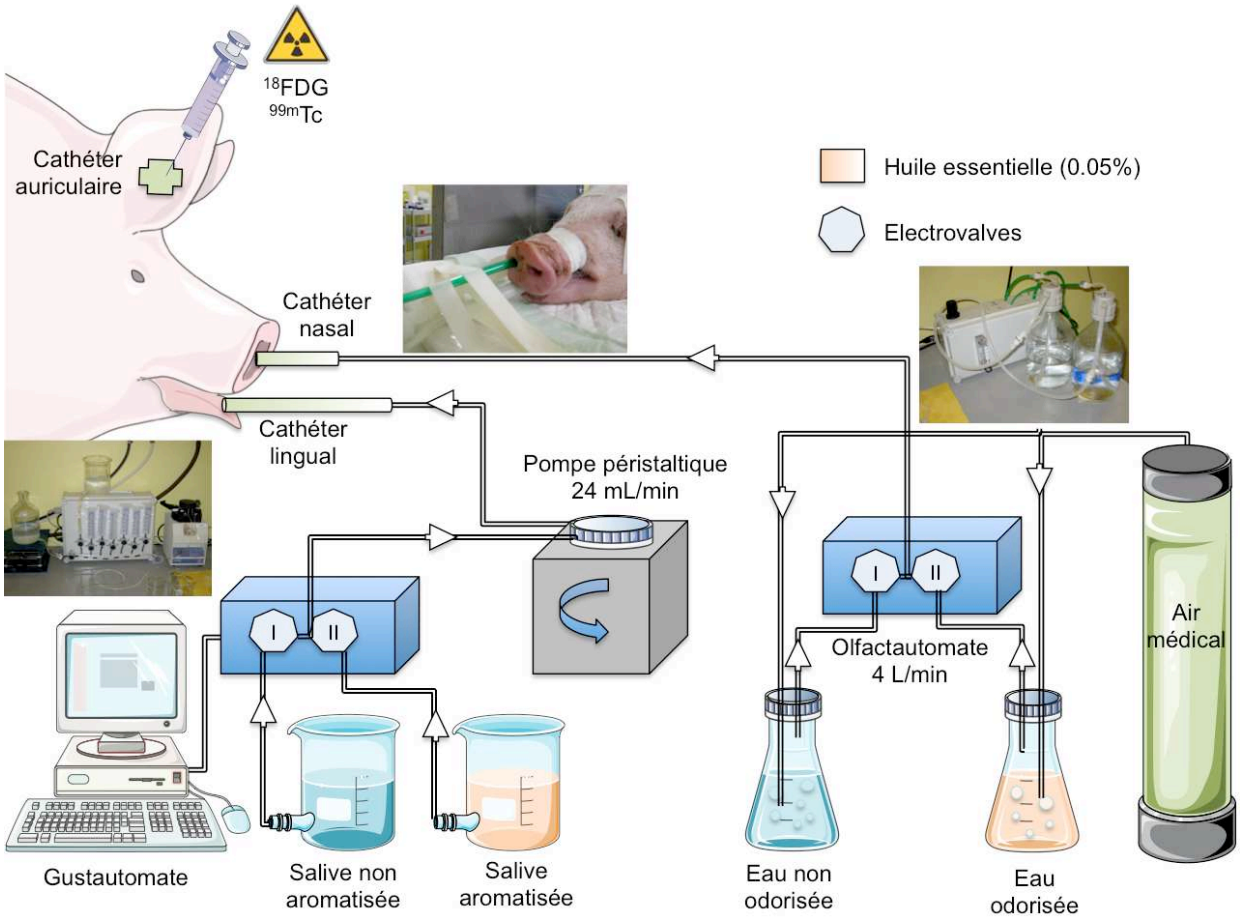

Figure 7. Schéma des dispositifs de stimulation olfactive (Olfactautomate) et gustative (Gustautomate). Les deux dispositifs pouvaient être utilisés de manière indépendante ou simultanément. Ces stimulations étaient couplées avec l'injection d'un marqueur radioactif (^{18}FDG ou ^{99m}Tc) pour la réalisation de séances d'imagerie cérébrale fonctionnelle (PET ou SPECT).

Paradigmes expérimentaux

Le paradigme de stimulation (durée et moment de la stimulation) était adapté aux besoins de l'étude. La stimulation débutait systématiquement par une stimulation neutre (air non odorisé et salive non aromatisée) afin d'habituer les thermo- et les mécanorécepteurs des muqueuses olfactives et gustatives. Puis, après une période de stimulation avec les flaveurs/goûts d'intérêt (air odorisé et salive aromatisée), la stimulation se terminait par un rinçage avec une stimulation neutre. La stimulation était systématiquement réalisée avant passage en imagerie cérébrale sur des animaux anesthésiés dans un environnement contrôlé (minimisation des stimulations visuelles et auditives par immobilisation des paupières avec une bande adhésive de pharmacie et obstruction des conduits auditifs avec de la ouate).

5.3. Techniques d'imagerie fonctionnelle

Deux types d'imagerie fonctionnelle ont été utilisés au cours de cette thèse : la **tomographie par émission positronique** (PET), et la **tomographie d'émission monophotonique** (SPECT) ou scintigraphie. Le principe de ces imageries tomographiques consiste à injecter un traceur radioactif dont on connaît les propriétés biologiques de fixation, puis à réaliser des images de coupe 2-dimensions (2D) sous différentes incidences angulaires (*i.e.* projections) à travers une structure, *i.e.* le cerveau dans notre cas, afin d'en réaliser une image 3-dimensions (3D) suite à une reconstruction tomographique.

5.3.1. Tomographie par émission positronique (Figure 8ab)

Le principe de cette technique d'imagerie cérébrale fonctionnelle est d'injecter au patient, par voie i.v., du **2-fluoro-2-désoxy-D-glucose**, une molécule marqué au fluor 18 (**^{18}F-FDG**). Ce marqueur, analogue du glucose, se fixe dans les tissus qui consomment du sucre, comme c'est le cas du cerveau, au *pro rata* de l'activité cérébrale, permettant ainsi, par une analyse de contraste d'images, d'obtenir une carte fonctionnelle de l'activité cérébrale suite à différentes stimulations sensorielles. Pour une PET cérébrale, l'activité administrée est de 200 MBq.

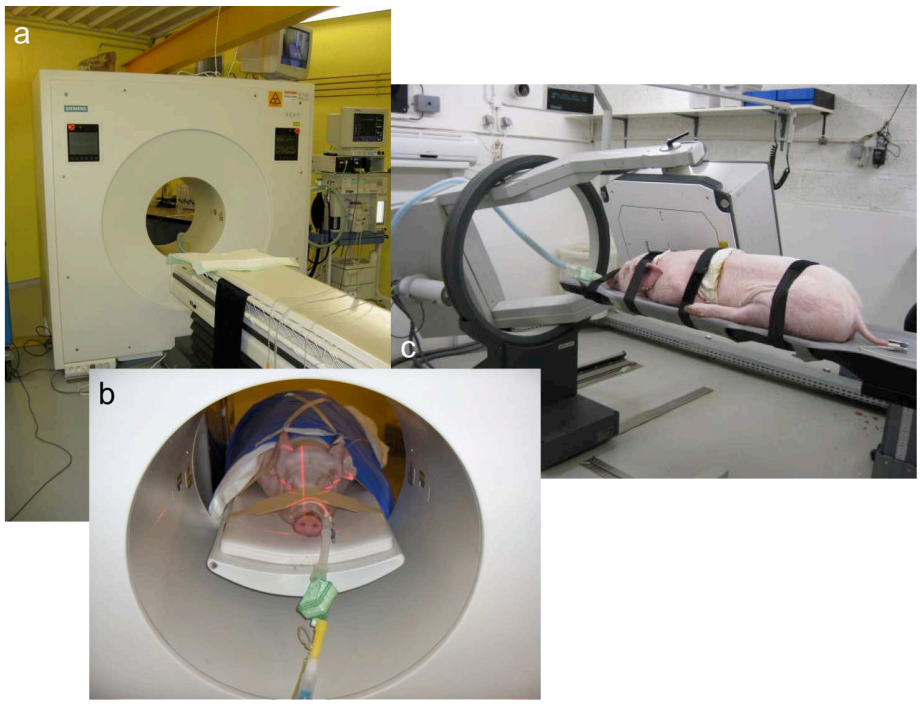

Figure 8. Dispositifs d'imagerie cérébrale fonctionnelle utilisés au cours de la thèse. (a) Tomographe d'émission positronique (PET). (b) Animal anesthésié installé dans l'oculus pour la réalisation d'un PET pour la réalisation d'une imagerie cérébrale fonctionnelle. (c) Animal anesthésié soumis à une tomographie par d'émission monophotonique (SPECT).

Le scanner PET (Siemens ECAT 962 HR+) utilisé au cours de cette thèse est constitué d'une vingtaine d'anneaux de détecteurs (collimateurs) constitués de cristaux scintillateurs et de photomultiplicateurs, avec environ 784 détecteurs par anneau. Le traceur marqué d'un atome radioactif (^{18}F), en se désintégrant, émet des positrons qui s'annihilent avec les électrons du milieu après un bref parcours, ce qui entraîne l'émission de deux photons gamma (511 keV) dans la même direction (*i.e.* le long d'une ligne de coïncidence), mais dans des sens opposés (**Figure 9a**). Les détecteurs situés sur le collimateur de la caméra PET détectent alors ces photons gamma d'annihilation qui arrivent en même temps (photons en coïncidence), permettant ainsi de localiser le lieu de la réaction d'annihilation et la concentration du traceur en ce point précis du cerveau (**Figure 9b**).

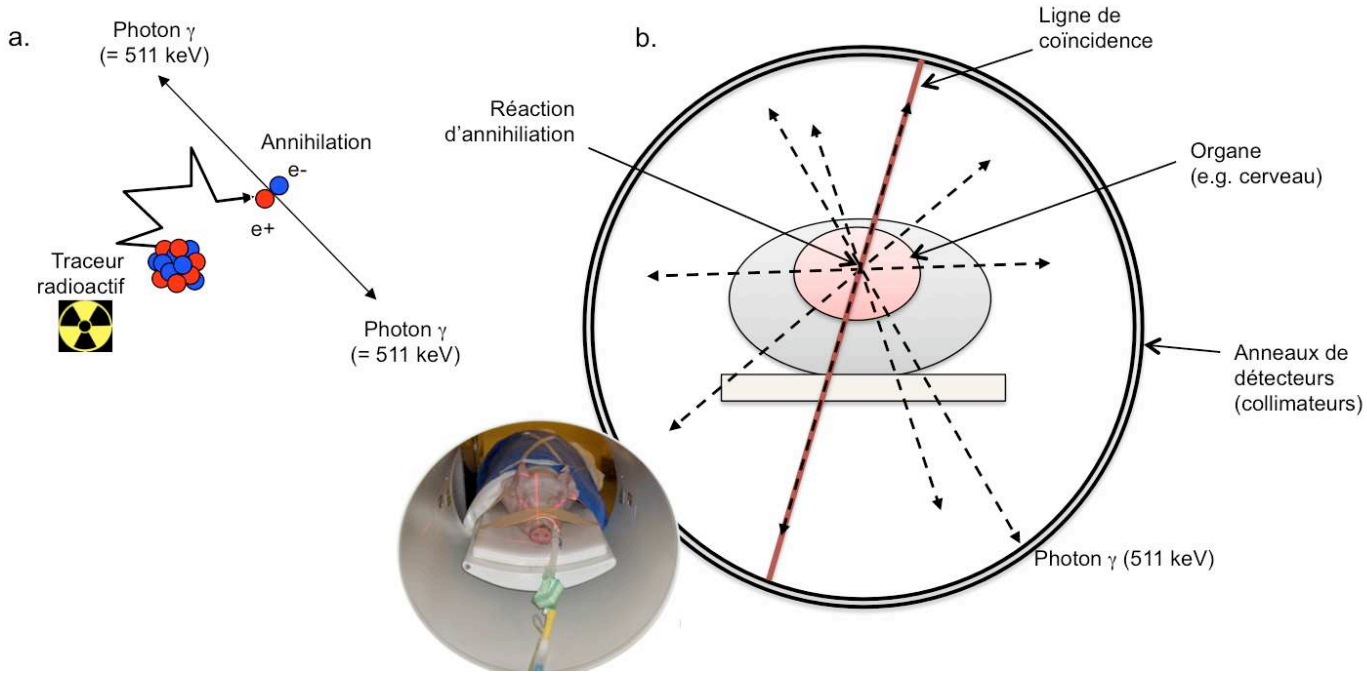

Figure 9. Principe de la tomographie par émission de positrons (PET). (a) Désintégration du positron et réaction d'annihilation. Le positron (e+) émis par le traceur radioactif (^{18}FDG) interagit avec un électron (e-) du milieu suivant une réaction d'annihilation au cours de laquelle sont libérés deux photons gamma de 511 keV, émis dans des sens opposés (d'après de Dreuille et al. 2004). (b) Examen tomographique par émission de positrons. La formation de l'image résulte de la détection par les anneaux de détecteurs des deux photons en coïncidence émis lors de la réaction d'annihilation.

Les informations relatives à la perception des photons en coincidence sont rassemblées dans une matrice appelée sinogramme et contenant l'ensemble des éléments de projection d'une coupe de la structure. L'obtention de l'image s'effectue ensuite *via* une étape de reconstruction tomographique, utilisant différents algorithmes de reconstruction et permettant d'obtenir une représentation 3D de la distribution du traceur dans l'organe à partir des sino-

grammes (de Dreuille *et al.*, 2002). Pendant l'acquisition, des mesures de transmission sont également réalisées au moyen d'un émetteur de position (^{68}Ge), et vont permettre de corriger les phénomènes d'atténuation du signal provoqué par les variations de composition et d'épaisseur des tissus traversés. Ces mesures de transmission seront ensuite prises en compte avant l'étape de quantification de la radioactivité au sein de la structure.

Dans le cadre de cette thèse, certaines expériences nécessitaient de réaliser des séances d'imagerie cérébrale après infusion i.d. de sucrose, modifiant ainsi de manière substantielle la glycémie au cours de la séance. Étant donné que la technique PET repose sur la fixation d'un analogue du glucose dans les zones cérébrales activées, elle n'est pas appropriée dans une situation de glycémie instable. Ainsi, dans ce cas précis, la technique SPECT a été privilégiée, bien que celle-ci présente une résolution moins importante (SPECT : 1 cm *vs* PET : 4-7 mm). Les caractéristiques de ces deux techniques sont rappelées et comparées à celles de l'IRM fonctionnel dans le **Tableau III** (d'après Otte et Halsband, 2006).

5.3.2. Tomographie par émission monophotonique (Figure 8c)

Le principe de cette technique d'imagerie cérébrale fonctionnelle est de marquer la microvascularisation cérébrale *via* l'injection au patient, par la voie i.v., d'un produit radiopharmaceutique composé d'un vecteur non-radioactif, l'HMPAO, et d'un isotope marqué, le **technétium 99m** (**^{99m}TC**) dont les rayonnements gamma émis sont captés par une gamma-caméra. Pour un SPECT cérébral, l'activité administrée est d'environ 740 MBq.

Dans notre étude, nous avons utilisé une gamma-caméra simple tête (Apex SP-6, Elscint, Tel-Aviv, Israël). Une gamma-caméra est essentiellement composée d'un collimateur et de têtes de détection de rayonnements gamma, composées chacune d'un cristal scintillateur d'iodure de sodium de grand diamètre (40 cm). Brièvement, les photons gamma (140 keV) émis par le radio-isotope vont percuter la face externe du cristal, ce qui va produire un photon de fluorescence. Les tubes photomultiplicateurs disposés sur la face interne du cristal vont détecter le signal lumineux émis (**Figure 10**).

La gamma-caméra réalise une rotation de 360° autour de la tête du sujet et va acquérir de multiples projections (128 projections dans notre cas), acquises en mode pas à pas, *i.e.* « step and shoot » (tous les 6° dans notre étude). Elle permet ainsi d'acquérir plusieurs images 2D (projections) sous différentes incidences angulaires. L'acquisition est de type dynamique, l'image étant constituée par accrétion des photons gamma reçus par la gamma ca-

méra durant un temps défini. Le temps d'acquisition pour une image unitaire est de 6 sec toutes les 2 min pour une durée totale de 120 min. Suite à cette acquisition, un algorithme de reconstruction tomographique est appliqué aux projections afin de reconstruire une image 3D.

Tableau III. Caractéristiques de deux techniques d'imagerie fonctionnelle utilisées au cours de la thèse pour la mesure de l'activité cérébrale (PET et SPECT), et comparaison avec une autre technique, le fMRI. PET : tomographie par émission de positrons, SPECT : tomographie par émission monophotonique, fMRI : imagerie par résonnance magnétique fonctionnelle. (*d'après* Otte et Halsband, 2006).

	PET	SPECT	fMRI
Paramètres évalués	Métabolisme du glucose	Flux sanguin	Mesure indirecte de l'oxygénation sanguine
Radiotraceur	Oui (^{18}F-FDG) ! vie courte (110 min)	Oui (^{99m}Tc) ! vie longue (6 h)	Non
Irradiation	Oui (10 mSv)	Oui (1-10 mSv)	Non
Précision quantitative	Oui	Non	Non
Résolution spatiale	4 mm	10-12 mm	1-1,5 mm
Résolution temporelle	35 min (^{18}FDG)	10 min	1,5-2 min
Résolution et sensibilité de l'image	+++	–	+++
Durée minimale de l'examen	15-30 min	15-20 min	1,5-2 min
Informations neuroanatomiques	Non scan additionnel nécessaire	Non scan additionnel nécessaire	Oui
Examen bruyant	Non	Non	Oui
Coût de l'examen	+++ (prix radiotraceur, cristaux scintillateurs)	++ (prix radiotraceur) 5! moins que PET	– (aucun radiotraceur)
Faisabilité	Nécessite un cyclotron pour l'obtention du radiotraceur + personnel qualifié	Obtention du radiotraceur simple et sans cyclotron	Aucun radiotraceur

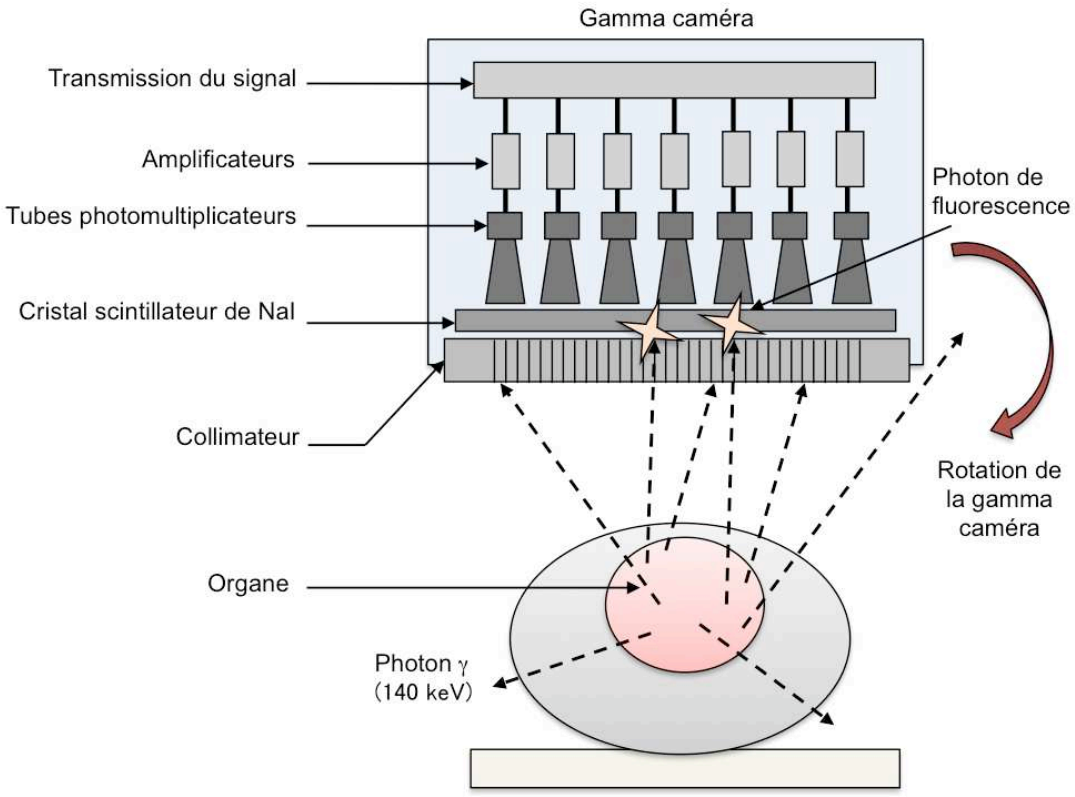

Figure 10. Principe de fonctionnement de la gamma caméra lors d'une tomographie par émission monophotonique (SPECT). Les rayonnements gamma émis par le radioisotope (^{99m}Tc) percutent la face externe du cristal de la gamma caméra, ce qui produit l'émission de photons de fluorescence. Ces photons de fluorescence sont alors détectés par les tubes photomultiplicateurs. Ces signaux seront ensuite traités pour le dispositif informatique inhérent.

5.4. Traitement et analyses des images cérébrales

L'ensemble des images cérébrales obtenues par la PET et le SPECT ont été traitées puis analysées avec le **logiciel SPM8** (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK), installé sous MATLAB 7.1 (The Mathwordks Inc., Natick MA, USA).

Avant la réalisation d'une analyse statistique des images, un pré-traitement complexe des images a été nécessaire. Brièvement, ce traitement initial consistait à rendre l'ensemble des images plus uniformes entre elles et vis-à-vis d'une image de référence, le template, en appliquant différentes étapes de transformations successives aux images (*i.e.* réorientation, réalignement, masquage, lissage, normalisation, etc.). L'ensemble des étapes de pré-traitement des images proposées par le logiciel SPM8 et utilisées au cours de cette thèse sont présentées dans le **Tableau IV**. Néanmoins, l'agencement des étapes entre elles étant dépen-

dant de la technique d'imagerie utilisée, les procédures précises utilisées pour chaque étude seront donc indiquées plus en détail dans les articles concernés. Les templates SPECT et PET, ou images de référence, ont été obtenus en réalisant des séances d'imagerie SPECT et PET dans des conditions similaires d'anesthésie, d'acquisition et de traitement sur un certains nombre d'animaux (template PET : n = 11 ; template SPECT : n = 16) de taille et d'âge équivalents à ceux utilisés au cours de cette thèse. Un template obtenu par imagerie par résonnance magnétique (IRM) a également été utilisé pour différentes estimations effectuées sous SPM et pour la localisation anatomique des différentes structures cérébrales d'intérêt. Les conditions d'acquisition et de prétraitement des images IRM, obtenues sur un appareil Siemens Avanto system (Siemens AG, Munich, Allemagne) avec un champ magnétique de 1,5 T, sont détaillées dans l'étude de Boubaker et al. (2012).

Une fois ces étapes de pré-traitement réalisées, l'analyse statistique consistait à comparer les images cérébrales en fonction des traitements expérimentaux grâce à des tests *t* pour données appariées. Ces comparaisons de contraste inter-traitement permettaient d'obtenir des cartes différentielles de l'activité cérébrale entre deux traitements ou conditions données (*e.g.* stimulus négatif *vs* stimulus neutre). L'analyse utilisée était l'analyse **« Small Volume Correction analysis »** (SVC). Cette analyse permet de réaliser des comparaisons voxel à voxel dans des régions d'intérêt (**Regions Of Interest, ROI**) sélectionnées sur la base d'hypothèses posées *a priori*. Les ROIs concernaient donc principalement les structures impliquées dans la régulation hédonique de la prise alimentaire et dans la caractérisation hédonique de stimuli olfacto-gustatifs et présentées dans la deuxième partie de l'introduction de thèse. L'analyse SVC avait donc pour objectif d'identifier, dans chacune de ces ROIs, les voxels pour lesquels l'activité était statistiquement différente entre les traitements (*i.e.* entre les images). La reconnaissance des structures cérébrales et la caractérisation anatomique des ROIs ont été réalisées *via* l'utilisation d'un atlas stéréotaxique numérique 3D mis au point dans notre laboratoire (Saikali *et al.*, 2010) exploité sous le logiciel 3DSlicer (http://www.slicer.org/). Pour l'ensemble des analyses, le seuil de significativité a été fixé à $P < 0,05$ (seuil étendu à 5 voxels).

Tableau IV. Description et principe des différentes étapes de pré-traitement des images issues des séances d'imagerie cérébrale fonctionnelle. Ces étapes de pré-traitement ont été agencées entre elles différemment selon les techniques d'imagerie utilisées. Ces étapes servent à homogénéiser la forme et l'orientation des images entre elles afin de faciliter les comparaisons réalisées par la suite lors des étapes d'analyses statistiques.

Étape	Principe
Reorient	Réoriente les images sources dans l'axe commissure antérieure-commissure postérieure pour qu'elles soient dans la même inclinaison que le template qui est l'image de référence. Consiste donc à appliquer des angles de rotation à l'image, puis à la recentrer par rapport à un point de référence dont les coordonnées x,y,z sont 0,0,0. Le point de référence choisi ici est la commissure postérieure.
Realign	Réaligne l'ensemble des images d'un même sujet sur une image de référence (template) en utilisant une approche "least squares" et une transformation sur 6 paramètres (i.e. déplacement, rotation, etc.). Le but est de supprimer les artéfacts de mouvement entre les séries d'images. Après le réalignement, les images sont découpées afin de correspondre avec l'image de référence, voxel à voxel.
Coregister	Étape sensiblement équivalente à l'étape « Realign ». Consiste à manipuler les images sources dans l'espace pour les faire correspondre au mieux à l'image de référence (template) qui, elle, reste stationnaire. Tout comme pour l'étape "Realign", les 6 paramètres de modifications des images correspondent à trois déplacements et trois rotations selon différents axes. Après le réalignement, les images sont découpées afin de correspondre avec l'image de référence, voxel à voxel.
Normalise	Consiste à appliquer des déformations sur les images sources jusqu'à l'obtention d'une forme qui permettra de faire correspondre au mieux la forme des images sources entre elles et à la forme de l'image de référence (template). Ces déformations consistent en fait à réaliser une transformation affine des images sources limitée à 12 paramètres, ce afin d'éviter des déformations trop importantes des images originales.
Mask	Supprime la matière extracérébrale entourant le cerveau grâce à l'application d'un masque adapté à la taille du cerveau. Pendant le masquage, le programme cherche parmi les séries d'images les voxels qui doivent être échantillonnés à l'extérieur de l'image originale. La valeur du voxel est ainsi fixée à 0 pour l'ensemble des images et le voxel ne sera pas pris en compte lors des analyses ultérieures.
Smooth	Permet de lisser les images *via* l'application d'un filtre Gaussian d'une largeur spécifiée. Ce procédé permet de supprimer les bruits de fond et les effets dus à des différences anatomiques et fonctionnelles résiduelles entre les individus.

CHAPITRE I

Préférences et Aversions Alimentaires Conditionnées par des Renforcements Viscéraux : Réponses Comportementales et Cérébrales

CHAPITRE I : PRÉFÉRENCES ET AVERSIONS ALIMENTAIRES CONDITIONNÉES PAR DES RENFORCEMENTS VISCÉRAUX : RÉPONSES COMPORTEMENTALES ET CÉRÉBRALES

Ce premier chapitre est consacré au développement d'un modèle de l'aversion et de la préférence alimentaire conditionnée chez le porc. Dans un premier temps, grâce à des techniques de conditionnement pavlovien, l'étude a porté sur l'impact de l'infusion i.d. d'une substance émétique (chlorure de lithium) ou calorique (glucose 15%) pendant un repas sur la consommation et sur les choix alimentaires ultérieurs. L'hypothèse de travail était que l'expérience acquise au contact de l'aliment, *i.e.* les conséquences post-ingestives, modifie durablement les choix alimentaires et la prise alimentaire chez ces animaux. Dans un second temps, les travaux ont porté sur les réponses cérébrales provoquées par la perception de flaveurs alimentaires aux valeurs hédoniques contrastées. L'hypothèse était que la perception de stimuli alimentaires dont les valeurs hédoniques sont différentes entraînent des patterns d'activité cérébrale contrastés dans les structures connues pour être impliquées chez l'Homme dans la caractérisation de la palatabilité des aliments, dans le traitement des informations sensorielles, dans la motivation et l'apprentissage, et plus généralement dans la régulation hédonique du comportement alimentaire (*cf. Partie 2 de la synthèse bibliographique*).

Partie 1. Expérience préliminaire : choix des concentrations des flaveurs dans l'aliment

1. Contexte et objectifs

La réalisation d'expériences de conditionnement alimentaire nécessite l'utilisation d'aliments expérimentaux aromatisés dont la flaveur sera conditionnée positivement, dans le cas d'une préférence, ou négativement, dans le cas d'une aversion. Ainsi, il est nécessaire de s'assurer au préalable que les animaux ne déclarent pas de préférences spontanées pour les aliments aromatisés avant l'apprentissage alimentaire, deux flaveurs distinctes étant susceptibles, pour une même concentration, d'avoir une palatabilité différente. Dans cette thèse, trois flaveurs ont été utilisées pour confectionner les aliments expérimentaux : une flaveur de thym, une de cannelle et une autre d'orange (Laboratoires Phodé, Terssac, France). L'objectif de cette série d'expériences préliminaires était donc de déterminer les taux de dilution de thym, de cannelle et d'orange pour lesquels les animaux n'exprimeraient pas de préférences spontanées, suggérant que les trois aliments auraient des palatabilités similaires. Différents taux de dilution ont donc été testés en appliquant des tests de triple choix alimentaire.

2. Matériels et méthodes

2.1. Animaux et logement

Au total, trois lots de huit animaux ont été utilisés dans le cadre de cette étude préliminaire. Les animaux, des femelles juvéniles de race Piétrain × (Large White × Landrace) d'environ 30 kg, étaient logées en cages individuelles et avaient libre accès à l'eau. La pièce était maintenue à une température avoisinant 24°C avec un cycle jour/nuit de 13 heures/11 heures. Tous les animaux étaient naïfs en début d'expérience, c'est-à-dire qu'ils n'avaient jamais été mis en contact avec les aliments expérimentaux aromatisés.

2.2. Aliments expérimentaux

Pour chaque lot, trois aliments expérimentaux ont été fabriqués à partir d'huiles essentielles de thym (T), de cannelle (C) et d'orange (O ; Laboratoires Phodé, Terssac, France). Les huiles essentielles étaient diluées à des taux différents (**Tableau V**) dans une même base d'huile végétale, et incorporées dans l'aliment à raison de 10 ml/kg d'aliment. Les huiles étaient ajoutées dans un aliment 2ème âge granulé inconnu des animaux au début de l'expérience (*cf.* **article n°2** pour la composition de l'aliment).

Tableau V. Taux de dilutions testées pour chaque huile essentielle de thym, cannelle et orange. Les huiles essentielles ont été diluées aux taux indiqués dans une base d'huile végétale et incorporées dans l'aliment à raison de 10 ml/kg d'aliment.

	Concentrations des huiles essentielles		
Lot expérimental	Thym	Cannelle	Orange
LOT 1	0,2%	0,2%	0,1%
LOT 2	0,4%	0,2%	0,1%
LOT 3	0,4%	0,1%	0,1%

2.3. Tests comportementaux

À raison d'un test par jour, les animaux ont été soumis à des tests de triple choix alimentaire pour évaluer leurs préférences pour les différents aliments aromatisés. Pour le lot 1, les tests ont été répétés deux jours consécutifs, pour le lot 2, les tests ont été répétés cinq jours consécutifs, et enfin, pour le lot 3, les tests ont été répétés quatre jours consécutifs. Pendant ces tests, une auge tripartite contenant 1 kg de chaque aliment expérimental T, O ou C était présentée aux animaux à jeun, à 9 h et pendant 30 min, puis l'auge était retirée et les refus étaient pesés. La distribution des aliments dans les auges tripartites était alternée entre les animaux et entre les répétitions pour éviter tout biais méthodologique.

2.4. Analyses statistiques

Les données ont été analysées avec le logiciel R 2.14.1 (The R Foundation for Statistical Computing, 2011). Les quantités d'aliments consommées pendant les tests de choix ont été comparées par des analyses de variance de type ANOVA à un facteur sur mesures répétées, suivie éventuellement de comparaisons deux à deux par le test *t* de Student pour données appariées. Le seuil de significativité était fixé à 5% et une correction était appliquée pour les comparaisons multiples d'après la méthode de Benjamini et Yekutieli (2001). La normalité des résidus a été vérifiée par des tests de Kolmogorov-Smirnov.

3. Résultats

Les niveaux de consommation pendant les tests sont présentés dans la **Figure 11**. Des différences de consommation ont été mises en évidence dans le lot 1 ($F(2,14) = 8,93$, $P < 0,01$) Les comparaisons deux à deux révèlent que l'aliment T était davantage consommé que l'aliment C ($P < 0,01$), et que l'aliment O ($P < 0,05$), alors que la quantité d'aliment O consommée ne différait pas de celle de l'aliment C. Aucune différence de consommation n'a été mise en évidence dans les lots 2 ($F(2,14) = 1,88$, $P > 0,1$) et 3 ($F(2,14) = 1,25$, $P > 0,1$).

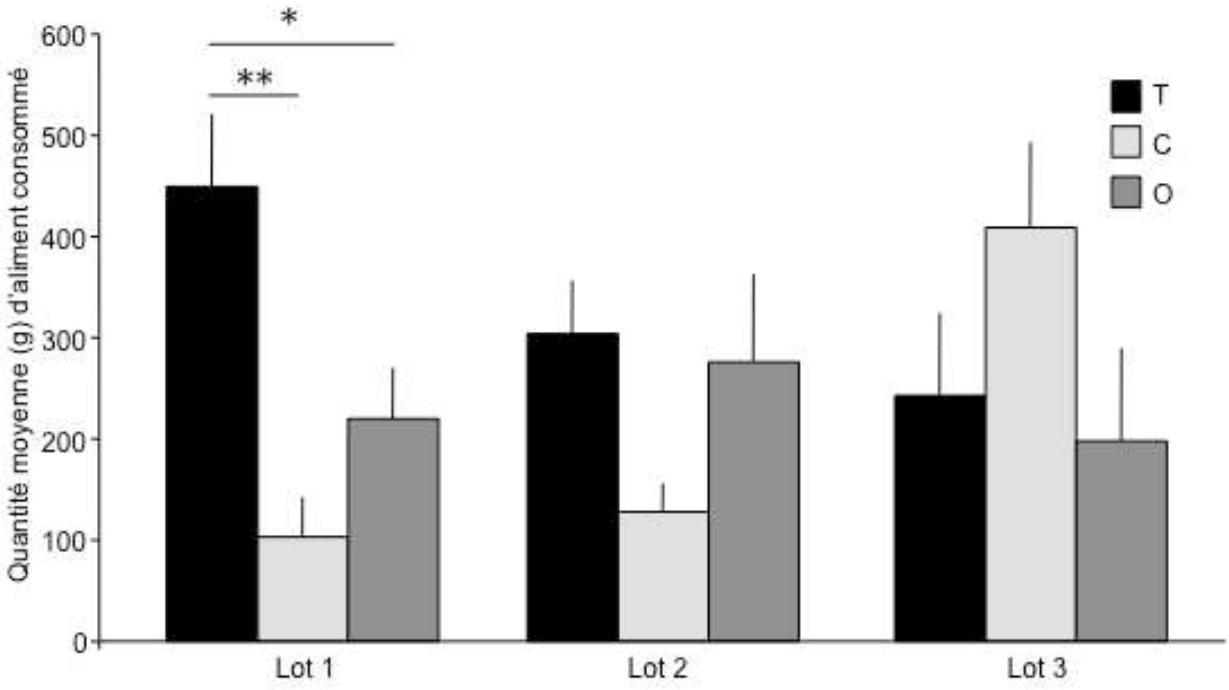

Figure 11. Quantité moyenne (en g, S.E.M.) d'aliment consommé pendant les tests de triple choix alimentaires. T : thym, C : cannelle, O : orange. Les concentrations des flaveurs dans les aliments changent selon les lots expérimentaux. Lot 1 : T 0,2%, C 0,2%, O 0,1% ; Lot 2 : T 0,4%, C 0,2%, O 0,1% ; Lot 3 : T 0,4%, C 0,15%, O 0,1%. * $P < 0,05$; ** $P < 0,01$.

4. Conclusions

L'absence de différence de consommation dans les lots 2 et 3 suggère que les animaux n'ont montré de préférence spontanée pour aucun des trois aliments ainsi aromatisés, *i.e.* thym à 0,4%, orange à 0,1% et cannelle à 0,1% ou 0,2%. À la vue des profils de consommation, il a cependant été décidé d'utiliser la cannelle diluée à 0,15% pour équilibrer au maximum les choix alimentaires lors des expériences de cette thèse.

Partie 2. Modifications des choix alimentaires et des réponses cérébrales induites par différents renforcements intra-duodénaux (article n°2)

1. Contexte et objectifs

De nombreux organismes ont la capacité d'associer la flaveur d'un aliment spécifique avec les conséquences de son ingestion et de moduler la consommation de cet aliment *via* l'établissement de préférences ou d'aversions alimentaires conditionnées. Expérimentalement, les préférences et les aversions alimentaires peuvent être induites en associant une flaveur avec un apport calorique (*e.g.* infusion i.d. de glucose) ou une substance provoquant un malaise gastrique (*e.g.* infusion i.d. de chlorure de lithium), respectivement. Ces mécanismes de régulation de la prise alimentaire sont sous l'influence de structures cérébrales qui ont été bien caractérisées chez les rongeurs, les primates non-humains et l'Homme, et récemment chez le porc. L'objectif de cette étude était donc : (1) de moduler la prise alimentaire et les préférences alimentaires de porcs juvéniles en associant un aliment aromatisé avec des infusions i.d. dont les conséquences post-ingestives étaient contrastées (apport calorique, malaise intestinal) ; (2) d'évaluer, *via* la technique du PET cérébral, les modifications du métabolisme cérébral provoquées par l'exposition à des flaveurs dont les valeurs hédoniques étaient contrastées, plus particulièrement dans les structures caractérisées chez l'Homme pour leurs implications dans la régulation hédonique de la prise alimentaire.

2. Méthodes

Au total, douze porcs juvéniles ont été soumis à quatre sessions de trois jours de conditionnement. Durant trois jours successifs, ils recevaient pendant 30 min trois aliments aromatisés (thym, orange, cannelle) associés à des infusions i.d. de glucose 15% (F_{Glu}), de chlorure de lithium (F_{LiCl}) ou de sérum physiologique (F_{NaCl}, contrôle). La semaine suivant le conditionnement, les animaux ont été soumis pendant 30 min à trois tests de double choix alimentaires (thym *vs* cannelle, thym *vs* orange, orange *vs* cannelle) pendant trois jours consécutifs afin d'évaluer l'acquisition d'une préférence ou d'une aversion alimentaire. Les mêmes tests ont été répétés un mois après la fin du conditionnement afin de s'assurer du maintien de l'apprentissage à moyen-terme. Entre les deux sessions de tests de choix, les animaux anesthésiés ont été soumis à trois sessions d'imagerie fonctionnelle cérébrale (^{18}FDG PET) couplée à une stimulation olfacto-gustative avec les flaveurs conditionnées (F_{Glu}, F_{NaCl}, F_{LiCl}) pour évaluer le métabolisme cérébral pendant la perception des flaveurs.

3. Résultats

L'analyse de variance (ANOVA) a mis en évidence l'effet de l'interaction session × traitement ($P < 0,001$) sur les quantités ingérées pendant le conditionnement, caractérisées par une diminution des quantités de F_{LiCl} ingérées pendant les sessions 3 ($P < 0,01$) et 4 ($P < 0,001$) comparées à la session 1, et durant la session 4 comparée à la session 2 ($P < 0,001$). Aucune différence d'activité comportementale n'a été mise en évidence après que les animaux ont reçu le traitement NaCl ou Glu ($P > 0,05$). Après le traitement LiCl, les animaux passaient moins de temps debout et plus de temps couché ($P < 0,016$) comparativement aux traitements NaCl ou Glu. Ils passaient également plus de temps inactifs et moins de temps dans des activités d'exploration ($P < 0,016$). Durant les tests de choix réalisés une semaine et un mois après le conditionnement, les animaux consommaient plus d'aliment F_{NaCl} ou F_{Glu} que d'aliment F_{LiCl} ($P < 0,001$). Les animaux exprimaient également une préférence pour F_{NaCl} comparé à F_{Glu} mais seulement une semaine après le conditionnement ($P < 0,05$). Les analyses des sessions d'imagerie cérébrale ont permis de mettre en évidence trois résultats principaux. La perception de la flaveur aversive (F_{LiCl} *vs* F_{Glu} ou F_{NaCl}) et de la flaveur moins préférée (F_{Glu} *vs* F_{NaCl}) entraîne une activation moindre dans des structures du cortex préfrontal (APFC, OFC) et un pattern d'activation latéralisé dans les noyaux de la base (*e.g.* NAcc, PUT, CAU). L'exposition à la flaveur aversive (F_{LiCl} *vs* F_{Glu} ou F_{NaCl}), mais pas à la flaveur moins préférée (F_{Glu} *vs* F_{NaCl}), entraîne une activation plus importante dans le cortex cingulaire postérieur et dans l'AMY.

4. Conclusions

Nos résultats ont montré que des stimulations i.d. post-ingestives pouvaient moduler la valeur hédonique de l'aliment et les choix alimentaires ultérieurs. La perception de flaveurs aux valeurs hédoniques contrastées ont induit des différences du métabolisme cérébral dans des structures connues pour être impliquées, chez l'Homme, dans la caractérisation de la palatabilité des aliments, dans l'identification des stimuli olfactifs et gustatifs et dans la régulation hédonique de la prise alimentaire. Ces similitudes considérables entre le métabolisme cérébral du porc et de l'Homme montrent que le porc est un modèle prometteur pour l'étude des déterminants comportementaux et neurobiologiques de la prise alimentaire. Des études complémentaires sont cependant nécessaires pour comprendre la difficulté rencontrée dans le développement d'un modèle de préférence alimentaire conditionnée par le glucose.

Article n°2

Exposures to conditioned flavours with different hedonic values induce contrasted behavioural and brain responses in pigs

Clouard C., Jouhanneau M., Meunier-Salaün M.C., Malbert C.H., Val-Laillet D., 2012.

PLoS ONE, 7(5): e37968

(Accepted 27 04 2012)

OPEN ACCESS Freely available online

PLoS one

Exposures to Conditioned Flavours with Different Hedonic Values Induce Contrasted Behavioural and Brain Responses in Pigs

Caroline Clouard[1,2,3], Mélanie Jouhanneau[1,2,3], Marie-Christine Meunier-Salaün[2,3], Charles-Henri Malbert[1], David Val-Laillet[1]*

1 INRA, UR1341 ADNC (Alimentation & Adaptations Digestives, Nerveuses et Comportementales), Saint Gilles, France, **2** INRA, UMR1348 PEGASE (Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage), Saint Gilles, France, **3** Agrocampus Ouest, UMR1348 PEGASE (Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage), Rennes, France

Abstract

This study investigated the behavioural and brain responses towards conditioned flavours with different hedonic values in juvenile pigs. Twelve 30-kg pigs were given four three-day conditioning sessions: they received three different flavoured meals paired with intraduodenal (i.d.) infusions of 15% glucose (F_{Glu}), lithium chloride (F_{LiCl}), or saline (control treatment, F_{NaCl}). One and five weeks later, the animals were subjected to three two-choice feeding tests without reinforcement to check the acquisition of a conditioned flavour preference or aversion. In between, the anaesthetised pigs were subjected to three ^{18}FDG PET brain imaging coupled with an olfactogustatory stimulation with the conditioned flavours. During conditioning, the pigs spent more time lying inactive, and investigated their environment less after the F_{LiCl} than the F_{NaCl} or F_{Glu} meals. During the two-choice tests performed one and five weeks later, the F_{NaCl} and F_{Glu} foods were significantly preferred over the F_{LiCl} food even in the absence of i.d. infusions. Surprisingly, the F_{NaCl} food was also preferred over the F_{Glu} food during the first test only, suggesting that, while LiCl i.d. infusions led to a strong flavour aversion, glucose infusions failed to induce flavour preference. As for brain imaging results, exposure to aversive or less preferred flavours triggered global deactivation of the prefrontal cortex, specific activation of the posterior cingulate cortex, as well as asymmetric brain responses in the basal nuclei and the temporal gyrus. In conclusion, postingestive visceral stimuli can modulate the flavour/food hedonism and further feeding choices. Exposure to flavours with different hedonic values induced metabolism differences in neural circuits known to be involved in humans in the characterization of food palatability, feeding motivation, reward expectation, and more generally in the regulation of food intake.

Citation: Clouard C, Jouhanneau M, Meunier-Salaün M-C, Malbert C-H, Val-Laillet D (2012) Exposures to Conditioned Flavours with Different Hedonic Values Induce Contrasted Behavioural and Brain Responses in Pigs. PLoS ONE 7(5): e37968. doi:10.1371/journal.pone.0037968

Editor: John I. Glendinning, Barnard College, Columbia University, United States of America

Received December 22, 2011; **Accepted** April 27, 2012; **Published** May 25, 2012

Funding: This study was supported by a grant from INRA (Department of Human Alimentation; Department of Animal Physiology and Livestock Systems) and the Brittany Region (Région Bretagne), and especially by the CRITT Santé Bretagne (Centre Régional d'Innovation et de Transfert de Technologies). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: david.val-laillet@rennes.inra.fr

Introduction

Flavours are perceived during food consumption [1] and result from the combination of a taste and odour, through the stimulation of the gustatory system as well as the orthonasal and retronasal olfactory systems respectively [2,3]. All animal species have the ability to associate the flavour of a specific food with the consequences of its ingestion and can modulate further food intake via the establishment of conditioned food preferences [4,5] or aversions [4,6]. Conditioned food/flavour preference and aversion have been widely studied, especially in rats. Usually, preference conditioning is experimentally induced by pairing an unknown flavour with abundant caloric supply, e.g. gastric glucose infusions [7–11], while aversion conditioning is induced by pairing an unfamiliar flavour with a visceral infusion of emetic substances, e.g. lithium chloride [12–16].

In rats, numerous brain lesion studies investigated the brain structures involved in the acquisition of flavour preference and aversion, such as the amygdala (AMY) [10,17–19] and the insular cortex (IC) [20–23]. Unfortunately, those studies often focused on the only first steps of preference and aversion learning processes, i.e., detection of the conditioned stimulus (CS), detection of the visceral unconditioned stimulus (US), association between the US and the CS (e.g., role of the parabrachial nucleus [24,25]). Athough Touzani et al. [10,11] reported that the AMY and the lateral hypothalamus were not involved in the expression of conditioned flavour preferences in rats, less is known about the brain structures that are involved in the last steps of those processes, i.e., retrieval of the learning when the CS is further encountered and expression of the appropriate behaviour. Consequently, there is a need for studies to investigate the brain structures that are involved in the recall of conditioned learning during subsequent exposure to the conditioned flavour (CS).

Thanks to the emergence of functional brain imaging techniques, several brain structures involved in the processing of hedonic information during olfactogustatory stimulations have

been characterized in rats, primates and humans. Both the IC [26] and the AMY [27] are known to be involved in the evaluation of food stimuli hedonism, but with contradictory data. Some papers reported that the IC is activated during pleasant odour exposure [28,29] and the AMY during aversive stimuli [30–32], while others noticed some activation of the two structures during both unpleasant and aversive food/taste stimuli exposure [33–36]. The basal nuclei also play an important role during the processing of food hedonism. For instance, the nucleus accumbens (NAcc) is activated by pleasant food stimuli [37], while cerebral responses in the caudate (CAU) and the putamen (PUT) decrease with decreasing reward value of stimuli in humans [38]. Additionally, the dorsolateral prefrontal cortex (DLPFC) [39], the orbitofrontal cortex (OFC) [28,36,40–42], the cingulate cortex [26,28,31,37,38,42] and the parahippocampal cortex (PHC) [38] are involved in the processing of the hedonic value of taste and/or odour stimuli. Lastly, the temporal gyrus participates in the recognition of food-related vs. food-unrelated stimuli and is involved in the perception of pleasant taste [39,43].

Gaultier et al. [29] performed the very first study aimed at exploring the pigs' brain metabolism during exposure to flavours with contrasted hedonic values acquired after aversive or positive flavour conditioning using Single Photon Emission Computed Tomography (SPECT). Compared to a control condition (no flavour), the perception of a preferred or an aversive flavour triggered brain responses in the prefrontal cortex, the temporal gyrus, some limbic structures, the IC, as well as the AMY and several basal nuclei. These findings suggest that similar structures are involved in the recognition of food-related flavours with different hedonic values in pigs and humans. However, two major limitations can be pointed out in the study of Gaultier et al. [29]. First, the only positive reinforcement during conditioning was the positive postingestive consequences (food hedonism and caloric supply) provided by the CS (i.e., the meal), but there was no additional positive oral or visceral reinforcement. Second, during the imaging sessions, the control stimulation was provided by exposure to unflavoured air and saliva. As the control stimulation was not a food-related stimulus, it is difficult to determine whether the differences of brain metabolism recorded after the perception of the conditioned flavours compared to the control stimulation were triggered by the perception of flavours with contrasted hedonic values, or by the perception of a food-related flavour compared to a non-food condition.

To continue and complete this work, three main objectives were defined in the present study: 1) to modulate food intake of pigs by pairing a flavoured meal with intraduodenal (i.d.) infusions with different putative hedonic values, 2) to check the acquisition of the conditioning and its persistence a month after learning by studying animals' food preference during repeated two-choice feeding tests with the flavoured meals, and 3) to investigate, via brain Positron Emission Tomography (PET), the brain activity patterns in some predefined structures during subsequent exposure to flavours with contrasted hedonic values in anaesthetized animals. We hypothesized that: 1) the animals would exhibit contrasted behavioural patterns after different visceral reinforcement during conditioning, 2) food preferences would be shaped by conditioning on a long-term basis, and 3) subsequent exposure to the conditioned flavours would induce contrasted activity patterns in some brain structures involved in the processing of hedonic judgment and discrimination during sensory stimulations, and especially during gustatory and/or olfactory stimulations.

Materials and Methods

Ethics Statement

The experiments presented in this paper were conducted in accordance with the current ethical standards of the European Community (Directive 86/609/EEC), Agreement No. A35–622 and Authorizations No. 01894 and No. 35–88. The Regional Ethics Committee in Animal Experiment of Brittany has validated the entire procedure described in this paper (b–2009–DVL–01).

Animals and Housing

A total of twelve 30-kg Large White × Piétrain female pigs were used in this study. The pigs were individually housed in pens (150×60×80 cm) and had free access to water. A chain was suspended in each pen to enrich the environment of the animals and fulfil their natural disposition to play. The room was maintained at approximately 24°C with a 13:11-h light-dark cycle. The animals were fed daily at 09:00 with 1 kg of pelleted meal (3.63 kcal/g) composed of 30% barley, 30% wheat, 25% hulled oat, 6% bran, 5% molasses, 1.5% bi-calcic phosphate, 1.5% calcium carbonate, 0.5% salt and 0.5% vitamin complement. In order to accustom the animals to the experimental oiled meal, the pelleted meal was supplemented with 10 mL of vegetable oil (Phodé Laboratories, Terssac, France) per kg of food, the vehicle enabling the adjunction of essential oils in the food during conditioning (see *Experimental procedure* section).

Surgery

After a 24-h fasting period, the pigs were preanaesthetised with an intramuscular injection of ketamine (15–20 mg/kg, Mérial, Lyon, France), then put on isoflurane (3–5% v/v, Isoflurane Belamont, Nicholas Piramal, London, UK) anaesthesia and subjected to a tracheal intubation. A surgical level of anaesthesia was maintained by isoflurane (2–3% v/v) delivered by a mechanical ventilator and analgesia was obtained by intravenous injection of a morphinic agent (Fentanyl 4 mL, 1.4 mL/min, Renaudin, Paris, France). Heart rate was continuously monitored throughout surgery using a pulse oxymeter (Ohmeda oxymeter, GE Healthcare Clinical Systems, Limonest, France). Normocapnia was controlled by an infrared capnometer (Amstrong capnometer, Gambo Engström, Bromma, Sweden). A midline laparotomy was performed under aseptic conditions. A catheter was inserted into the proximal duodenum, tunnelled under the skin and exteriorized between the shoulders for further i.d. infusions during food conditioning. The animals were allowed one week to recover from surgery before the beginning of the experiments. During the recovery week, the animals were exclusively fed with the oiled meal and were accustomed to eat their meal in 30 min.

Conditioned and Unconditioned Stimuli Preparation

The conditioned stimuli were flavoured meals. The three flavoured meals were elaborated by the adjunction in pelleted meal of essential oils of thyme (T; 0.4%), orange (O; 0.15%), or cinnamon (C; 0.1%) diluted in vegetable oil (Phodé Laboratories, Terssac, France), with 10 mL of additive per kg of meal. At these dilutions, the animals normally consume as much thyme-, orange- and cinnamon-flavoured meal [29]. The unconditioned stimuli were produced by an i.d. injection of glucose (Glu), lithium chloride (LiCl) or saline (NaCl) 5 min before the end of a 30-min meal. The putative positive reinforcement was induced by an i.d. injection of 150 mL of glucose 15% (90 kcal; Glu treatment). The negative reinforcement was induced by an i.d. injection of 50 mL of LiCl 8%, followed by 100 mL of saline – NaCl 0.9% (LiCl

treatment). As NaCl has no particular postingestive effect, the control treatment was induced by an i.d. injection of 150 mL of saline – NaCl 0.9% (NaCl treatment). Solutions were injected with a peristaltic pump connected to the duodenal catheter, and the injection rate was 10 mL/min.

Experimental Procedure

The study was carried out in three successive batches, each of them lasting seven weeks and composed of a conditioning period, a testing period and a brain imaging period. Four animals were studied in each batch, in which the presentation order of the flavours was counterbalanced to avoid any bias.

Conditioning sessions. After the recovery week, the animals were subjected to four three-day conditioning sessions with the flavoured meals. For each conditioning session, on day 1, the animals were fed during 30 min the cinnamon- (batch 1), thyme- (batch 2) or orange-flavoured meal (batch 3), on day 2, the animals were fed the thyme- (batch 1), orange- (batch 2) or cinnamon-flavoured meal (batch 3) and on day 3, the animals were fed the orange- (batch 1), cinnamon- (batch 2) or thyme-flavoured meal (batch 3). Each day, a third of the pigs received the LiCl treatment, a third of the animals received the Glu treatment, and the last third received the NaCl treatment. Consequently, at the end of the conditioning period, the animals have been subjected to a total of four repetitions of each kind of conditioning, that is a treatment (LiCl, Glu, NaCl) paired with a specific flavoured meal (T, C, O; e.g. $C_{LiCl}/T_{NaCl}/O_{Glu}$ or $C_{Glu}/T_{LiCl}/O_{NaCl}$ or $C_{NaCl}/T_{Glu}/O_{LiCl}$). Each day of conditioning, the meal was removed after 30 min and refusals were weighed.

Two-choice feeding test sessions. After two weeks of conditioning, the pigs were subjected to three two-choice feeding tests to assess their preferences for the different flavoured meals (F_{LiCl}, F_{Glu} and F_{NaCl}). On day 1, 2 and 3, the animals could choose between the thyme- and the cinnamon-flavoured meals, the thyme- and the orange-flavoured meals, and the orange- and the cinnamon-flavoured meals, respectively. The two different meals were presented in a two-part trough containing 1 kg each. They were presented at 09:00 to the animals, and during 30 min. Then, the two-part trough was removed and refusals were weighed. No i.d. injection was given during these preference tests. Meal distribution in the troughs was interchanged over days and animals to avoid any bias. The same three two-choice feeding tests were repeated one month after the end of the conditioning to ensure that the conditioned learning did not extinguish before the end of the brain imaging sessions. Meal distribution in the trough was interchanged compared to the first testing session.

Behavioural Analyses

During the conditioning sessions, behavioural observations were carried out during the 30 min following the end of the meal. Behaviours were recorded using the scan-sampling method (1 observation every 30 sec) and the Pocket Observer® software (Noldus, Wageningen, Nederland) installed in a pocket PC (iPAQ 214, Hewlett-Packard, Palo Alto CA, USA). The behavioural repertoire was adapted from the study of Gaultier et al. [29]: bars-focused activity (bites or licks the pen's bars), ground-focused activity (licks, paws, rubs the ground), self-directed activity (scratches or licks its own body), chain-focused activity (chews or plays with the chain), trough-focused activity (bites or licks the trough although there is no food in it), drinks, vomits, urinates/defecates, chews (with no food in the mouth), no activity and other activities. Additionally, four postures were recorded: standing, sitting, kneeling down and lying. Behavioural observations were also carried out during the 30-min two-choice test meals. The same method and the same items were used, with one additional item "eats" (chews with the head at the trough when there is still food in it). The trough used by the animal when eating was systematically specified in order to determine the time spent in each trough during the meal.

Brain Imaging Procedure

After the first session of two-choice food tests, the animals (three out of four per batch) underwent three brain imaging sessions to investigate the brain metabolism during flavour exposure (F_{LiCl}, F_{Glu} and F_{NaCl}). The brain imaging modality used to investigate the cerebral glucose metabolism (CGM) was the PET of ^{18}F-fluorodesoxyglucose (^{18}FDG, CIS bio international, France).

Animal preparation and olfactogustatory stimulation. After a 24-h fasting period, the animals were anaesthetized and subjected to a tracheal intubation following the same procedure as that described above (see *Surgery* section). The animals were placed in a Head First Prone position on the bed of a whole body, high-resolution PET and a venous catheter was inserted in their left ear in order to inject the radiolabel. The ears and eyes of the animals were sealed with cotton and surgical tape respectively, in order to minimize auditory and visual stimulations. Animals' body temperature was maintained at least at 37°C by using a heating blanket.

The olfactogustatory stimulation was performed with computer-assisted automats designed in our laboratory (Figure 1). The olfactory stimulation consisted in diffusing a nonodorized or an odorized air (0.05% essential oil) into the pig's right nostril (4 L/min). As the animals were intubated and mechanically ventilated, the diffused air could not come out from the mouth. Consequently, the olfactory stimulation was performed via one of the two nostrils to let the air flow through the nasal cavity. The choice to perform the stimulation via the right nostril rather than the left nostril, however, has been done arbitrarily. A tube was inserted in the right nostril of the animal and connected to a device composed of a medical air cylinder connected to a flow meter and a two-way circuit of bottles equipped with a system of electronic valves. One of the bottles contained unodorized tap water and the other contained odorized water (0.05% essential oil). The gustatory stimulation consisted in irrigating the pig's tongue (24 mL/min) with an unflavoured or a flavoured artificial saliva (0.05% essential oil; for the saliva composition, see [44]). A tube was positioned on the middle of the tongue and connected to a computer-operated automat developed in our laboratory (Gustautomat, INRA, St Gilles, France, see [29]) and inspired by the Taste–o–Matic designed by Hellekant's group [45]. The animals were subjected to a neutral olfactogustatory stimulation (i.e., nonodorized air and unflavoured saliva) for 5 min to accommodate the mucosa thermoreceptors and mechanoreceptors to the stimulation. Then, the diffusion of odorized air and flavoured saliva was performed for 15 min. The stimulation was ended by a 15-min neutral stimulation.

Data acquisition. The radiolabel (^{18}FDG, 200MBq) was injected 5 min after the beginning of the olfactogustatory stimulation procedure. PET data were acquired on a CTI/Siemens HR+ Scanner in 3D mode (Siemens ECAT, 962, HR+). A 30-min 3-dimensional (3D) emission scan was performed 45 min after the radiolabel injection using an axial FOV of 15.52 cm. It was corrected by a 15-min transmission scan using rotating ^{68}Ge rods. Following scatter, dead time and random corrections, PET transaxial images were obtained by iterative reconstruction using a ramp filter (Kernel FWHM = 6 mm) providing 63 contiguous slices. Spatial resolution after reconstruction was 0.64 mm per

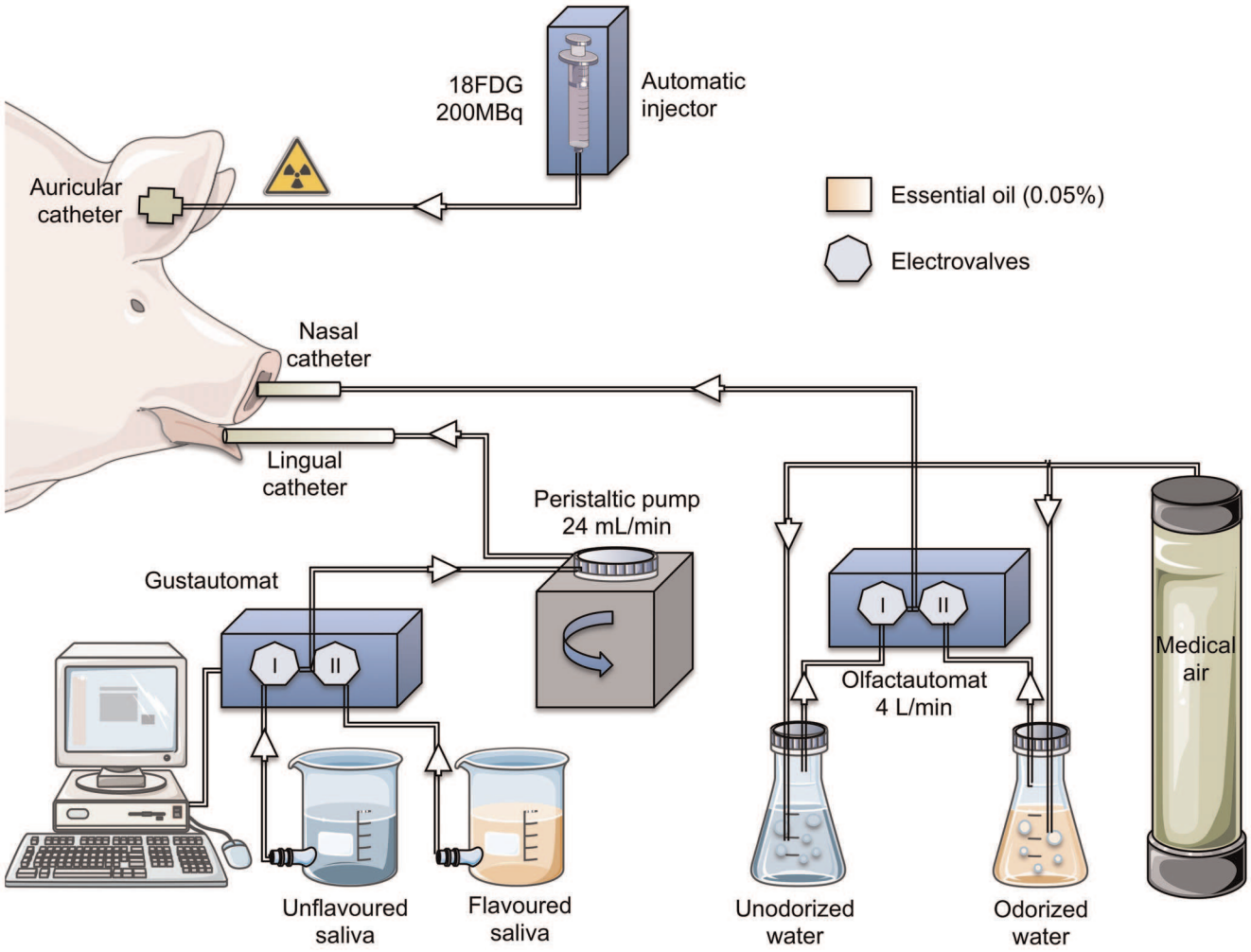

Figure 1. Experimental device and paradigm designed to perform olfactogustatory stimulations for brain imaging in anaesthetised pigs. The illustrations used to make this figure were obtained from the "Servier Medical Art" website, http://www.servier.fr/servier-medical-art.
doi:10.1371/journal.pone.0037968.g001

pixel in the x and y directions and 2.42 mm per pixel in the z axis. Pixel depth encoding was performed using the Standard Uptake Value (SUV) method.

Image processing. The data were analyzed with statistical parametric mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB 7.1 (The Mathworks Inc., Natick MA, USA). The pre-processing of the PET images obtained in this study was realized in 6 steps. The images were first manually reoriented (pitch $= -1.57077$, roll $= 3.14159$). The spatial coordinates were then centered compared to a reference point (x_0, y_0, z_0, posterior commissure). The images were masked to remove the extracerebral matter, and the coordinates were realigned on a PET template. The images were then spatially normalized and the normalization was restricted to 12-parameter affine transformations in order to minimize deformations of the original images. A second narrower masking was then performed to eliminate more finely the extracerebral matter. Finally, spatially normalized images were smoothed using a Gaussian filter set at 4×4×4 mm full width at half maximum. Eleven male and female pigs of approximately 35 kg different from those used in this experiment were used to build the PET template. PET images were acquired and processed as described above.

Statistical Analysis

Statistical behaviour analysis. Data were analyzed with the Statview software 4.57 (Abacus Concepts Inc., USA). When Kolmogorov-Smirnov tests showed that the data presented a Gaussian distribution, parametric tests were performed. The conditioning consumption data were thus analysed using two-way repeated measures ANOVA followed by simple main effects tests when appropriate. The consumption data obtained during the two-choice tests were analysed using paired *t*-tests. The behavioural activity data were analysed using non parametric Wilcoxon tests. When multiple comparisons were performed, a Bonferroni correction was applied. Otherwise, the significant level for all analyses was set at $P<0.05$.

Statistical image analysis. The regional ^{18}FDG uptake was standardized to the mean global uptake using proportional scaling in order to minimize interindividual differences in global CGM. The F_{NaCl}, F_{Glu} and F_{LiCl} PET images were compared together using paired *t*-tests. The three contrasts ($F_{Glu} - F_{NaCl}$, $F_{LiCl} - F_{NaCl}$ and $F_{LiCl} - F_{NaCl}$) presented hereafter show the bidirectional differences of brain metabolism, that is both higher and lesser CGM responses of one treatment compared to another. For practical reason and in each contrast, we systematically decided to compare the brain metabolism triggered by the perception of the

less preferred flavour to that triggered by the perception of the more preferred flavour relatively to the behavioural responses during two-choice tests. Variances were considered unequal. The dependency and heteroscedasticy induce different error covariance components that were estimated using REML (Restricted Maximum Likelihood) and used to adjust the statistics and degrees of freedom during inference. By default, SPM uses weighted least squares to produce Gauss-Markov or Maximum likelihood estimators using the non-sphericity structure specified at this stage (SPM8 User Manual). The error variances were 1.075 for F_{NaCl} images, 0.966 for F_{Glu} images and 0.955 for the F_{LiCl} images, and the error covariances were 0.423 for the F_{NaCl}/F_{Glu}, 0.37 for the F_{NaCl}/F_{LiCl}, and 0.282 for the F_{Glu}/F_{LiCl}. A Small Volume Correction (SVC) analysis was performed with SPM8 on the regions of interests (ROIs) selected upon the *a priori* hypotheses presented in the introduction. With this analysis, that allows for voxel to voxel comparisons within restricted ROIs, we managed to identify the voxels for which the activity was statistically different between treatments in the ROIs. An uncorrected value of $P = 0.05$ was set as the threshold (extent threshold of 5 voxels).

Regression analyses were also performed to investigate a possible relationship between the brain metabolism in the ROIs obtained for the F_{Glu}, F_{NaCl} and F_{LiCl} stimulations and the food consumption data. Two sets of consumption data were used for these regression analyses: 1) the amount of the F_{Glu}, F_{NaCl} and F_{LiCl} food consumed during the last session of conditioning, and 2) the total amount of the F_{Glu}, F_{NaCl} and F_{LiCl} food consumed during the two-choice tests performed one week after conditioning. These data were used to calculate a regression with the images obtained during the F_{Glu}, F_{NaCl} and F_{LiCl} stimulations (each image was associated with the amount of food consumed during the conditioning or the two-choice tests). An uncorrected value of $P = 0.05$ was set as the threshold (extent threshold of 5 voxels).

The statistical analysis with SPM8 produced a listing of voxels for which the activation (CGM) differed between treatments. Each voxel was associated with a set of coordinates (x y z) corresponding to its spatial location in the CA-CP (*commissura anterior-commissura posterior*) plane with CP set as the origin. The ROIs chosen for the SVC analysis were anatomically identified on the basis of a 3D digitized pig brain atlas developed in our laboratory [46], and selected upon the *a priori* hypotheses presented in the introduction. Consequently, the ROIs included the structures (bilaterally) that are known to be involved in the evaluation of sensory stimuli valence, that is some prefrontal and frontal structures (the OFC, the DLPFC and the anterior prefrontal cortex (APFC)), the cingulate cortex, the PHC, the IC, the temporal gyrus, the AMY, and the basal nuclei (the CAU, the globus pallidus (GP), the NAcc and the PUT).

Results

One out of the 12 animals was excluded from the study because it showed a generalized aversion for food, regardless of the flavour or the treatment associated with the meal, after only one pairing between the meal and the LiCl injection. A total of 11 and 9 animals were used for behavioural and brain imaging analyses, respectively.

Behavioural Results

Before conditioning, there was no difference in the average amount of each flavoured food consumed (O: 834±85 g, T: 781±75 g, C: 874±45 g, $F(2,10) = 0.92$, $P = 0.42$).

Consumption and behaviour during conditioning sessions. The food consumption data are presented in Figure 2. The two-way within subjects ANOVA showed no global effect of the treatment ($F(2,20) = 1.82$, $P = 0.19$), but a significant global effect of the conditioning session ($F(3,30) = 6.32$, $P<0.01$) in that the pigs consumed more food during the first session than during the fourth ($P<0.05$) session; other comparisons were not significantly different. There was also a significant session-treatment interaction ($F(6,60) = 9.48$, $P<0.001$). Simple mean effect tests revealed that the F_{LiCl} food intake decreased over sessions in that the pigs consumed less of the F_{LiCl} food during the third ($P<0.01$) and fourth ($P<0.001$) sessions than during the first session, and during the fourth session than during the second session ($P<0.001$). The pigs also consumed less of the F_{LiCl} food than of the F_{NaCl} food ($P<0.05$) during the third and fourth sessions, and than the F_{Glu} food ($P<0.05$) during the fourth session only.

There was no difference in the general activity exhibited by the animals after they received the NaCl or the Glu treatments ($P>0.05$). After the LiCl reinforcement, the animals spent less time standing (NaCl: $z = 2.76$, $P<0.016$; Glu: $z = 2.5$, $P<0.016$) and more time lying (NaCl: $z = 2.76$, $P<0.016$; Glu: $z = 2.67$, $P<0.016$) than after the NaCl or the Glu reinforcements (Figure 3a). They also spent more time inactive (NaCl: $z = 2.93$, $P<0.016$; Glu: $z = 2.85$, $P<0.016$) and less time in exploratory and playing activities (bars-focused, chain-focused or trough-focused activities) than after the NaCl or the Glu treatments (Figure 3b). The animals also spent 2% of their time vomiting whereas this behaviour was not expressed after the NaCl or the Glu treatments. A total of 2.1±0.4 vomiting occurrences were observed during the 30 min following the LiCl injection, with the first occurrence being observed 11.5±1.2 min after the beginning of the injection.

Consumption and behaviour during the two-choice feeding tests. During the two-choice feeding tests performed one week after conditioning (Figure 4a), the animals consumed significantly more of the F_{NaCl} ($t(10) = 32.52$, $P<0.001$) or F_{Glu} food ($t(10) = 14.16$, $P<0.001$) than of the F_{LiCl} food. The animals also consumed more of the F_{NaCl} food than of the F_{Glu} food ($t(10) = 2.65$, $P<0.05$). The animals spent significantly less time with the head in the trough containing the F_{LiCl} food than in the trough containing the F_{NaCl} (F_{LiCl}: 2 ± 1%, F_{NaCl}: 92 ± 3%, $z = 2.93$, $P<0.01$) or the F_{Glu} food (F_{LiCl}: 1 ± 1%, F_{Glu}: 86 ± 5%, $z = 2.93$, $P<0.01$). The animals also had a tendency to spend more time with the head in the trough containing the F_{NaCl} food than in the trough containing the F_{Glu} food (F_{NaCl}: 61 ± 6%, F_{Glu}: 38 ± 6%, $z = 1.96$, $P<0.1$). During the two-choice feeding tests performed one month after the conditioning (Figure 4b), the animals consumed significantly more of the F_{NaCl} ($t(10) = 9.56$, $P<0.001$) or F_{Glu} food ($t(10) = 13.36$, $P<0.001$) than of the F_{LiCl} food, but they did not consumed more of the F_{NaCl} food than of the F_{Glu} food anymore ($t(10) = 0.85$, $P = 0.42$).

Brain imaging results

The results of the SVC analysis in some brain regions for which differences of CGM were found for the three types of contrast are summarized in Tables 1 and 2.

F_{LiCl} compared to F_{NaCl} or F_{Glu}. The APFC was significantly less activated in the F_{LiCl} condition than in the F_{NaCl} (Figure 5) or F_{Glu} (Figure 6) conditions, and the OFC in the F_{LiCl} condition than in the F_{NaCl} condition. Conversely, the PHC, the posterior cingulate cortex (PCC) and the AMY were more activated, while the anterior cingulate cortex (ACC) and the CI were globally less activated in the F_{LiCl} condition than in the F_{NaCl} or F_{Glu} conditions. As for the basal nuclei, the right NAcc, GP and PUT were more activated, whereas the left PUT and GP were less activated in the F_{LiCl} condition than in the F_{NaCl} or F_{Glu}

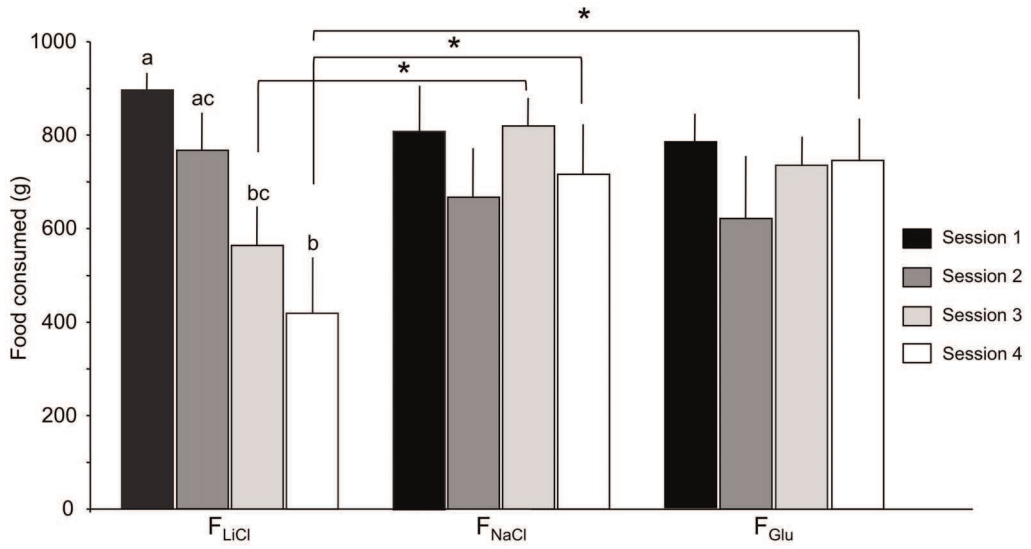

Figure 2. Quantity of food (g) consumed during the four conditioning sessions. During the conditioning period, the animals were given a 30-min flavoured meal associated with NaCl, LiCl or Glucose (Glu) duodenal injection. Data are presented with means and standard errors. Significant simple mean effects are indicated with asterisks and letters. An asterisk indicates a significant difference between two treatments during a single conditioning session (* $P<0.05$). Two different letters indicate a significant difference between two conditioning sessions for the same treatment ($P<0.01$).
doi:10.1371/journal.pone.0037968.g002

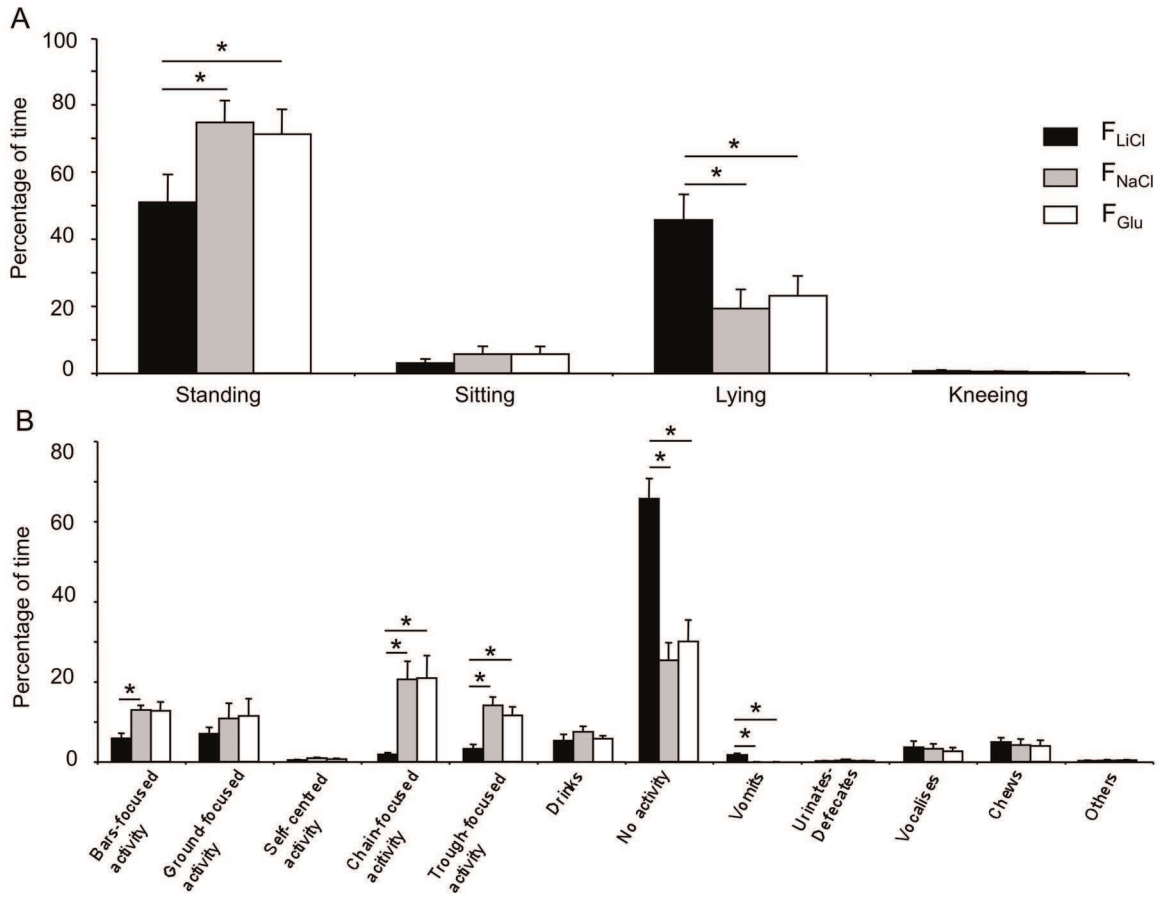

Figure 3. Behavioural observations performed during the conditioning sessions. Body postures (A) and behavioural activity (B) recorded during 30 min after a meal associated with NaCl, LiCl or Glucose (Glu) duodenal injection. Data are presented with means and standard errors. Significant differences between two treatments ($P<0.05$) are indicated with an asterisk.
doi:10.1371/journal.pone.0037968.g003

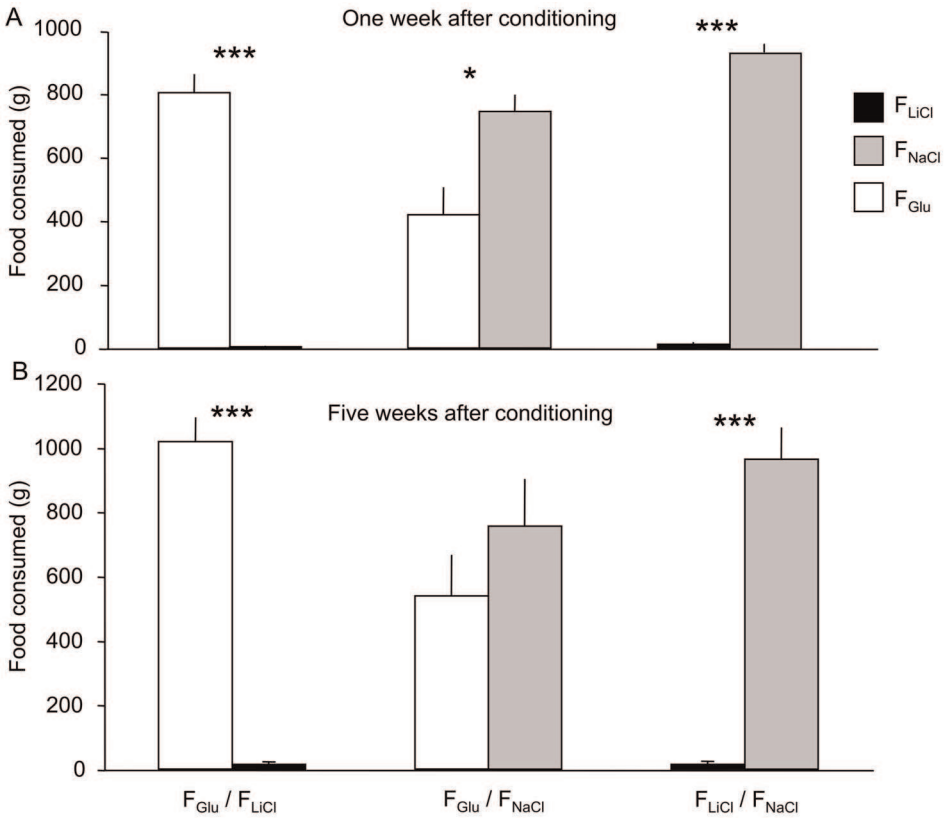

Figure 4. Quantity of flavoured food (g) consumed during the 30-min two-choice tests. The tests were carried out one week (A) and five weeks (B) after conditioning. Data are presented with means and standard errors. The following symbols are used * $P<0.05$; ** $P<0.01$.
doi:10.1371/journal.pone.0037968.g004

Table 1. Regions that were more activated in the F_{LiCl} condition than in the F_{NaCl} and F_{Glu} conditions, and in the F_{Glu} condition than in the F_{NaCl} condition.

		$F_{LiCl} - F_{NaCl}$	$F_{LiCl} - F_{Glu}$	$F_{Glu} - F_{NaCl}$
Middle temporal gyrus	L	2.47 (−18 −12 10)	1.94 (−18 −12 10)	
Inferior temporal gyrus	L	2.12 (−18 3 3)		2.31 (−22 5 9)
Inferior temporal gyrus	R		2.11 (18 3 3)	
Superior temporal gyrus	L	4.02 (−16 −8 12)	2.58 (−18 −9 11)	3.87 (−18 −3 15)
Parahippocampal cortex	L	2.00 (−16 −3 −2)		
Parahippocampal cortex	R		2.35 (14 −6 6)	
Dorsal posterior cingulate cortex	R	2.44 (4 −4 21)	2.42 (4 −5 19)	
Ventral posterior cingulate cortex	L		1.74 (−2 −5 14)	
Insular cortex	L			2.63 (−12 28 9)
Insular cortex	R		2.53 (22 7 13)	
Nucleus accumbens	R	1.78 (4 19 −4)	1.93 (2 18 −0)	
Caudate nucleus	R		2.20 (8 11 8)	
Globus pallidus	R	2.14 (8 17 −1)	2.62 (10 11 5)	
Putamen	L			1.86 (−6 27 −2)
Putamen	R	2.00 (8 19 −1)	2.36 (12 9 7)	
Amygdala	L	1.98 (−16 4 3)		
Amygdala	R		2.74 (16 4 2)	

The threshold for significance was set at $P<0.05$ (uncorrected). The *t*-value of the peak of maximal intensity is indicated for each cluster. The stereotaxic coordinates (x y z, in mm) of the peak in the CA-CP (*commissura anterior-commissura posterior*) plane with CP set as the origin are indicated in parentheses. L, left; R, right.
doi:10.1371/journal.pone.0037968.t001

Table 2. Regions that were less activated in the F_{LiCl} condition than in the F_{NaCl} and F_{Glu} conditions, and in the F_{Glu} condition than in the F_{NaCl} condition.

		$F_{LiCl} - F_{NaCl}$	$F_{LiCl} - F_{Glu}$	$F_{Glu} - F_{NaCl}$
Dorsolateral prefrontal cortex	L			1.79 (−4 31 9)
Anterior prefrontal cortex	L	2.40 (−6 30 2)	2.81 (−6 30 1)	1.85 (−0 29 −0)
Anterior prefrontal cortex	R	1.98 (4 32 2)	1.94 (4 35 2)	1.85 (0 29 −0)
Orbitofrontal cortex	L	1.92 (−0 23 3)		
Orbitofrontal cortex	R	1.87 (0 23 3)		1.82 (0 21 3)
Inferior temporal gyrus	L		1.80 (−22 5 9)	
Inferior temporal gyrus	R			2.57 (18 3 3)
Superior temporal gyrus	R	1.86 (22 −8 16)		
Parahippocampal cortex	L	2.72 (−8 −10 4)		1.96 (−10 −10 5)
Parahippocampal cortex	R			2.61 (14 −6 5)
Dorsal posterior cingulate cortex	L			3.42 (−4 1 17)
Dorsal posterior cingulate cortex	R	3.28 (2 4 17)		3.09 (0 3 18)
Dorsal anterior cingulate cortex	L			2.03 (−2 29 8)
Ventral anterior cingulate cortex	L	2.56 (−2 2 15)		2.42 (−2 1 15)
Ventral anterior cingulate cortex	R	2.62 (2 3 15)		
Insular cortex	L	2.30 (−8 31 3)	2.40 (−8 30 3)	
Insular cortex	R	1.83 (14 27 10)	2.10 (14 27 10)	2.10 (22 9 13)
Caudate nucleus	R			2.18 (8 11 8)
Globus pallidus	L	2.42 (−12 12 3)	2.00 (−6 15 1)	
Globus pallidus	R			2.05 (10 12 5)
Putamen	L	2.31 (−12 12 4)	2.48 (−6 27 −2)	
Putamen	R			2.11 (16 7 5)
Amygdala	L	2.05 (−12 11 −0)		2.10 (−10 11 −1)
Amygdala	R			3.07 (16 4 3)

The threshold for significance was set at $P<0.05$ (uncorrected). The *t*-value of the peak of maximal intensity is indicated for each cluster. The stereotaxic coordinates (x y z, in mm) of the peak in the CA-CP (*commissura anterior-commissura posterior*) plane with CP set as the origin are indicated in parentheses. L, left; R, right.
doi:10.1371/journal.pone.0037968.t002

conditions. The right CAU was also more activated in the $F_{LiCl} - F_{Glu}$ contrast (Figure 6). Compared to F_{NaCl} and F_{Glu}, the perception of F_{LiCl} induced higher CGM responses in the left (superior, middle and inferior) temporal gyrus.

F_{Glu} compared to F_{NaCl}. The APFC, the right OFC, the left DLPFC, the PHC and some parts of the cingulate cortex were less activated in the $F_{Glu} - F_{NaCl}$ contrast (Figure 7). The right CAU, GP and PUT were less activated, while the left PUT was more activated in the F_{Glu} condition than in the F_{NaCl} condition (Figure 7). The left inferior and superior temporal gyrus was more activated and the right temporal gyrus was less activated in the F_{Glu} condition than in the F_{NaCl} condition (Figure 7).

Regression analyses between behavioural and brain imaging data. The brain metabolism in 18 and 11 structures out of 34 was correlated with the quantity of food consumed during the last session of conditioning and food consumption during the preference tests performed 1 week after conditioning, respectively. Hereafter, we focused on the ROIs for which regression analysis was significant at $P<0.01$ for at least one voxel – the stereotactic coordinates [x y z] of the voxel with the highest *t*-value are indicated. Five out of the 6 voxels for which the metabolism was correlated with consumption data were located in the left hemisphere. The amount of food consumed during conditioning was significantly correlated with metabolism in the left ([−4 30 −2], $t=2.5$, $P=0.009$; Figure 8) and right ([2 34 −2], $t=2.6$, $P=0.005$) APFC, the left DLPFC ([−4 41 9], $t=2.6$, $P=0.008$) and the left CAU ([−6 9 9], $t=2.7$, $P=0.007$). The amount of food consumed during preference tests was significantly correlated with metabolism in the left APFC ([−6 30 3], $t=2.8$, $P=0.005$) and the left IC ([−8 30 3], $t=2.7$, $P=0.007$).

Discussion

Flavour Preference and Aversion Conditioning

Behavioural data showed that after the LiCl conditioning, the animals spent more time lying and inactive and less time expressing exploratory, rooting and playing activities than after the Glu and NaCl (control) reinforcements. A reduction of activity and an increase of the time spent lying are known to be indicative of discomfort and to reflect the expression of a malaise in various species [47–49]. Similarly, as playing behaviour has been suggested to be a positive indicator of welfare in juvenile individuals [50], a decrease of the chain-focused activity in our study is likely to indicate a decrease of well-being. According to these behavioural indicators, we assume that the LiCl treatment induced a state of ill-being in the pigs, which resulted in the development of a robust and persistent aversion for the associated flavoured meal. This aversion was confirmed by the systematic avoidance of the F_{LiCl} food during the subsequent two-choice feeding tests, a result that confirms previous data indicating that LiCl infusions induced strong food aversions in pigs [29]. On the

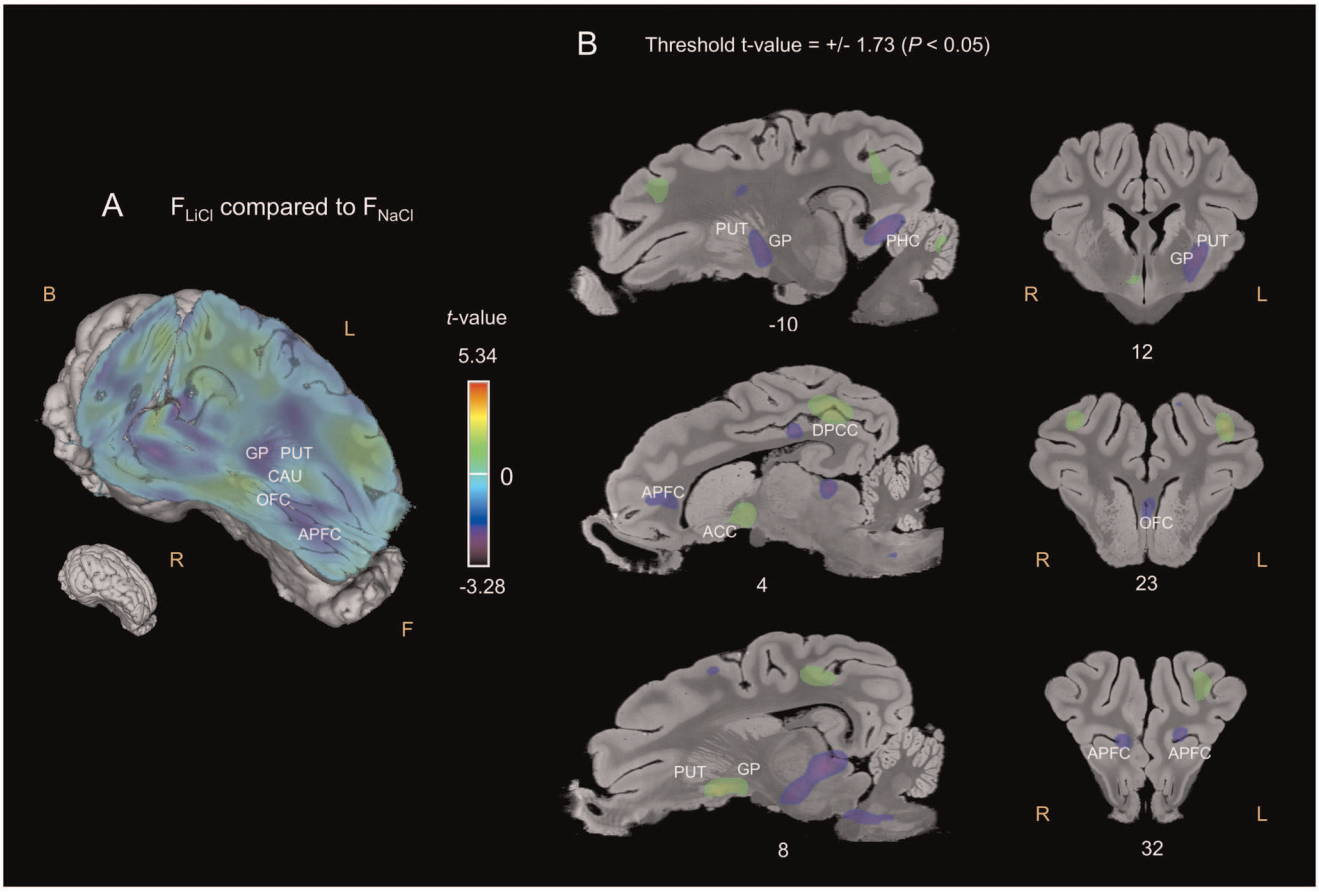

Figure 5. Cerebral glucose metabolism (CGM) differences obtained for the F_{LiCl} flavour compared to the F_{NaCl} flavour. (A) Three-dimensional skinned representation of the pig's brain with global CGM differences found in the F_{LiCl} *vs* F_{NaCl} contrast. The (x y z) coordinates are indicated below the representation. (B) Sagittal and coronal MRI sections showing significant CGM differences in the F_{LiCl} *vs* F_{NaCl} contrast. The threshold for significance was set at $P<0.05$ (uncorrected). The x or y coordinates are indicated below each section. Positive *t*-values (green, yellow and red) indicate more activation in the F_{LiCl} condition than in the F_{NaCl} condition, while negative *t*-values (blue and purple) indicate more deactivation in the F_{LiCl} condition than in the F_{NaCl} condition. F, Front; B, Back; R, Right; L, Left; APFC, anterior prefrontal cortex; OFC, orbitofrontal cortex; CAU, caudate nucleus; GP, globus pallidus; PUT, putamen; DPCC, dorsal posterior cingulate cortex.
doi:10.1371/journal.pone.0037968.g005

other hand, no difference in the behavioural activities and food consumption was reported between the Glu and the NaCl treatments during conditioning, which might suggest that the glucose infusion was not more reinforcing than the saline infusion. Besides, preference tests indicated that the F_{NaCl} food was significantly preferred over the F_{Glu} food, at least one week after conditioning. Though unexpected, the lower preference found for the F_{Glu} in the present study suggests that a visceral glucose infusion might be perceived as a relatively negative reinforcement by pigs.

Different hypotheses might explain our inability to condition a glucose-induced preference. First, we injected a fixed dose of glucose, regardless of the quantity of food consumed. Although some studies also induced strong preferences for flavoured solutions paired with fixed doses of glucose (6 mL: [8]; 10 mL: [51]), several studies rather used a glucose amount directly proportional to the quantity of solution consumed, with a fixed ratio of 1:1 [7,10,11,52,53]. Therefore, this suggests that the infusion of a dose of glucose adapted to the quantity of food consumed would have come to better results. Second, the amount of glucose injected might have been insufficient to induce a preference only based on energy supply. In the present study, the energy supply provided by 15% glucose infusions represented approximately 3 kcal/kg, while, in average, the amount of 8 or 16% glucose injected in rodents represented approximately 7 to 8 kcal/kg (e.g., [8,52]). Consequently, the amount of energy injected was approximately 2.5 times lesser than in previous studies in rodents. Moreover, the conditioned stimulus was a caloric flavoured meal, not a non-caloric flavoured beverage. The postingestive reinforcing effect of glucose might have been in competition with or just overlapped by the stronger postingestive reinforcing effects of food and may explain our inability to develop a preference for the F_{Glu} food compared to the F_{NaCl} food, which was reinforcing in itself. Further trials with a greater amount of glucose injected would likely result in a successful preference conditioning.

Neurobiological Determinants

In the second part of the study, we investigated, in predetermined ROIs, the differences of brain metabolism triggered by exposure to the conditioned flavours, i.e., the aversive flavour (F_{LiCl}), the less preferred flavour (F_{Glu}) or the preferred flavour (F_{NaCl}). Three main findings emerged from our study: 1) exposure to aversive and less preferred flavours triggered lesser activation in the prefrontal lobe and 2) a lateralized pattern of activity in the basal nuclei and, in a lesser extent, in the temporal gyrus and, 3)

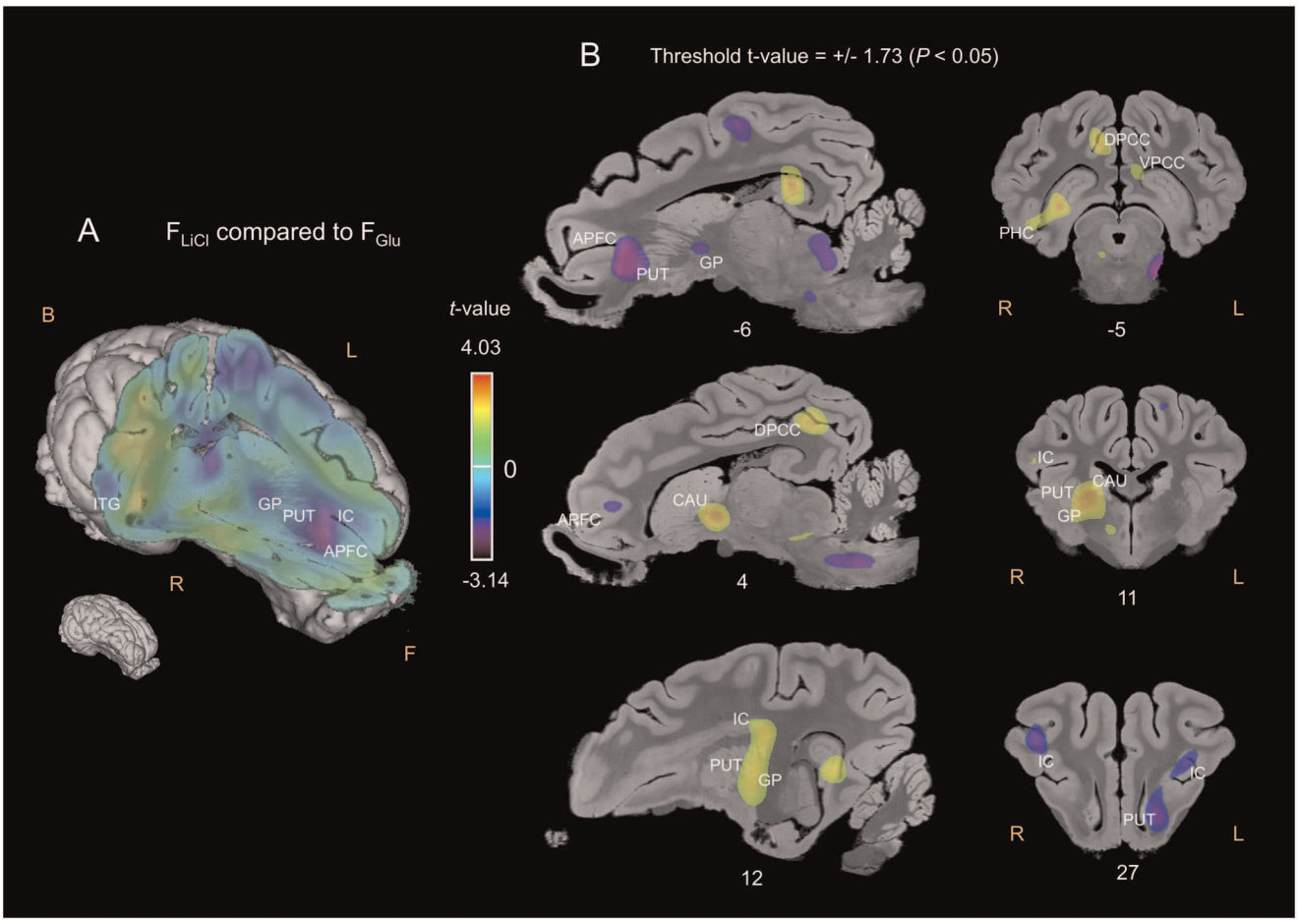

Figure 6. Cerebral glucose metabolism (CGM) differences obtained for the F_{LiCl} flavour compared to the F_{Glu} flavour. (A) Three-dimensional skinned representation of the pig's brain with global CGM differences found in the F_{LiCl} *vs* F_{Glu} contrast. The (x y z) coordinates are indicated below the representation. (B) Sagittal and coronal MRI sections showing significant CGM differences in the F_{LiCl} *vs* F_{Glu} contrast. The threshold for significance was set at $P<0.05$ (uncorrected). The x or y coordinates are indicated below each section. Positive *t*-values (green, yellow and red) indicate more activation in the F_{LiCl} condition than in the F_{Glu} condition, while negative *t*-values (blue and purple) indicate more deactivation in the F_{LiCl} condition than in the F_{Glu} condition. F, Front; B, Back; R, Right; L, Left; IC, insular cortex; ITG, inferior temporal cortex. Other abbreviations: see Figure 5.
doi:10.1371/journal.pone.0037968.g006

exposure to the aversive but not to the less preferred flavour triggered higher activation in the PCC and the left AMY.

Negative flavour perception triggered lesser activation in the prefrontal cortex. The APFC was bilaterally less activated during exposure to both the aversive and less preferred flavours. Some authors reported that the APFC [29], as well as the OFC [30,31,33], was activated by both aversive and pleasant flavour perception in pigs and humans, suggesting that the prefrontal cortex might be involved in the recognition of food-related flavours rather than in the characterization of flavour palatability. The OFC, however, is known to be involved in the passive perception of odours but also in the active smelling of odours (hedonic and familiarity judgments; [28,54]) and is implicated in the processing of reward [55]. As activation in the OFC is correlated with pleasantness ratings of the stimuli in humans [42], a lesser activation during exposure to a less preferred and/or aversive flavour was expected. In humans, Rolls et al. [28] also demonstrated that pleasant odours induced more activation in the medial OFC than unpleasant odours, while Gaultier et al. [29] reported that the deactivation in the OFC was larger during aversive than during preferred flavour perception in pigs.

Previous studies in humans reported activation in the left DLPFC during perception of a pleasant taste [39,56], which is consistent with its lesser activation during the perception of a less preferred flavour (F_{Glu}) in our study. The prefrontal cortex, and especially the left DLPFC, is involved in the treatment of feeding signals and is known to modulate food intake by sending inhibitory inputs to the orexigenic network to suppress hunger [57–59]. These results suggest that the perception of a flavour with a relatively low hedonic value is likely to modulate the inhibitory inputs sent to the orexigenic system, as well as further food intake. As the perception of the aversive flavour did not trigger similar brain responses, further investigation is needed to understand to what extent the level of aversiveness of the stimuli is determinant in the modulation of the DLPFC activity.

Negative flavours triggered lateralized patterns of activity in specific brain structures. We demonstrated that the perception of the aversive flavour induced lesser activation in the left basal nuclei compared to the control and less preferred conditions, while the perception of the less preferred flavour triggered lesser activation in the right basal nuclei compared to the control condition. As the basal nuclei are an integrant part of the

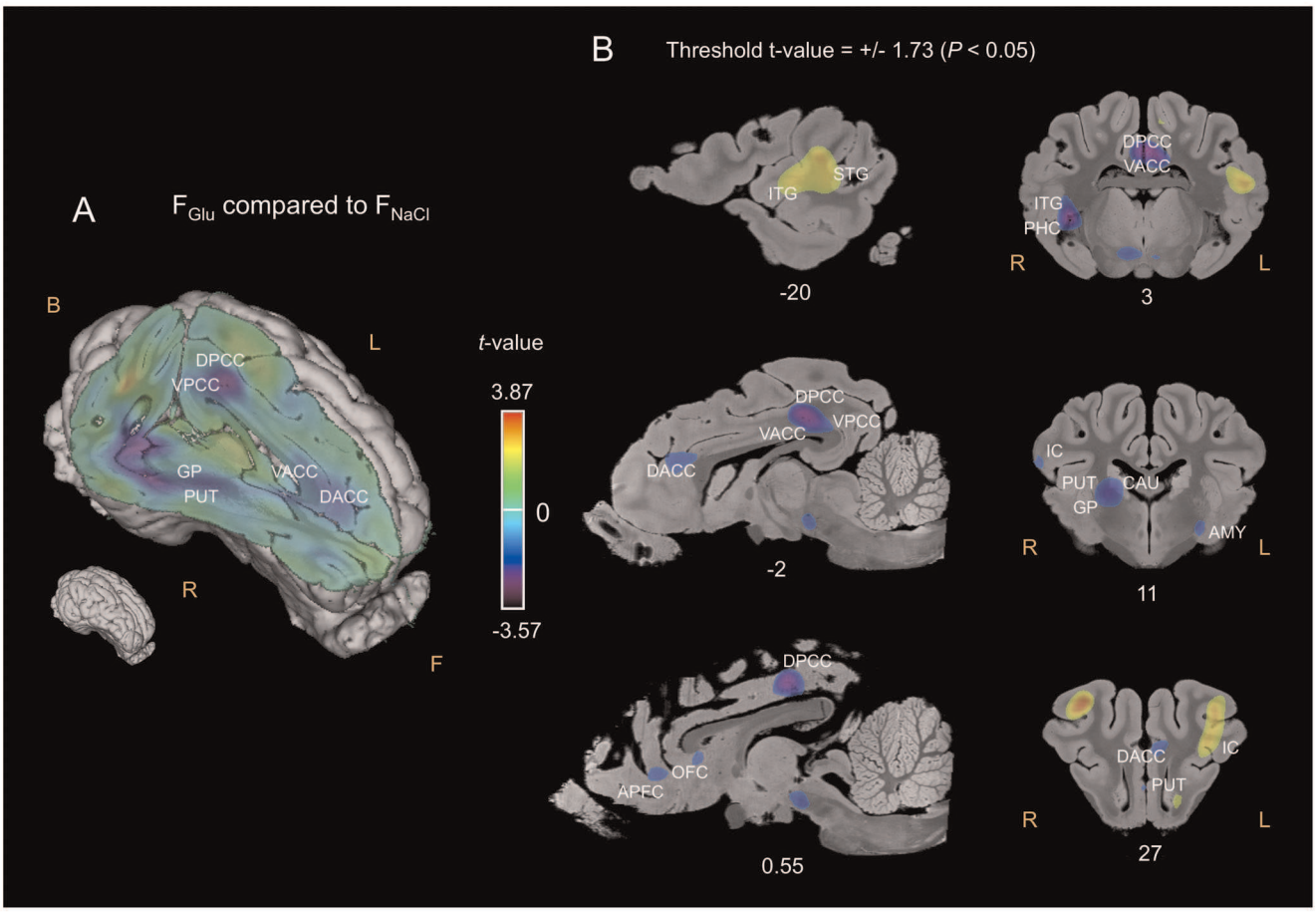

Figure 7. Cerebral glucose metabolism (CGM) differences obtained for the F_{Glu} flavour compared to the F_{NaCl} flavour. (A) Three-dimensional skinned representation of the pig's brain with global CGM differences found in the F_{Glu} *vs* F_{NaCl} contrast. The (x y z) coordinates are indicated below the representation. (B) Sagittal and coronal MRI sections showing significant CGM differences in the F_{Glu} *vs* F_{NaCl} contrast. The threshold for significance was set at $P<0.05$ (uncorrected). The x or y coordinates are indicated below each section. Positive *t*-values (green, yellow and red) indicate more activation in the F_{Glu} condition than in the F_{NaCl} condition, while negative *t*-values (blue and purple) indicate more deactivation in the F_{Glu} condition than in the F_{NaCl} condition. F, Front; B, Back; R, Right; L, Left; OFC, orbitofrontal cortex; AMY, amygdala; STG, superior temporal gyrus; PHC, parahippocampal cortex; DACC, dorsal anterior cingulate cortex; VACC, ventral anterior cingulate cortex; VPCC, ventral posterior cingulate cortex. Other abbreviations: see Figures 5 and 6.
doi:10.1371/journal.pone.0037968.g007

reward system and mediate numerous goal-directed behaviours, including emotions, motivation, and cognition [55], lesser CGM during exposure to negative stimuli was quite expected. Besides, Small et al. [38,60] reported that activation in the CAU and the PUT was correlated with pleasantness ratings of the stimuli and/or the motivation to eat (e.g., chocolate). Surprisingly, in our study, the perception of the aversive flavour also triggered higher activation in the right NAcc, GP, CAU and PUT, while the perception of the less preferred flavour triggered higher activation in the left PUT.

Numerous studies reported asymmetric brain activity during exposure to pleasant or unpleasant stimuli, although the results are not consistent. Henkin and Levy [61] reported that the smell of odours considered as unpleasant generally triggered greater activity in the right than in the left hemisphere, which is concordant with higher activation of the right basal nuclei found in our study during the perception of an aversive flavour. Gaultier et al. [29] found that the perception of a preferred flavour compared to an aversive flavour triggered activation in the left CAU, PUT, and GP in pigs, which is consistent with our finding that an aversive stimulation triggered lesser activation in left PUT or GP. Moreover, in our study, the correlation found between food consumption and brain metabolism, especially in left brain structures including the APFC, DLPFC, CAU and left IC, supports general knowledge admitting that the left hemisphere is involved in emotional processing of odours and hedonic judgments, while the right hemisphere is rather involved in the processing of odour familiarity and recognition [62]. Although we found that the perception of an aversive and/or a less preferred flavour mostly induced higher CGM responses in the left temporal gyrus, some studies in humans showed that activation during food or pleasant taste stimulation is higher in the left cortical regions, such as the superior temporal cortex [36,39] known to be involved in the perception of taste [39]. As exposed here, scientific data are quite contradictory as for the lateralization of brain responses to sensorial stimulations [58,63] and further studies are needed to extricate the relationships between brain lateralization and the processing of stimuli with contrasted hedonic values.

The perception of an aversive flavour triggered specific brain activations. The perception of the highly aversive flavour induced higher CGM responses in the AMY, the PHC and the PCC, whereas the perception of the less preferred flavour

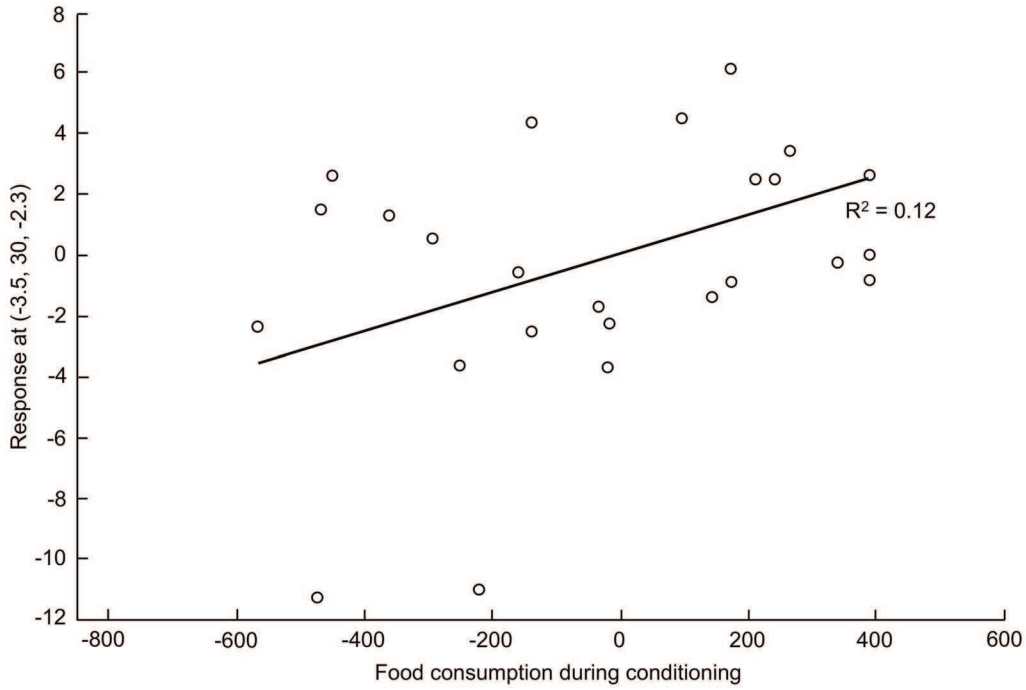

Figure 8. Relationship between the quantity of food consumed during the last conditioning session and brain metabolism for the voxel with the highest *t*-value (2.52) in the left anterior prefrontal cortex. Least-square regression line: $R^2 = 0.11709$. The (x, y, z) coordinates of the voxel are indicated in the y-axis legend. The statistical value for the voxel is $P = 0.009$. The open circles indicate the adjusted data (% error) for the subjects.
doi:10.1371/journal.pone.0037968.g008

did not. In humans, the AMY, which is involved in the hedonic processing of olfactory and gustatory stimuli [30,64], has been found to be activated during exposure to aversive odorants [30] and tastes [31], and it appeared that the amplitude of activation in the left AMY is correlated with the level of perceived aversiveness in humans [40]. In their meta-analysis, Costafreda et al. [32] reported that the AMY is activated by aversive rather than positive stimuli. All sensory stimuli with strong emotional value, however, are likely to induce AMY activation, regardless to the valence of the stimuli (pleasant and aversive) [33,64,65], although the responses are often less consistent with positive stimuli than with aversive stimuli [35]. As for the PCC, Small et al. [38] reported that it was more activated when patients eating chocolate rated it as highly pleasant or highly aversive than when they rated it neutral. According to Maddock [66], they concluded that the PCC was rather activated by stimuli with a high (positive or negative) emotional valence than by stimuli with a low or neutral emotional valence. Consequently, our results seems to corroborate the finding of Small et al. [38] in humans.

It is worth noting that we also found that different part of the cingulate cortex (e.g. the ACC), as well as the PHC, were less activated during perception of both aversive and less preferred flavours. Those structures are involved in the processing of olfactory perception [67] and in the emotional evaluation of sensory stimuli [26,38], and the activation of the ACC is correlated with the pleasantness ratings of odours [28,37,42]. In their review, Haber and Knuston [55] reported that the ACC is highly associated with reward and strongly connected to the basal nuclei and consequently considered as an integrant part of the reward circuit. Deactivation in the PHC and the ACC during perception of the aversive and less preferred flavours was thus expected, especially since Reiman et al. [68] found that the ACC was involved in the experience of unpleasant emotions, while the PHC was rather activated by pleasant taste [39].

Lastly, we noticed that the IC was predominantly less activated during the perception of aversive or less preferred flavours. The IC is a multimodal structure receiving projections from the olfactory system (in monkeys: [69]), and is considered as the primary taste cortex [33,36,70]. Some studies reported that the IC is activated in response to olfactory stimulations [67], and especially, but not exclusively, to pleasant odour perception [28], though, other studies mentioned that the IC is rather activated during unpleasant and aversive gustatory stimulations [31,33]. All together, these findings suggest that the IC might be involved in the recognition of flavours rather than in the processing of the stimulus hedonism or, that distinct parts of the IC are differentially implicated in the processing of aversive or pleasant sensory stimuli.

Conclusion

In conclusion, we demonstrated that postingestive visceral stimuli can modulate the flavour/food hedonism and further feeding choices. We performed here one of the first studies highlighting considerable similarities in the pig's and human's brain metabolism during the processing of the hedonic value of sensory stimuli. As expected, exposure to flavours with different hedonic values induced some metabolism differences in neural circuits that have been identified in humans to be involved in the characterization of food palatability, flavour identification and more generally, in the regulation of food intake. The present study also complemented a previous study published by our group [29], which was the very first to describe unconscious brain responses during flavour exposure in pigs. These results are promising in terms of biomedical research applied to human nutrition and show that the pig is a good model to study the behavioural and neurobiological determinants of food intake. However, our study

has some limitations requiring further investigations. First, while LiCl i.d. infusions induced a strong long-lasting flavour aversion, 15% glucose infusions failed to condition a flavour preference. As sweet taste enhances the effect of caloric supply [71], adding glucose directly in the food may enable to enhance its reinforcement value and condition a clear flavour preference to study the specific cerebral responses triggered by the perception of a highly pleasant flavour in pigs. Second, in the present study, the small number of animals prevented us from finding brain metabolism differences when correction was made for multiple comparisons. A complementary study using an improved paradigm and an increased number of pigs should result in the establishment of a persistent conditioned flavour preference and in a substantial improvement of the statistical power for brain imaging analyses.

Acknowledgments

The authors wish to thank Phodé Laboratories (Terssac, France) for providing the essential oils used in this study and Servier Medical Art for providing some of the illustrations used in this paper. The authors gratefully acknowledge the efforts and cooperation of the scientific and technical staff, and especially Sylvie Guérin for participating in this study, Paul Meurice for contributing to the brain analyses, and Alain Chauvin, Francis Le Gouevec and Loïc Gaillard for taking care of the animals.

Author Contributions

Conceived and designed the experiments: CC DVL MCMS. Performed the experiments: CC MJ DVL. Analyzed the data: CC MJ DVL. Contributed reagents/materials/analysis tools: CHM DVL. Wrote the paper: CC DVL MCMS CHM.

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CHAPITRE II

Préférences Conditionnées Induites par Différents Renforcements :

Comparaison entre Stimuli Caloriques et/ou Gustatifs

CHAPITRE II : PRÉFÉRENCES CONDITIONNÉES INDUITES PAR DIFFÉRENTS RENFORCEMENTS : COMPARAISON ENTRE STIMULI CALORIQUES ET/OU GUSTATIFS

Dans le chapitre précédent, nous avons validé un modèle porcin de l'aversion alimentaire conditionnée induite *via* des injections i.d. de chlorure de lithium, qui se caractérise par une modification drastique des préférences alimentaires, du profil comportemental ainsi que par des réponses cérébrales spécifiques. Au contraire, l'utilisation d'infusion i.d. de glucose 15% ne s'est pas révélée efficace pour l'établissement d'une préférence alimentaire conditionnée. Une des hypothèses pour expliquer cet échec serait qu'un apport énergétique n'est pas suffisant à lui seul pour conditionner une préférence robuste, notamment lorsque l'aliment utilisé pour le conditionnement (CS) représente à lui seul un apport calorique non négligeable. Ceci suggère soit qu'une stimulation gustative positive comme un goût sucré pourrait être plus efficace pour le conditionnement d'une préférence alimentaire, soit que l'association entre apport énergétique et stimulation gustative positive pourrait être indispensable à l'établissement d'une préférence. Une deuxième hypothèse pourrait être que le glucose n'est tout simplement pas le meilleur candidat pour l'établissement d'une préférence robuste chez le porc, suggérant alors que d'autres composés énergétiques pourraient s'avérer plus efficaces.

L'objectif principal du *chapitre II* était de développer un modèle de préférence alimentaire conditionnée chez le porc. Nous avons donc testé les hypothèses issues du *chapitre I* en comparant l'efficacité de différents composés sucrés et/ou énergétiques (saccharine, maltodextrine et saccharose) pour le développement de préférences alimentaires conditionnées. Afin de nous affranchir totalement de l'apport énergétique inhérent à l'aliment utilisé pour le conditionnement et de maximiser les effets potentiellement positifs d'un renforcement calorique, les procédures de conditionnements ont été réalisées dans un premier temps sur des eaux de boisson aromatisées non énergétiques dans lesquelles ont été ajoutés les renforcements caloriques et/ou sucrés. Dans un second temps, la possibilité d'une transposition de ces préférences à un aliment solide aromatisé a été testée. Les hypothèses de ce travail sont qu'un renforcement sucré et/ou calorique dans l'eau de boisson provoquera le développement d'une préférence pour la flaveur conditionnée, caractérisée notamment par une augmentation des quantités ingérées de l'eau de boisson ou de l'aliment contenant cette flaveur lors des présentations futures.

Préférences conditionnées induites par différents renforcements : comparaison entre stimuli caloriques et/ou gustatifs (article n°3)

1. Contexte et objectifs

Dans les élevages porcins, les animaux sont soumis à de nombreuses périodes de transitions alimentaires, en particulier au sevrage ou à la transition gestation-lactation, durant lesquelles leur activité alimentaire peut être fortement perturbée. Lors de l'exposition à des aliments nouveaux, les animaux expriment souvent une réponse néophobique qui se caractérise par une réduction de la prise alimentaire et qui peut être accompagnée d'une diminution du gain de poids. L'établissement de préférences alimentaires conditionnées pourrait représenter un outil efficace pour réduire cette réponse néophobique et maintenir une prise alimentaire stable durant ces phases de transition. L'objectif principal de cette étude était donc de développer un modèle porcin de la préférence alimentaire conditionnée. Dans un premier temps, nous avons donc testé l'établissement de préférences alimentaires conditionnées sur l'eau de boisson *via* l'utilisation de différents renforcements agissant sur la sphère gustative (goût sucré) et/ou sur la sphère viscérale (apport énergétique). Nous avons tenté ainsi de déterminer quel facteur, *i.e.* gustatif et/ou calorique, est le plus déterminant pour l'acquisition de préférences alimentaires robustes. Dans un second temps, la possibilité d'une transition de ces préférences induites *via* l'eau de boisson sur un aliment solide a également été testée.

2. Méthodes

Dans l'expérience 1A, neuf porcs juvéniles ont été soumis à trois sessions de deux jours de conditionnement durant lesquels ils recevaient une eau de boisson aromatisée additionnée de saccharose 1,125% (calories + goût sucré, F+$S_{1,125}$) et une autre eau de boisson aromatisée sans additif (F–). Dans l'expérience 1B, neuf porcs juvéniles ont été soumis à six sessions de deux jours de conditionnement durant lesquels ils recevaient une eau de boisson aromatisée additionnée de saccharose 10% (calories + goût sucré, F+S_{10}) et une autre eau de boisson aromatisée sans additif (F–). Dans l'expérience 2, neuf porcs juvéniles ont été soumis à quatre sessions de trois jours de conditionnement. Ils recevaient trois eaux de boisson aromatisées et additionnées de maltodextrine 2,25% (calories, F+m), de saccharine 0,37% (goût sucré, F+s) ou sans additif (F–). Après le conditionnement, les animaux étaient soumis à des tests de double choix sur les eaux de boisson aromatisées sans renforcement pour tester l'acquisition des préférences, et à des tests de double choix sur des aliments solides aromati-

sés pour tester la possibilité de transposition des préférences sur un aliment solide aux caractéristiques organoleptiques plus complexes.

3. Résultats

Durant les tests de double choix sur l'eau de boisson, les porcs n'ont exprimé aucune préférence pour la solution $F+_{1,125}$ comparée à la solution F– ($P > 0,1$). Au contraire, la solution $F+_{10}$ était significativement préférée à la solution F– ($P < 0,05$), mais seulement pendant la première répétition du test de choix, suggérant que les animaux développent une préférence à court terme pour l'eau de boisson associée au saccharose 10% mais pas au saccharose 1,125%. Dans l'expérience 2, aucune préférence significative n'a émergé durant les tests de double choix sur l'eau de boisson ($P > 0,1$). Cependant, la consommation de F+m était 107% et 35% plus importante que la consommation de F– et F+s, respectivement. Dans les trois expériences, malgré l'émergence de préférences pour la solution renforcée (expérience 1B), aucune préférence significative n'a été mise en évidence durant les tests de double choix sur l'aliment solide ($P > 0,1$).

4. Conclusions

Le saccharose 10% semble être le meilleur candidat pour le conditionnement de préférences alimentaires induites sur l'eau de boisson chez le porc. L'efficacité du saccharose 10% à conditionner une préférence, au détriment de la saccharine ou de la maltodextrine, pourrait souligner l'importance de la combinaison entre renforcement gustatif et renforcement calorique pour l'acquisition d'une préférence alimentaire. Cependant, les résultats des tests de choix laissent à penser qu'un apport calorique seul *via* la maltodextrine pourrait également s'avérer efficace pour le développement de préférences conditionnées. Des études supplémentaires à base d'édulcorants artificiels comme l'aspartame pourraient permettre de déterminer si l'échec du conditionnement à la saccharine est dû à l'inefficacité d'un goût sucré à conditionner des préférences en l'absence d'apport énergétique ou à l'incapacité des porcs à percevoir le goût sucré de la saccharine. L'incapacité à transposer les préférences conditionnées sur l'eau de boisson à un aliment solide pourrait s'expliquer : (1) par la complexité des caractéristiques organoleptiques de l'aliment (texture, goût, odeur) qui peut changer de manière considérable la perception de la flaveur dans l'aliment comparée à sa perception dans l'eau ou, (2) par la valeur calorique renforcatrice intrinsèque de l'aliment qui pourrait avoir minimisé/masqué la valeur ajoutée par le renforcement calorique dû au sucre.

Article n°3

Flavour preference acquired* via *beverage-induced conditioning and its transposition to solid food: Sucrose but not maltodextrin or saccharin induced significant flavour preferences in pigs

Caroline Clouard, Mathieu Chataignier, Marie-Christine Meunier-Salaün and David Val-Laillet*

Applied Animal Behaviour Science , 2012, 136(1): 26-36

(Accepted 6 November 2011)

INRA, UMR Systèmes d'Élevage Nutrition Animale et Humaine, F-35590 Saint Gilles, France / Agrocampus Ouest, Systèmes d'Élevage Nutrition Animale et Humaine, F-35000 Rennes, France

*Corresponding author: david.val-laillet@rennes.inra.fr

Abstract

When exposed to novel food during food transitions, growing pigs often elicit a neophobic response that is responsible for decreased food consumption. Flavour preference conditioning may represent an interesting way to reduce neophobia and improve food intake in growing pigs. The present series of experiments investigated the pig's conditioned preference for a flavoured beverage added with different carbohydrates and sweeteners and the possible transition of those beverage-induced preferences to flavoured solid food. In Experiment 1A, nine juvenile pigs were given three two-day conditioning sessions: they received a flavoured beverage added with 1.125% sucrose ($F+S_{1.125}$) and a second flavoured beverage with no additive (F-). In Experiment 1B, nine juvenile pigs were given six two-day conditioning sessions: they received a flavoured beverage added with 10 % sucrose ($F+S_{10}$) and a second flavoured beverage with no additive (F-). In subsequent two-choice drinking tests, the pigs exhibited no clear-cut preference for $F+S_{1.125}$, whereas $F+S_{10}$ was preferred compared to F- ($P < 0.05$) but only during the first subsequent two-choice drinking tests, suggesting that pigs developed a short-term preference for the flavour previously paired with 10% but not 1.125% sucrose. The Experiment 2 was conducted to assess the independent effects of visceral (caloric intake) and gustative (sweet taste) reinforcement in flavour preference conditioning. Nine juvenile pigs were subjected to four three-day conditioning sessions: they received flavoured beverages added with 2.25% maltodextrin (F+m, caloric intake), 0.37% saccharin (F+s, sweet taste), or no additive (F-). During further two-choice drinking tests, no clear-cut preference emerged, but the consumption of F+m was 107% and 35% higher than that of F- and F+s, respectively. Despite pigs exhibited some conditioned flavour preferences during two-choice drinking tests in experiment 1B and 2, no clear-cut preference was observed during two-choice feeding tests with flavoured solid food. Overall, these findings highlight the importance of the combination and/or synergy between gustative and visceral reinforcements

for conditioned flavour preference and suggest that a visceral reinforcement via maltodextrin might be sufficient to condition such a preference. Moreover, the absence of clear-cut preference during two-choice feeding test illustrates the difficulty to transpose a flavour preference acquired via a sweet beverage to solid food. Further studies are needed to investigate the failure of saccharin-conditioned preference in pigs.

Keywords: pigs, flavour/taste conditioning, flavour/nutrient conditioning, preference, sweet taste, caloric intake

1.Introduction

In livestock production, pigs have to face many sensitive periods during which their feeding activity is strongly disrupted due to unfamiliar feeding and environmental conditions (Campbell, 1976; Dong and Pluske, 2007; Meunier-Salaün and Picard, 1996; Rutherford et al., 2006). When exposed to novel food during food transitions, growing pigs often elicit a neophobic response that is caused by the fear of novelty and is reinforced by the associated novel environment (Hursti and Sjödén, 1997). This neophobic response is responsible for decreased food consumption (Oostinger et al., 2010), as well as behavioural changes that can reflect a decreased welfare (Meunier-Salaün and Bergeron, 2005). This reduced food intake is also a significant issue for farmers as it may lead to decreased productivity, such as a loss of body weight or a growth decrease in piglets (Forbes 1995). Some studies have shown that reducing neophobia via the pre-exposure to a familiar flavour before food transition is efficient to increase food intake, and especially at weaning. For instance, pre- and post-natal exposure to flavours through sow's diet during gestation and lactation, i.e. through maternal amniotic liquid and milk, increased postweaning feed intake and enhanced the health and welfare of piglets (Landendijk et al., 2007; Oostinger et al., 2010). Similarly, during growth, flavour

preference conditioning may represent an alternative and interesting way to reduce neophobia and improve food intake in growing pigs.

Animals and humans learn to associate the flavour of foods with their appetibility and post-ingestive consequences to modulate further food intake. When food intake generates positive appetitive and/or post-ingestive consequences (e.g. high hedonic value and/or strong energy supply), the organism learns to preferentially consume this particular food, as referred as conditioned food preference. Although the ingestion of high palatable food (e.g. sweet food) is often paired with an energy supply (caloric intake; Myers and Sclafani, 2006), two types of conditioned learning, operating independently, are reported. The flavour/taste conditioning consists in the association between the flavour of an unfamiliar food and a flavour/taste that is familiar and/or already has a high hedonic value (e.g. sweet taste). This kind of association has been widely studied in rats (Sclafani and Ackroff, 1994; Warwick and Weingarten, 1994, 1996) as well as in humans (Brunstrom and Fletcher, 2008; Mobini et al., 2007). In contrast, the flavour/nutrient conditioning is induced by pairing the flavour of an unfamiliar food with an energy supply, i.e. positive post-ingestive consequences (Myers and Sclafani, 2006). Flavour/nutrient learning has been studied in humans (Brunstrom and Mitchell, 2007; Mobini et al., 2007; Zeinstra et al., 2009) and rats (Lucas et al., 1997; Lucas and Sclafani, 1998; Sclafani, 2001; Sclafani and Ackroff, 1994; Warwick and Weingarten, 1994).

The addition of carbohydrates as reinforcement in the flavoured solution emerges as a particularly efficient way to experimentally condition a flavour preference, especially because several mammal species, including rats, humans and other primates, are very attracted to the sweet taste of sugars (for a review, see Berridge 2000; Steiner et al., 2001). Sucrose (Bonacchi et al., 2008; Gilbert et al., 2003; Sclafani et al., 1997; Warwick and Weingarten, 1996), fructose (Sclafani and Ackroff, 1994) and glucose (Sclafani and Ackroff, 1994; Warwick and

Weingarten, 1994) are the most common sweet compounds used to induce classical flavour preference conditioning via a beverage since these compounds induce high energy supply (4kcal/g) and have an important hedonic value (i.e. sweet taste). Saccharin, an artificial calorie free sweetener has been used to condition flavour/taste preference (Sclafani and Ackroff, 1994), whereas maltodextrin, an almost flavourless carbohydrate, has been used to condition flavour/nutrient preference (Dwyer, 2008; Sclafani et al., 1997).

Some electrophysiogical studies have shown that pigs are able to detect some sweet compounds in water (Hellekant and Danilova, 1996, 1999), while behavioural studies have shown that pigs exhibit a high preference over water for compounds known to be intense sweeteners in humans, including sucrose, fructose or saccharin (Baldwin, 1976; Glaser et al., 2000; Nofre et al., 2002). Consequently, there are several indications that such conditioning may be feasible in pigs. No study, however, investigated the ability of pigs to develop a preference to a flavoured-beverage added with carbohydrates or sweeteners. The possibility to develop such conditioned preference may represent interesting opportunities of application in an animal production perspective. Coupled with the possibility to transpose those beverage-induced conditionings to a solid food, the addition, in the novel food, of a flavour that has been previously positively conditioned via beverage-induced conditioning may increase the acceptation of a novel food in feeding transition periods.

The aim of our study was threefold: (1) To develop a model of conditioned preference toward a flavoured beverage in pigs, (2) to compare three types of flavour conditionings, that is flavour/taste-nutrient, flavour/nutrient and flavour/taste conditionings, by using different sweet compounds, i.e. sucrose, maltodextrin and saccharin, respectively and; (3) to test the possible transition of those beverage-induced conditionings to solid food. We hypothesised that the addition of a sweet compound in a flavoured-beverage during a sufficient conditioning period

would: (1) increase the further intake of this flavoured-beverage and; (2) increase the intake of a unfamiliar solid food added with the same flavour of interest compared to an unfamiliar unflavoured solid food, i.e. reduce the neophobia exhibited by the animals during the presentation of a novel solid food.

2. General materials and methods

The experiments presented in this paper were conducted in accordance with the current ethical standards of the European Community (Directive 86/609/EEC), Agreement No. A35-622 and Authorization No. 35-88. The Regional Ethics Committee in Animal Experiment of Brittany has validated the entire procedure described in this paper (R-2010-CC-01).

2.1. Animals

Three batches of 9 Large White/Landrace x Large White female pigs of 34.1 ± 2.5 kg at the beginning of the study were used. The animals were fed daily at 10:00 h with 1.2-1.5 kg of a pelleted meal composed of 23.2% wheat, 25% corn, 22.8% barley, 24,3% soybean meal, 0,4% vegetal oil, 1% carbonate, 1.11% bi-calcic phosphate, 0.4% salt, and 0.5% vitamin complement. During the days free of behavioural tests, the animals had free access to water. During the experimental days, the animals had access to running water from 16:00 h to 08:00 h only. From 09:00 h to 16:00 h, the running water circuit was turned off and animals could only drink the experimental beverages from two specific troughs independent from the running water circuit.

2.2. Apparatus

The pigs were housed in individual pens (126 cm x 75 cm x 86 cm) modified for the implementation of two-choice feeding/drinking tests. Each pen was equipped with a removable two-party trough on the front wall (i.e. the door) to perform two-choice feeding tests. On the

back wall of the pen, one drinking trough was connected to the standard running water circuit. Two supplementary troughs were installed on both sides of the pen to distribute the experimental solutions. These two lateral troughs were connected with silicone pipes to plastic tanks equipped with a valve to turn on/off the water flow. These bottles were removable and were placed high up, above the door. If necessary, silicone grease (Dow Corning, USA) was applied between the valve and the pipe to avoid water leak. The room was maintained under controlled conditions: temperature was kept at 25 ± 2°C with a natural day/night cycle.

2.3. Experimental solutions and meals

The conditioned solutions were tap water flavoured with 0.025% essential oils of thyme (T), cinnamon (C) or orange (O) provided by Phodé Laboratories (Terssac, France). At this concentration, the effect of neophobia towards the novel flavours is minimized but flavours are perceptible by pigs and there is no spontaneous preference for one of the flavours over the others (unpublished preliminary study). During conditioning (i.e. one-tank training sessions) in Experiment 1, the positive reinforcement (F+) was induced by the addition of sucrose in the flavoured solution. In Experiment 1A, 1.125% sucrose (33 mM, 45 kcal/L) was used as a reinforce ($F+S_{1.125}$, since some studies reported that pigs (Baldwin, 1976; Glaser et al., 2000) and rats (Bonacchi et al., 2008) exhibited preference for 0.5-1% and 2% sucrose solutions over water, respectively. In Experiment 1B, 10% sucrose (0.3 M, 400 kcal/L) was used as a reinforce ($F+S_{10}$). During conditioning in Experiment 2, the positive reinforcement was induced by the addition of maltodextrin (F+m, 2.25%, 0.125 M, 90 kcal/L, flavour-nutrient conditioning) or saccharin (F+s, 0.37%, 20 mM, flavour-taste conditioning) in the flavoured solutions. The control treatment was represented by an unsweetened (i.e. no sweet compound) flavoured solution (F-). At these concentrations, saccharin is preferred over water by pigs (Baldwin, 1976) and maltodextrin is normally efficient to induce flavour preference (un-

published preliminary study). The specific flavours paired with each treatment were counter-balanced between animals (e.g. some were positively conditioned to thyme, others to cinnamon or orange). After conditioning, during two-choice tests, flavoured solutions were free of any sweet/carbohydrate compound. Flavoured meals were also prepared to perform two-choice feeding tests. The three flavoured meals were made by the addition in pelleted standard meal of essential oils of thyme (T; 0.4%) cinnamon (C; 0.1%) or orange (O; 0.15%) diluted in vegetable oil (Phodé Laboratories, Terssac, France), with 10 ml of oils per kilogram of meal. At these dilutions, the animals normally consume as much thyme-, cinnamon- and orange-flavoured meals (unpublished preliminary study).

2.4. Statistical analysis

Statistical analyses were performed with the Statview software (SAS Institute, USA). The consumption of the flavoured meals and solutions as well as the behavioural activity were compared between treatments using non-parametric Wilcoxon tests. When multiple comparisons were performed, a Bonferroni correction was applied.

3. Experiment 1

In this experiment, the combined effects of calories and sweet taste for the acquisition of flavour preference were assessed by using sucrose at different concentrations as a stimulus.

3.1. Experiment 1A.

3.1.1. Experimental paradigm.

3.1.1.1. Habituation. During the first week, the pigs were accustomed to the pens and to the experimental drinking troughs. During the five first days, from 09:00 h to 16:00 h, the running water circuit was turned off and the animals had access to tap water via the two lateral

troughs connected to the tanks. Each tank contained 4 L of tap water. At 16:00 h, the tanks were removed, the running water circuit was turned on and water refusals were weighted in order to establish a baseline water consumption. As numerous animal species are known to show lateralised behavioural responses in various tasks (e.g. horses: Austin and Rogers, 2007; chicks: Mascetti et al., 1999; monkeys: Laurence et al., 2011), the absence of a laterality bias (i.e. no preference for the left or the right drinking trough) was also checked during this step of the study.

3.1.1.2. Conditioning sessions. During one week, the pigs were given 6 one-tank training sessions (from 09:00 h to 16:00 h) with the CS solutions. On odd-numbered days, animals received orange-flavoured solutions and on even-numbered days, they received thyme-flavoured solutions. The quantity of beverage available during conditioning sessions has been adjusted according to the mean amount of water consumed during the habituation period to ensure that the animals were not in a water-deprived situation at the end of the conditioning session. Each day, half of the animals received the $F+S_{1.125}$ treatment (sucrose added to the flavoured solution) and the other half received the F- treatment (no sucrose addition). At 16:00 h, the tanks were removed and refusals were weighted.

3.1.1.3. Two-choice tests. The week following conditioning, the animals were subjected to two-choice tests. The first day, a two-choice drinking test was conducted. During this test, the pigs were given the choice between the orange-flavoured solution and the thyme-flavoured solution from 09:00 h to 16:00 h. During this time, the pigs had no access to the running water circuit but only to the two experimental drinking troughs connected to the tanks containing 4 L of flavoured solutions. No sweet compound was added to the solutions during the tests. At 16:00 h, the tanks were removed and refusals were weighted. For the next two days, two-choice feeding tests were conducted. The pigs were given the choice between the or-

ange-flavoured meal and the thyme-flavoured meal. A two-party trough containing 1 kg of each meal was presented at 09:30 h to the animals. After 30 min, the trough was removed and refusals were weighted. The animals had free access to tap water during these tests. Meal and beverage distribution in the troughs and tanks were counterbalanced over days and between animals to avoid any laterality bias.

3.1.2. Results

During the habituation phase, the animals consumed 2.37 ± 0.20 L of water per day. No laterality bias was found since there was no difference in the average amount of water drunk from the right or left drinking trough (right trough: 1.28 ± 0.18 L, left trough: 1.09 ± 0.62 L, z = 0.65, $P = 0.51$). There was no difference in the average amount of each flavoured solution consumed during conditioning (Orange: 0.65 ± 0.21 L, Thyme: 0.63 ± 0.12 L, z = 1.01, $P = 0.31$).

3.1.2.1. Consumption during conditioning sessions. In average, the animals consumed the same total amount of each flavoured beverage, regardless of the associated reinforcement ($F+S_{1.125}$ = 0.62 ± 0.09 L vs. F- = 0.56 ± 0.09 L, z = 1.01, $P = 0.31$). During the first training session, the animals tended to consume more of the flavoured solution associated with sucrose than the control flavoured solution ($F+S_{1.125}$ = 0.92 ± 0.34 L vs. F- = 0.41 ± 0.07 L, z = 1.72, $P = 0.09$), whereas during the last training session, they tended to consume more of the control flavoured solution than the flavoured solution associated with sucrose ($F+S_{1.125}$ = 0.55 ± 0.17 L vs. F- = 0.69 ± 0.22 L, z = 1.18, $P = 0.09$). However, there was no significant evolution of the consumption of each kind of beverage all along the conditioning sessions.

3.1.2.2. Consumption during two-choice tests. Only one two-choice drinking test was performed in this experiment. There was no significant difference between the consumption of

the control flavoured solution and that of the flavoured solution previously associated with sucrose during conditionings ($F+S_{1.125}$ = 0.63 ± 0.19 L vs. F- = 0.49 ± 0.16 L, z = 0.28, *P* = 0.78; Figure 1a). Though, the animals consumed in average 27% more flavoured solution previously associated with sucrose during conditioning than control flavoured solution (56% vs. 44% of the total amount consumed). During the first two-choice feeding test, the animals preferred the meal that was added with the flavour previously associated with sucrose ($F+S_{1.125}$) during conditioning over the meal that was added with the control flavour ($F+S_{1.125}$ = 0.60 ± 0.10 L vs. F- = 0.37 ± 0.92 L, z = 2.38, *P* = 0.02; Figure 1b). However, this difference of consumption disappeared from the second two-choice feeding test.

In conclusion, three one-bottle conditioning sessions with 1.125% sucrose (45kcal/l) did not induce a clear-cut flavour/nutrient-taste preference. During two-choice drinking tests, however, the animals consumed in average 27% more flavoured solution previously associated with sucrose during conditioning than control flavoured solution. An additional study is thus needed to further investigate the efficiency of sucrose to condition flavour preference in pigs.

3.2. Experiment 1B.

Two factors might explain our failure to condition a preference induced by sucrose in Experiment 1A: (1) the sucrose concentration was too low and/or (2) the length of the conditioning period was too short. As a result, the same experiment as Experiment 1A was repeated in Experiment 1B with a higher concentration of sucrose and an increased number of conditioning sessions.

3.2.1. Experimental paradigm.

3.2.1.1 Habituation. The same procedure as in experiment 1A was followed.

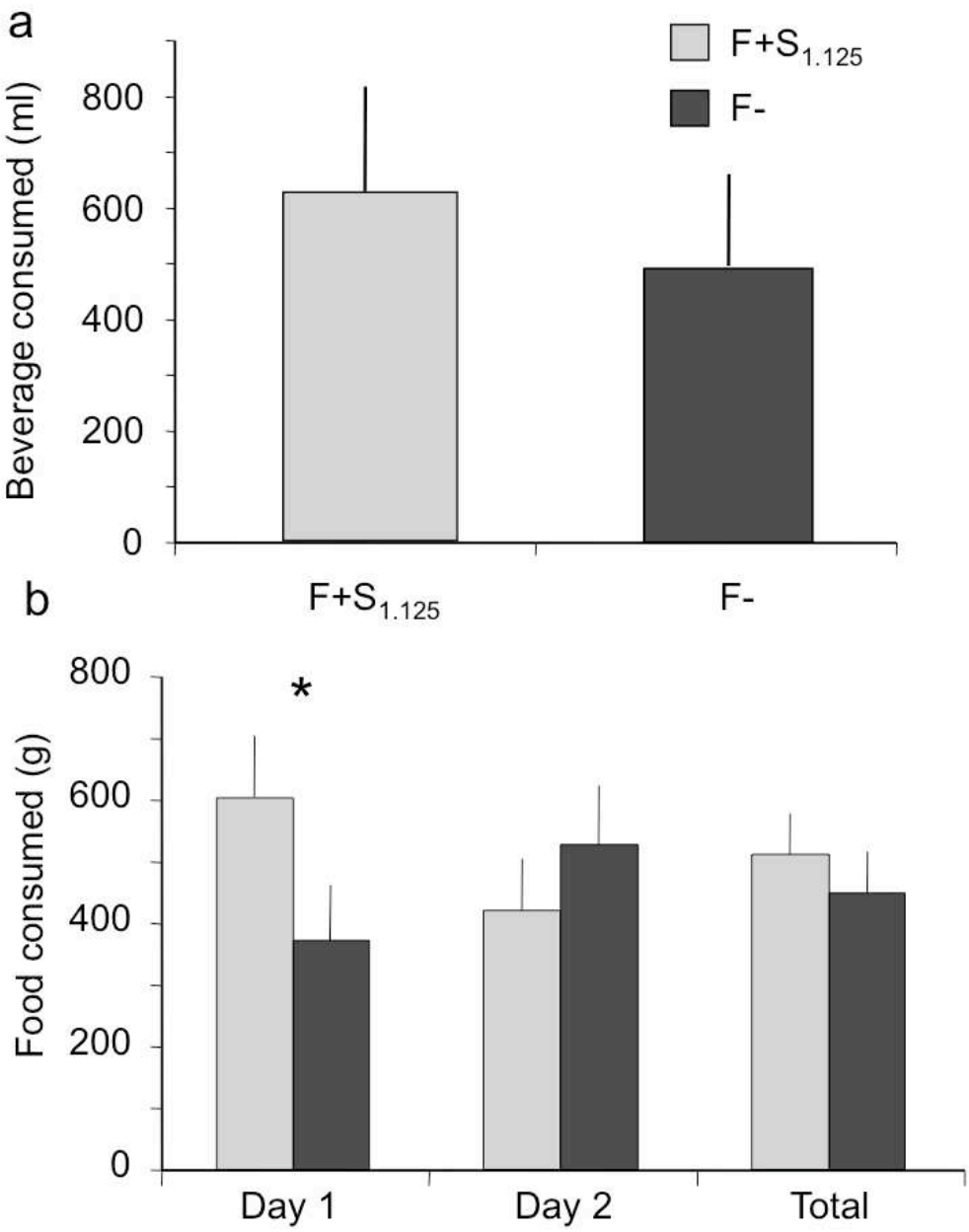

Figure 1. Quantity of beverage (ml) and food (g) consumed during the two-choice drinking (a) and feeding tests (b), respectively. These tests were carried out the week following the conditioning period in Experiment 1A (the $F+S_{1.125}$ flavour was associated with 1.125% sucrose during conditioning while the F- flavour was presented alone in the water). Flavoured beverage and meal were presented in the absence of reinforcement during two-choice tests. Data are presented with means and standard errors. An asterisk indicates a significant difference between two treatments ($P < 0.05$).

3.2.1.2. Conditioning sessions. The same procedure as in experiment 1A was followed but the pigs were subjected to12 one-tank training sessions during 2 weeks.

3.2.1.3. Two-choice tests. The same procedure as in experiment 1A was followed but the two-choice feeding tests were carried out before the two-choice drinking tests. This permutation was performed in order to avoid the fact that pigs could noticed the absence of sucrose in the flavoured solutions during the two-choice drinking test and unlearn their preference for the sucrose-reinforced flavour. Moreover, two instead of one two-choice drinking test were carried out.

3.2.2. Results.

One out of the 9 animals used in this experiment exhibited an abnormal drinking behaviour. It often emptied the tanks, thus consuming the maximal amount of solution regardless of the treatment. Consequently, this animal has been excluded from the present experiment. During the habituation phase, the animals consumed 2.15 ± 0.10 L of water per day. No laterality bias was found since there was no difference in the average amount of water drunk from the right or left drinking trough (right trough: 1.04 ± 0.10 L, left trough: 1.11 ± 0.13 L, $z = 0.14$, $P = 0.89$). There was no difference in the average amount of each flavoured solution consumed during conditioning (Orange: 0.82 ± 0.13 L, Thyme: 0.98 ± 0.15 L, $z = 0.84$, $P = 0.40$).

3.2.2.1. Consumption during conditioning sessions. In average, over the 6 conditioning sessions, there was no significant difference between the total consumption of each flavoured beverage, regardless of the associated reinforcement ($F+S_{10}$ = 1.11 ± 0.09 L vs. F- = 0.69 ± 0.14 L, $z = 1.54$, $P = 0.12$). Despite non-significant results, the consumption of the flavoured solution positively reinforced by sucrose was approximately 61% higher than the consumption of the control flavoured solution ($F+S_{10}$: 62% vs. F-: 38% of the total amount

consumed). The flavoured solution reinforced with sucrose was significantly less consumed during the first training session than during further sessions (Figure 2). The consumption of the control flavoured solution did not change across sessions, except during the last training session where the animals consumed significantly more solution than during the first training session. However, these differences did not resist a Bonferroni correction. During the third conditioning sessions, the animals tended to consume the positive reinforced solution more than the control solution. This difference was only significant for the fourth and fifth sessions (Figure 2).

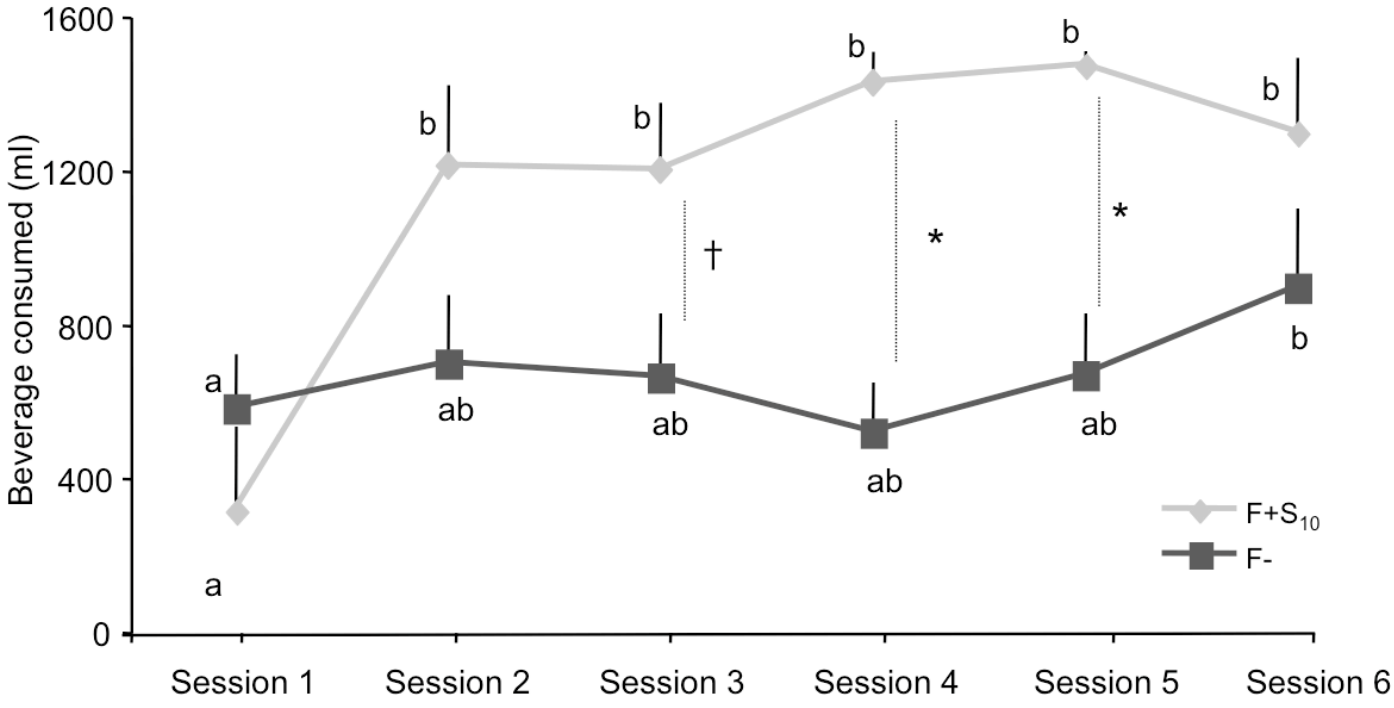

Figure 2. Quantity of beverage (ml) consumed during the six conditioning sessions of Experiment 1B. During the conditioning period, the animals were given a flavoured beverage added with 10 % sucrose ($F+S_{1.125}$) or no additive (F-). The following symbols are used to indicate a significant difference between two treatments during a particular conditioning session (i.e. inter-treatments/intra-sessions comparison): † $P < 0.1$, *$P < 0.05$. Two different letters indicate a significant difference between two conditioning sessions for the same treatment (i.e. intra-treatments/inter- sessions comparison; $P < 0.05$ before Bonferroni correction).

3.2.2.2. Consumption during two-choice tests. On Day 1 of the two-choice drinking tests, the animals significantly preferred the flavoured solution that was associated with sucrose during conditioning over the control solution (Figure 3a) The consumption of the flavoured solution previously reinforced with sucrose was approximately 141% higher than the consumption of the control flavoured solution ($F+S_{10}$: 71% vs. F-: 29% of the total amount consumed). However, on Day 2, the animals exhibited no clear-cut preference anymore. Over the two days of tests, the average consumption of the flavoured solution previously reinforced with sucrose during conditioning was approximately 56% higher than the consumption of the control flavoured-solution ($F+S_{10}$: 61% vs. F-: 39% of the total amount consumed), but this difference was not significant. On Days 1 and 2 of the two-choice feeding tests, as well as in average over the two days, there was no difference of consumption between the flavoured meal previously associated with sucrose during conditioning and the control flavoured meal (Figure 3b). Despite non-significant results, the consumption of the $F+S_{10}$ meal was 34%, 23% and 28% higher than the consumption of the F- meal on Day 1 (F+: 57% vs. F: 43%), on Day 2 (F+: 55% vs. F-: 45%) and on average of Days 1 and 2 (F+: 56% vs. F-: 44%), respectively.

4. Experiment 2

In Experiment 1, we demonstrated that 10% sucrose, a high-caloric sweet compound, induced a flavour preference in pigs. In this second experiment, two kinds of conditioning were compared to assess whether the preference acquisition depends on the visceral (caloric intake) or the gustatory (sweet taste) reinforcement: a flavour/nutrient conditioning was induced with maltodextrin (i.e. caloric intake but no sweet taste) and a flavour/taste conditioning was induced with saccharin (i.e. no caloric intake but sweet taste).

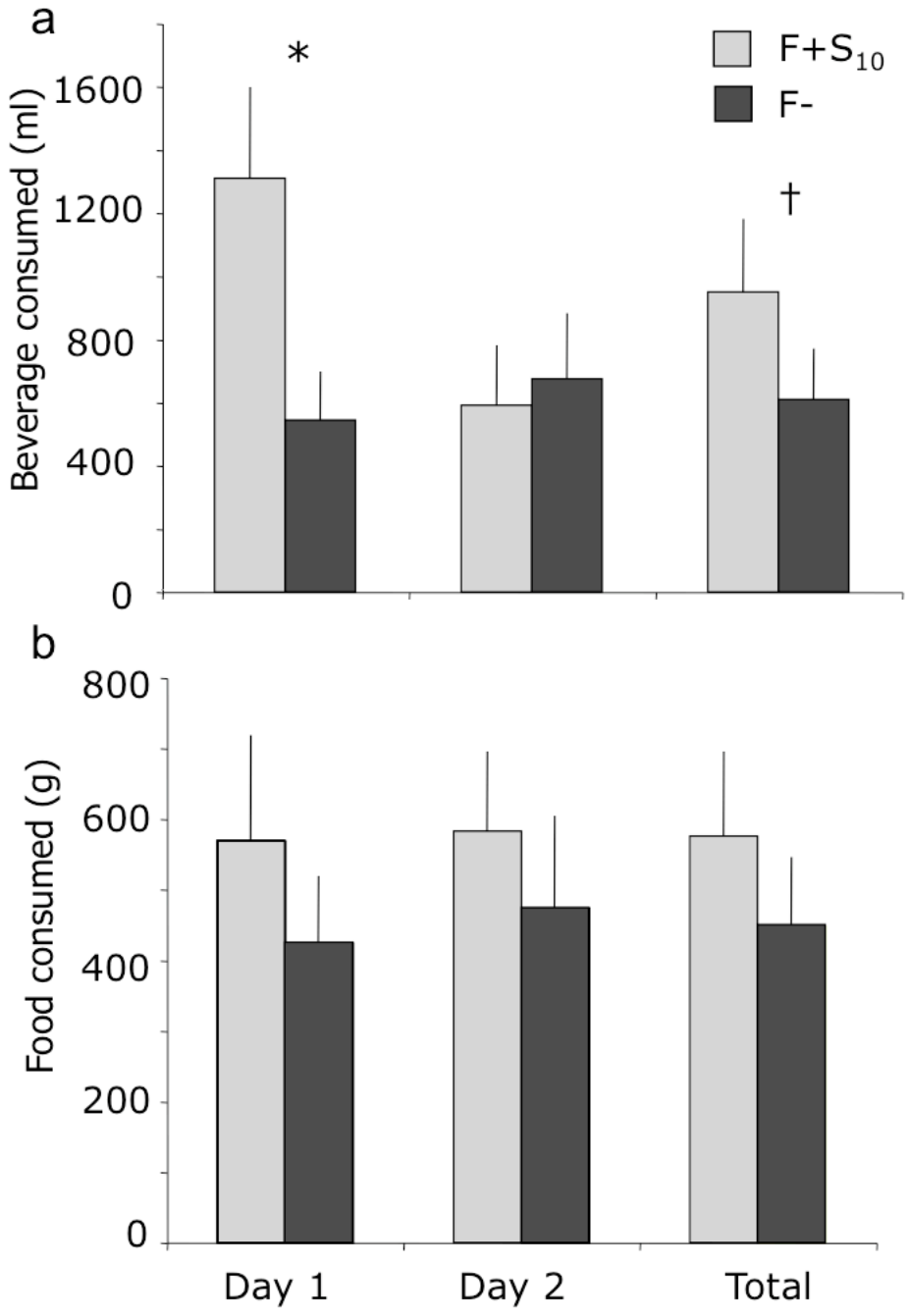

Figure 3. Quantity of beverage (ml) and food (g) consumed during the two-choice drinking (a) and feeding tests (b), respectively. These tests were carried out during the week following the conditioning period in Experiment 1B (the $F+S_{10}$ flavour was associated with 10% sucrose during conditioning while the F- flavour was presented alone in the water). Flavoured beverages and meals were presented in the absence of reinforcement during the two-choice tests. Data are presented with means and standard errors. An asterisk indicates a significant difference between two treatments ($P < 0.05$).

4.1. Experimental paradigm.

2.1.1. Habituation. The same procedure as in Experiment 1 was followed.

4.1.2. Conditioning sessions. The pigs were subjected to 12 one-tank training sessions (from 09:00 h to 16:00 h) with the flavoured solutions. On the first day, the animals received orange-flavoured solutions, on the second day, they received cinnamon-flavoured solutions and on the third day, they received thyme-flavoured solutions. Each day, a third of the animals received F+m treatment (maltodextrin added in the flavoured solution), a third of the animals received F+s treatment (saccharin added in the flavoured solution), and the other third received F- treatment (no substance added). The quantity of beverage available during conditioning sessions has been adjusted according to the mean amount of water consumed during the habituation period to ensure that the animals were not in a water-deprived situation at the end of the conditioning session. At 16:00 h, the tanks were removed and refusals were weighted.

4.1.3. Two-choice tests. The week following conditioning, the animals were subjected to two-choice tests. For the first three days, two-choice feeding tests were conducted. On Day 1, the pigs were given the choice between the orange-flavoured meal and the thyme-flavoured meal. On Day 2, they received the cinnamon-flavoured meal and the orange-flavoured meal. On Day 3, they were given the choice between the thyme-flavoured meal and the cinnamon flavoured-meal. A two-party trough containing 1 kg of the two meals was presented at 09:30 h to the animals. After 30 min, the trough was removed and refusals were weighted. The animals had free access to water. For the next three days, two-choice drinking tests were conducted. On day 4, the pigs were given the choice between the orange-flavoured solution and the thyme-flavoured solution from 09:00 h to 16:00 h. On Day 5, they received the cinnamon-flavoured solution and the orange-flavoured solution. On Day 6, they were given the

choice between the thyme-flavoured solution and the cinnamon-flavoured solution. During the drinking tests, the pigs had no access to the running water circuit but only to the two experimental drinking troughs connected to the tanks that contained 4 L of flavoured solutions. No sweet compound was added in the solutions during the tests. At 16:00 h, the tanks were removed and refusals were weighted. Meal and beverage distribution in the troughs and tanks were counterbalanced over days and between animals to avoid any laterality bias.

4.2. Results

During the habituation phase, the animals consumed 2.18 ± 0.21 L of water per day. No laterality bias was found since there was no difference in the average amount of water consumed from the right or left drinking trough (right trough: 1.27 ± 0.11 L, left trough: 0.91 ± 0.1 L, z = 1.01, $P = 0.31$). There was no difference in the average amount of each flavoured solution consumed during conditioning (Orange: 2.27 ± 0.18 L, Thyme: 2.21 ± 0.19 L, Cinnamon: 1.95 ± 0.24 L, $Chi^2 = 1.56$, $P = 0.46$).

4.2.1. Consumption during conditioning sessions. In average, the flavoured solution reinforced with saccharin (F+s: 1.56 ± 0.14 L; Figure 4) was significantly less consumed than the flavoured solution reinforced with maltodextrin (F+m: 2.53 ± 0.04 L, z = 2.67, $P = 0.008$) and the control flavoured solution (F-: 2.34 ± 0.07 L, z = 2.66, $P = 0.008$). The flavoured solution reinforced with maltodextrin tended to be more consumed than the control flavoured solution (z = 1.72, $P = 0.09$). For each conditioning session, the consumption of the flavoured solution associated with maltodextrin was not significantly different from the consumption of the control flavoured solution, whereas the flavoured solution associated with maltodextrin treatment was significantly more consumed than the flavoured solution associated with saccharin during the first and the last conditioning session (Figure 5). The flavoured solution

associated with saccharin was significantly less consumed than the control solution, but only during the first conditioning session.

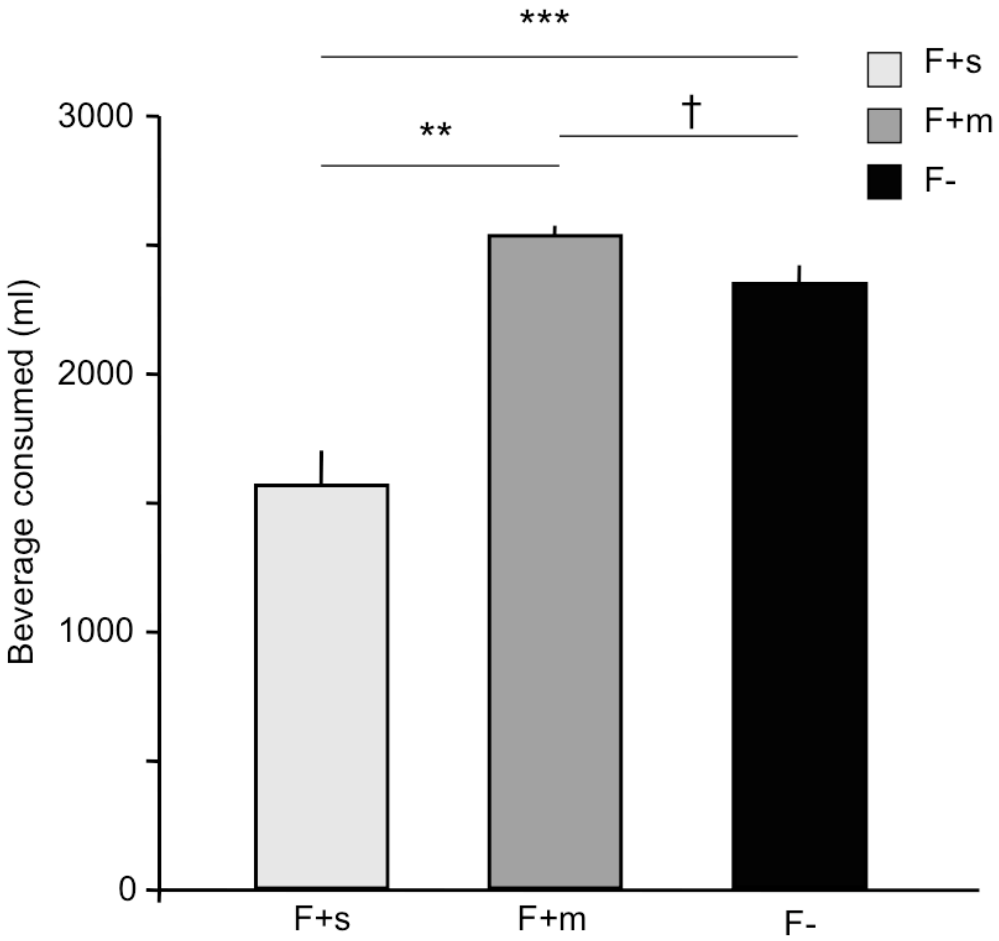

Figure 4. Mean amount of beverage (ml) consumed during the conditioning period. During the conditioning period, the animals were given a flavoured beverage added with 0.37 % saccharin (F+s), 2.25 % maltodextrin (F+m) or no additive (F-). Data are presented with means and standard errors. The following symbols are used: † $P < 0.1$, ** $P < 0.01$, ***$P < 0.001$.

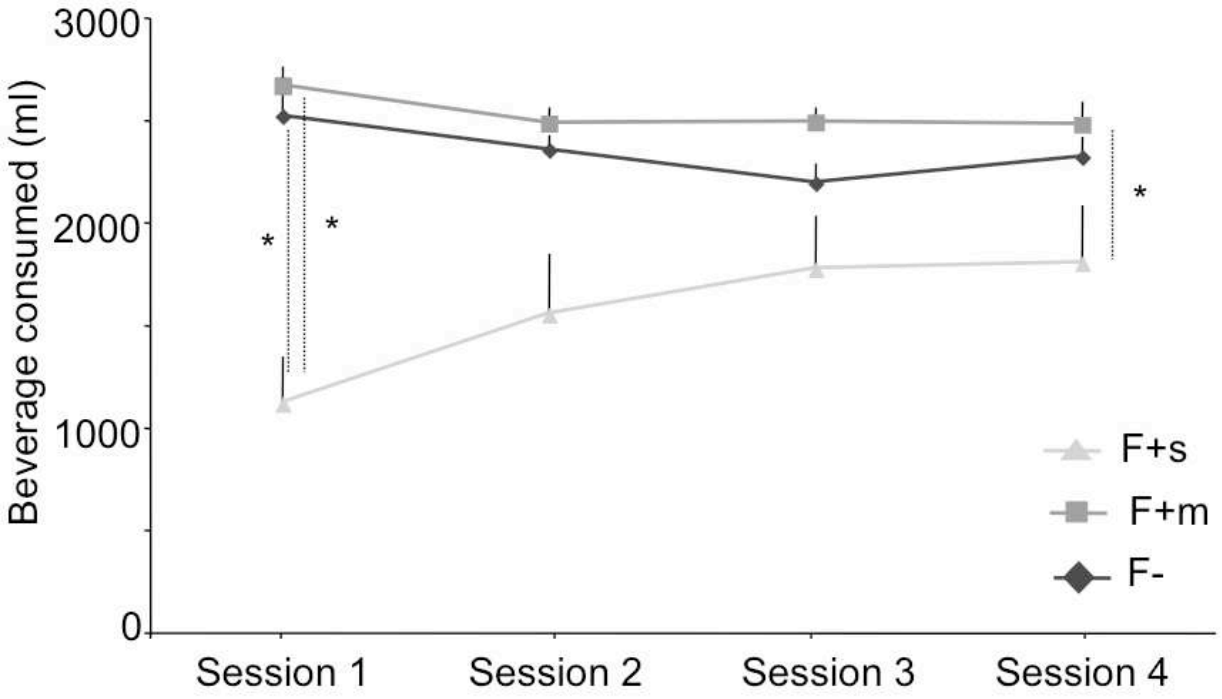

Figure 5. Quantity of beverage (ml) consumed during the four conditioning sessions. During the conditioning period, the animals were given a flavoured beverage added with 0.37 % saccharin (F+s), 2.25 % maltodextrin (F+m) or no additive (F). Data are presented with means and standard errors. The following symbols are used to indicate a significant difference between two treatments during a particular conditioning session (i.e. inter-treatments/intra-sessions comparison): *$P < 0.02$. No significant differences was found between two conditioning sessions for the same treatment (i.e. intra-treatments/inter- sessions comparison; $P < 0.02$). Bonferroni corrections were applied for both statistical analyses.

4.2.2. Consumption during two-choice tests. In average, over the three days of two-choice drinking tests, the animals exhibited no clear-cut preference (Figure 6a). We noted however that the flavoured solution previously reinforced with maltodextrin (F+m) during conditioning tended to be preferred over the control solution (F-) since the consumption of F+m was approximately 107% higher than the consumption of F- (F+m: 67% vs. F-: 33% of the total

amount consumed). Similarly, F+m tended to be preferred over the flavoured solution associated with saccharin during training, since the consumption of F+m was approximately 35% higher than the consumption of F+s (F+m: 57% vs. F+s: 43% of the total amount consumed). In average, over the three days of two-choice feeding tests, the animals exhibited no clear-cut preference (Figure 6b).

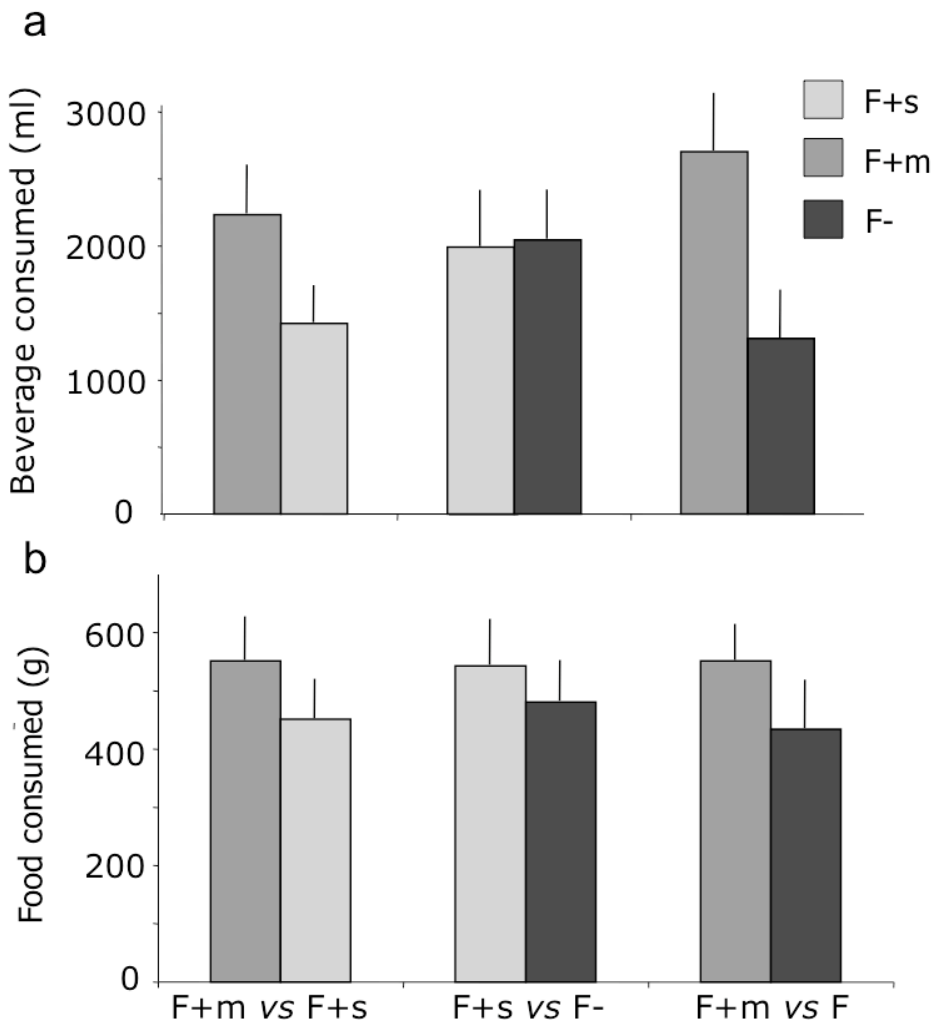

Figure 6. Quantity of beverage (ml) and food (g) consumed during the two-choice drinking (a) and feeding tests (b), respectively. These tests were carried out during the week following the conditioning period in Experiment 2. During the conditioning period, the animals were given a flavoured beverage added with 0.37% saccharin (F+s), 2.25% maltodextrin (F+m) or no additive (F-). Flavoured beverage and meal were presented in the absence of reinforcement during two-choice tests. Data are presented with means and standard errors.

5. General Discussion

5.1. Combination of taste and nutrient reinforcements is more reinforcing that taken separately in flavour preference conditioning.

In Experiment 1, six one-tank conditioning sessions with 10% sucrose (400 kcal/l) but not three one-tank conditioning sessions with 1.125% sucrose (45 kcal/l) induced a flavour/nutrient-taste preference. Moreover, the 10% sucrose solution was consumed significantly more than the unsweetened solution from the second conditioning session, and this preference persisted all along the following five conditioning sessions. This result may indicate that the determinant factor for the induction of a preference by sucrose is the concentration rather than the number of conditioning sessions, which is in accordance with previous studies reporting that conditioned preferences can be acquired rapidly (Ackroff et al., 2009; Bonacchi et al., 2008; Gilbert et al., 2003), even with a single and brief pairing of a novel flavour with the reinforcer (Myers, 2007). Consequently we assume that 10% but not 1.125% sucrose induces a flavour preference in pigs. These results are in accordance with studies in rats, which reported a clear-cut preference during two-choice tests for a flavour that has been paired with 2% to 11% sucrose (Bonacchi et al., 2008; Gilbert et al., 2003; Reilly and Trifunovic, 2000; Warwick and Weingarten, 1996). Ackroff and Sclafani (2011) also reported that rats elicited strong preference for water added with 11% sucrose over water during two-choice tests.

Sucrose being a sweet and high-caloric (4kcal/g) carbohydrate, its use as a reinforcer induces both gustative (sweet taste) and visceral (caloric intake) positive consequences and triggers the establishment of a flavour/nutrient-taste preference. In this case, it is difficult to determine which reinforcing pathway is the most important for the acquisition of a preference: caloric intake or palatability. To our knowledge, few studies investigated the relevance of the

sweet taste of sucrose to condition flavour preference in the absence of caloric intake, probably because of methodological limitations, and we cannot assert that the sweet taste of sucrose alone would be sufficient to enable the acquisition of a strong flavour preference. On the contrary, numerous studies used intragastric infusions of sucrose to investigate the efficiency of its caloric load in absence of any sweet taste. These studies have shown that rats preferred a flavoured solution that has been paired with intragastric infusions of 32% or 16% sucrose to a flavoured solution paired with intragastric infusions of water (Azzara and Sclafani, 1998; Sclafani et al., 2010). Sclafani and Glendinning (2005) found similar results with intragastric infusions of 16% sucrose in mice. These findings suggested that calories might be sufficient to condition a flavour preference using sucrose, even in the absence of sweet taste.

In the second experiment aimed at assessing the independent effects of palatability and caloric intake in flavour preference conditioning using saccharin (i.e. sweet taste but no caloric intake) and maltodextrin (i.e. caloric load but no sweet taste) respectively, the flavoured solution reinforced with saccharin was significantly less consumed during conditioning than the flavoured solution reinforced with maltodextrin and the control flavoured solution. Such result suggests that pigs can perceive the taste of 0.37% saccharin but that it is probably not perceived as sweet, which may explain that we failed to condition a flavour/taste preference. Some studies, however, showed that rats and mice elicit clear-cut preference for saccharin solutions over water at concentrations ranging from 0.002 to 0.62% (Le Pen et al., 2002; Sclafani et al., 2010), and learn conditioned flavour preference induced by 0.15% saccharin (Navarro and Cubero, 2003). Although electrophysiological studies in pigs reported that 0.03% saccharin elicits low chorda tympani (CT) and glossopharyngeal nerve (NG) response, indicating that saccharin is not well perceived at this concentration (Danilova et al., 1999; Hellekant and Danilova, 1996, 1999), pigs exhibit preference for saccharin solution over wa-

ter at concentrations ranging from 0.08 to 1.83% (Baldwin, 1976; Glaser et al., 2000). These findings suggest that 0.37% saccharin should be perceived as sweet by pigs and was supposed to be an attractive stimulus to condition flavour/taste preference.

Our inability to induce a flavour preference with 0.37% saccharin may be explained by methodological discrepancies between our paradigm and that of Baldwin (1976) or Glaser et al. (2000). In those studies, the saccharin solution and the water were unflavoured, whereas both the sweet and unsweetened solutions were flavoured in our study. One may hypothesise that the sweet compound was not sufficiently reinforcing to overcome the neophobia toward or the dislike of the flavour. In a complementary study, however, we demonstrated that a 0.37% saccharin solution was not preferred over water during two-choice tests (saccharin solution: 1.50 ± 0.32 L vs. water: 1.70 ± 0.25 L, unpublished data), suggesting that saccharin is not attractive even in the absence of flavour. Consequently, we assume that the failure to condition flavour/taste preference using saccharin is due to the unattractive taste of saccharin rather than to the neophobia for the flavours, suggesting that saccharin is not an efficient candidate to condition flavour/taste preference in pigs, contrary to rodents.

Although no significant preference was found during two-choice drinking tests, the consumption of the flavour that was associated with maltodextrin during conditioning was approximately 107 % higher than that of the control flavour. Some studies demonstrated that rats preferred a flavoured solution that was previously added with 16% but not with 8% or 2% maltodextrin, even after 12 one-bottle training sessions (Dwyer and Iordanova, 2010; Dwyer and Quirk, 2008). Dwyer (2008) also assessed the consumption of water added with maltodextrin at various concentrations and found that the consumption of 8% and 16% maltodextrin solutions was higher than that of 2% and 4% maltodextrin solutions. According to these results, it could be assumed that the concentration of maltodextrin used in the current study (i.e. 2.25%) was too low to condition a clear-cut preference, albeit a slight preference was

observed. As assumed by Dwyer and Quirck (2008), the consumption of maltodextrin solution during training may have been insufficient to support a conditioned preference based only on positive post-ingestive consequences. In our study, the mean amount of 2.25% maltodextrin solution consumed per conditioning session represented a total of 228 kcal. During these sessions, which lasted from 09:00 h to 16:00 h, the animals received a meal that represented a total of 2800-3500 kcal. As a result, the post-ingestive reinforcing effect of maltodextrin might have been in competition with or just overlapped by the stronger post-ingestive reinforcing effect of food and may explain the absence of clear-cut preference for maltodextrin during subsequent two-choice drinking tests. To avoid this interaction between the caloric load supplied by the reinforcer in itself and that supplied by the food, the paradigm should separate feeding time and conditioning sessions. This, associated with a higher maltodextrin concentration (e.g. 14-16%) would likely result in a successful flavour/nutrient preference conditioning.

Literature reports some discrepancies between species about the perception of maltodextrin. In rats, maltodextrin has a palatable taste but its taste alone is insufficient to condition flavour preference in the absence of post-ingestive effects (Dwyer, 2008), whereas in humans, maltodextrin is perceived as an almost flavourless compound. As the scientific data on the sensory perception of maltodextrin by pigs are lacking, it would be useful to ensure that maltodextrin does not taste sweet to pigs to determine whether or not the preference for the flavour paired with maltodextrin could be only attributed to caloric supply and be so called a flavour/nutrient conditioning, as suggested by Warwick and Weingarten (1994). An alternative efficient method to ensure that caloric load is responsible for the acquisition of a flavour preference in our study would be to pair the flavour with intragastric infusions of energy. This paradigm has been widely used in rats and enables to develop strong flavour/nutrient preferences (e.g. 16% maltodextrin intragastric infusions in Touzani and Sclafani, 2005,

2007), whilst avoiding taste interactions, as the response is only based on the energetic and not oral evaluation of the stimulus.

All together, the results of the present study indicate that: (1) the combination of sweet taste and caloric intake induces flavour preference in pigs (10% sucrose, Experiment 1B); (2) post-ingestive consequences might be sufficient to condition a flavour preference in the absence of sweet taste (maltodextrin, Experiment 2) but; (3) as saccharin appears to be unattractive to pigs in the current study, we cannot assert that sweet taste alone is sufficient to induce a flavour preference. Our results are in accordance with the study of Yeomans et al. (2008) who induced flavour/taste, flavour/nutrient and flavour/taste-nutrient preferences in humans, using aspartame, maltodextrin and sucrose, respectively. Increased intake of the flavours that were associated with sucrose (sweet taste and caloric load) and maltodextrin (caloric load) during conditioning was observed between pre- and post-conditioning two-choice tests, whereas no change was observed for the flavour associated with aspartame (sweet taste), suggesting that a caloric reinforcement is sufficient to induce a consumption increase of the conditioned flavour. More precisely, Warwick and Weingarten (1994) have shown that rats that consumed two flavoured solutions having equal caloric loads but different palatability (i.e. 20% glucose *vs* 20% glucose plus 0.4% acid citric), subsequently preferred the more palatable solution (i.e. glucose alone). This finding indicates that, in a situation where animals received similar caloric loads, a palatability advantage (e.g. sweet taste) induce a stronger flavour preference, suggesting that sweet taste enhances the effect of nutrient supply. This might explain that the pigs exhibit a preference that is more marked with 10% sucrose (sweet taste) than with maltodextrin (no sweet taste) in this study. Unfortunately, the mean amount of calories ingested per conditioning session in Experiments 1B and 2 were not equal (10% sucrose: 444 kcal *vs* maltodextrin: 228 kcal), and we cannot thereby determine precisely if the stronger preference for 10% sucrose compared to maltodextrin was due to a higher caloric intake or

to the combination between caloric intake and sweet taste, as suggested by Warwick and Weingarten (1994). Using maltodextrin and sucrose at the same concentration and during equivalent length of training would help to answer that question. Furthermore, the use of another artificial low-energy sweetener might provide new insights into the importance of sweet taste in flavour preference conditioning, although some studies reported that flavour/nutrient (ethanol) preferences were still stronger than flavour/taste (saccharin) preferences (Fedorchak and Bolles, 1987). For instance, Birch et al. (1990) managed to develop conditioned preference in human children. After eight pairs of conditioning trials, the infants increased their preference for a flavoured beverage previously paired with 14% low glucose maltodextrin (LGM) (155 kcal/150 ml) over a flavoured beverage paired with aspartame (3-5 kcal/150 ml).

5.2. Weak robustness of the preference makes difficult its transposition to solid food with complex organoleptic properties.

Although 10% sucrose and, to a lesser extent, 2.25% maltodextrin conditioned a preference for a flavoured beverage, two major issues appeared in the present study and may complicate the use of such conditioning as a method to decrease food neophobia during food transition in growing pigs.

During the first two-choice drinking test with non-reinforced flavoured solutions in Experiment 1B, the animals exhibited clear-cut preferences for the flavoured beverage that was previously associated with 10% sucrose. However, on the second test session, the preference did not persist, indicating that it was not robust and not persistent to extinction. Dwyer et al. (2009) showed that a flavour preference conditioned via 16% sucrose did not extinguish over repeated two- and one-bottle tests. Numerous studies also reported that conditioned flavour preferences, and especially flavour/nutrient conditioning, are very resistant to extinction and persist through several weeks of non-reinforced test sessions (Ackroff et al., 2009; Drucker et

al., 1994; Elizalde and Sclafani, 1990). According to the assumption that extinction is rather caused by new learning than by destruction of the acquired preference learning (Bouton, 2004; Delamater, 2004), the pigs may have learned that the reinforcement that was present in the flavoured solution during conditioning was absent in the subsequent two-tank tests, and consequently stopped responding in the tests. In his review, Bouton (2004) reported that there is a correlation between the extinction rate of a conditioned learning and the accumulated time of exposure with the non-reinforced solution during subsequent two-choice tests. That is, longer the two-choice test session with non-reinforced solutions lasts, quicker the animal stops responding (Haselgrove and Pearce, 2003). In the present study, as the test sessions were long lasting (from 09:00 to 16:00 h per session), the accumulated time of exposure with the non-reinforced solutions was high. As a result, the pigs stopped responding very quickly, that is after only one exposition with the non-reinforced solution.

As the animals of the present experiment received a meal at the beginning of the sessions, they were not hungry during conditioning and tests sessions. Some studies highlighted the impact of hunger state on the robustness of the conditioned flavour preference. In rats, the preference for a flavour previously paired with sucrose persists across repeated choice tests with non-reinforced solutions when the animals were sated during the conditioning or the test sessions (Delamater, 2007; Harris et al., 2004). On the contrary, after a training period where human participants received a flavoured solution added with sucrose and another non-supplemented flavoured solution, the liking of the sucrose solution increased more when the participants were trained and tested hungry than when trained and tested sated. As for flavour/taste learning, Mobini et al. (2007) reported that "liking" rating of humans for an aspartame solution increased regardless of whether the participants were hungry or sated, whereas Brunstrom and Fletcher (2008) reported that an increased liking of a flavour previously paired with saccharin only occurred in hungry participants. In rats, Fedorchak and Bolles

(1987) have found that hunger does not enhance the acquisition of a flavour/taste conditioned flavour preference induced by saccharin, but does enhance a flavour/nutrient preference conditioned by ethanol. Although contradictory, those findings suggest that hunger state plays a predominant role in the acquisition of a preference induced by taste and/or nutrient reinforcement and that further works are needed to investigate the role of hunger in animals for the onset of a conditioned preference.

Despite the pigs elicited preferences during two-choice drinking tests, no clear-cut preference was observed during two-choice feeding tests both in Experiment 1B and 2, illustrating the difficulty to transpose a flavour preference acquired via a beverage added with carbohydrate and/or sweetener to solid food. As the conditioning was not robust, it is possible that the reinforcing value of the food in itself was much greater than the reinforcing value of the conditioned flavour. Consequently, the flavour and the complex sensory characteristics of the food might have overshadow the conditioned flavour, leading the animals to show no preference. Our results may also indicate that animals' food choices may be influenced not only by the concentration of the compound added in food but also by the interactions between the food and the compound (Meunier-Salaün and Picard, 1996). Flavour perception is mediated by different sensory systems, including olfactory, gustatory and trigeminal (e.g. tactile, thermal) systems. As a result, oral processing of foods results from a complex interaction between texture, olfactory and gustatory stimuli (Roudnitzky et al., 2011), and each sensory characteristic of a particular food is likely to modify the perception of its other sensory features. For instance, some studies highlighted the relationship between food texture and odour intensity perception. Roudnitzky et al. (2011) have shown that the perception of odour intensity is enhanced by increasing levels of oral stimuli viscosity (milk thickened or not), while Weel et al. (2002) and Visschers et al. (2006) reported that the consumption of food with increased viscosity (water, custard, protein gels) decreased subsequent odour intensity perception. As a

result, the flavour may have been perceived quite differently whether it was added in water or in a solid meal, with higher viscosity.

6. Conclusions

To conclude, 10% sucrose appears to be the most efficient reinforcer compared to 2.25% maltodextrin or 0.37% saccharin for the acquisition of a clear-cut conditioned flavour preference induce via beverage in pigs. This finding highlights the importance of the combination between hedonic gustatory and visceral stimulations for the acquisition of conditioned preference, although a simple visceral reinforcement, via maltodextrin, may also be sufficient to condition a flavour preference in the absence of sweet taste. Using maltodextrin at a higher concentration and in a paradigm that separate feeding time and conditioning sessions might enable to enhance the reinforcing value of the caloric load and the strength of the preference. On the contrary, saccharin is obviously not a good candidate for the induction of a flavour/taste preference, as it appears to be unattractive to pigs. The use of another artificial low-energy sweetener might provide new insights into the importance of sweet taste in flavour preference conditioning. Finally, two hypotheses might explain our inability to transpose the beverage-induced preference to solid food: (1) the reinforcing value of the food in itself was much greater than the reinforcing value of the conditioned flavour and/or, (2) the flavour and the complex sensory characteristics of the food have overshadowed the conditioned flavour. Further studies are needed to investigate the complex interactions between the food sensory characteristics and enable the use of conditioned flavour preference as a method to reduce food neophobia exhibited by growing pigs during food transition.

Acknowledgements

The study was supported by a grant from the Brittany Region (Région Bretagne) and INRA (Department of Human Alimentation; Department of Animal Physiology and Livestock Sys-

tems). The authors wish to thank Phodé (Albi Terssac, France) for providing the essential oils used in this study. We are grateful to Sylvie Guérin for her participation to the study and to the staff of the swine facility, especially Patrick Touanel and Fabien Guérin for their cooperation in the study and for care of the animals.

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CHAPITRE III

Préférences Spontanées pour ou Conditionnées *via* le Saccharose :

Comparaison entre Stimulations Caloriques et/ou Gustatives

CHAPITRE III : PRÉFÉRENCES SPONTANÉES POUR OU CONDITIONNÉES VIA LE SACCHAROSE : COMPARAISON ENTRE STIMULATIONS CALORIQUES ET/OU GUSTATIVES

Le chapitre précédent a permis de mettre en évidence l'efficacité du saccharose 10% pour le développement de préférences alimentaires conditionnées, au détriment de la maltodextrine et de la saccharine. Ces résultats pourraient souligner l'importance d'une combinaison entre renforcement gustatif et renforcement calorique pour l'acquisition de préférences. Cependant, des questions restent en suspens. Bien que le saccharose combine apport énergétique et goût sucré, l'expérience ne permet pas d'affirmer que l'association de ces deux facteurs est nécessaire pour le conditionnement d'une préférence. En effet, nos résultats montrent également une légère augmentation de la consommation de la solution associée à la maltodextrine, suggérant qu'un apport énergétique pourrait suffire à induire une préférence. L'association entre apport énergétique et goût plaisant est-elle donc vraiment nécessaire à l'établissement d'une préférence conditionnée ou un apport énergétique seul est-il suffisant ?

Dans ce chapitre, nous tenterons de répondre à cette question *via* deux approches expérimentales complémentaires. Dans la *partie 1*, nous utiliserons le saccharose comme stimulus renforçateur (apport énergétique seul *vs* apport énergétique et goût sucré) dans une expérience de conditionnement préférentiel sur l'eau de boisson. Dans le *chapitre II*, les périodes de conditionnement s'étalaient sur une longue durée (*i.e.* 7 h/jour), et chevauchaient l'heure du repas. Il est donc possible que l'effet renforçateur des calories apportées par la maltodextrine ait été minimisé par l'apport calorique du repas. Afin de s'affranchir de l'apport énergétique dû à l'aliment, nous diminuerons la durée des sessions de conditionnement afin de séparer les périodes de conditionnement et de repas. En accord avec la littérature et les résultats du *chapitre II*, nous nous attendons à ce qu'un apport énergétique soit suffisant pour induire une préférence, mais qu'un apport calorique couplé à un goût sucré induise une préférence plus marquée et plus robuste. Ces préférences seront caractérisées par une augmentation des quantités ingérées et une modification des choix ultérieurs.

Dans une seconde partie, nous tenterons de déterminer dans quelle mesure les réseaux cérébraux impliqués dans la régulation hédonique du comportement alimentaire et dans le traitement central des informations olfacto-gustatives sont recrutés lors la perception orale et/ou viscérale de sucrose. Nous essayerons notamment de comparer l'effet d'une perception orale et viscérale simultanée à celle d'une exposition découplée, soit orale, soit viscérale, afin de déterminer si la synergie entre les signaux oraux et postoraux est nécessaire à une réponse cérébrale dans les réseaux cités plus haut.

Partie 1. Préférences alimentaires conditionnées *via* le saccharose : comparaison entre renforcement calorique et/ou gustatif (article n°4)

1. Contexte et objectifs

De nombreuses espèces animales ont la capacité d'associer la flaveur d'un aliment avec les conséquences de sa mise en bouche et/ou de son ingestion pour moduler sa prise alimentaire. Lorsque la prise d'un aliment génère des conséquences appétitives (*e.g.* goût plaisant) et/ou post-ingestives (*e.g.* satiété) positives, l'organisme apprend à consommer préférentiellement cet aliment par la suite ; c'est le phénomène de préférence alimentaire conditionnée. Des études suggèrent que les renforcements oral et viscéral ne sont pas aussi efficaces pour le conditionnement de préférences alimentaires, en fonction des espèces. L'objectif de l'étude était d'étudier l'acquisition de préférence alimentaire conditionnée chez le porc *via* l'administration par voie intestinale (apport calorique) ou orale (goût sucré et apport calorique) du saccharose 16% utilisé comme renforcement positif. Nous tenterons de déterminer si un apport calorique est suffisant pour induire une préférence alimentaire en l'absence de goût sucré, et de comparer la robustesse de cette préférence avec une situation où l'apport calorique est couplé avec le goût sucré du saccharose pendant le conditionnement.

2. Méthodes

Au total, neuf porcs juvéniles ont été soumis à quatre sessions de trois jours de conditionnement. Durant trois jours successifs, ils recevaient pendant 1 h trois solutions aromatisées (thym, orange, cannelle) comme stimulus conditionnés (CS). La solution CS++ était additionnée de saccharose 16% et couplée à une infusion i.d. d'eau, la solution CS+ était couplée avec une infusion i.d. de saccharose 16% et la solution CS– était associée à une infusion i.d. d'eau. Une et deux semaines après le conditionnement, les animaux ont été soumis pendant 1 h à trois tests de double choix alimentaires (thym *vs* cannelle, thym *vs* orange, orange *vs* cannelle) pendant trois jours consécutifs sur les solutions non renforcées. Pendant les périodes de conditionnement et les tests, les animaux étaient en état de privation d'eau. Les données de consommation, l'activité comportementale et les variables caractérisant la microstructure des séquences d'abreuvements ont été mesurées pendant l'ensemble de l'étude. L'osmolarité plasmatique a également été mesurée 15 min avant et 15 min après chaque session de conditionnement et de test pour vérifier l'impact de la privation d'eau et l'influence du traitement sur les variations d'osmolarité.

3. Résultats

Pendant le conditionnement, aucune différence de consommation entre CS++, CS+ et CS– n'a été mise en évidence $F(2,16) = 1.22$, $p = 0.32$). Les animaux ont passé moins de temps inactifs ($z = 2.66$, $p < 0.016$) et plus de temps debout ($z = 2.45$, $p < 0.016$) quand ils recevaient la solution CS++ comparée à CS+. Lorsqu'ils recevaient CS++, les porcs exploraient d'avantage l'abreuvoir que lorsqu'ils recevaient CS– ($z = 2.45$, $p < 0.016$). Bien que les résultats ne soient pas statistiquement significatifs, le nombre d'épisodes d'abreuvement, et le nombre de pauses intra-épisode étaient 36% et 49% moins important pendant le conditionnement CS++ comparé au conditionnement CS–. Aucun effet du traitement, de la prise d'eau de boisson ni de l'interaction n'a été mis en évidence pendant le conditionnement ($p > 0.05$). Pendant les tests de choix, les porcs n'ont montré aucune préférence significative. Néanmoins, pendant la 1ère session de test, les porcs montraient une préférence modérée pour la solution CS++ (57% de la consommation totale) comparée à CS+. Lors des tests CS++ *vs* CS– ou CS+, la durée moyenne des épisodes d'abreuvement dirigés vers CS++ représentait 64% de la durée des abreuvements. Lors des tests CS++ *vs* CS–, le temps total passé à boire CS++ représentait 57% du temps total passé à boire. Enfin, l'osmolarité était plus élevée après qu'avant l'heure d'accès à l'eau ($t(8) = 2.51$, $p < 0.05$).

4. Conclusions

Bien qu'aucune préférence alimentaire n'ait été mise en évidence durant les tests de double choix alimentaires, la perception orale du saccharose 16% pendant le conditionnement a induit des changements dans la distribution des activités comportementales, une augmentation des réponses motivationnelles et une modification de la microstructure des abreuvements. Ces résultats soulignent l'importance de la perception orale du sucre pour les processus de sélection alimentaire chez le porc, alors que l'apport calorique semble ne jouer qu'un rôle secondaire. De plus, il est possible que la complexité de l'apprentissage qui consistait à réaliser trois associations entre flaveur et renforcement pourrait avoir minimisé l'acquisition de la préférence pendant le conditionnement. La privation en eau pendant les tests de préférence, quant à elle, pourrait être responsable de l'absence d'expression de la préférence, les animaux privés en eau étant alors motivés pour boire sans considération pour la valeur hédonique des boissons présentées. Des études complémentaires sur des animaux non assoiffés et utilisant des apprentissages plus simples seraient donc nécessaires pour vérifier ces hypothèses et mettre en évidence l'acquisition et l'expression de préférences alimentaires chez le porc.

Article n°4

An attempt to condition flavour preference induced by oral and/or post-oral administration of 16% sucrose in pigs

Caroline Clouard[a,b,c], Florence Loison[a], Marie-Christine Meunier-Salaün[b,c], David Val-Laillet[a]

(Soumis le 04 septembre 2012 à *Physiology & Behavior*)

[a]*INRA, UR1341 Alimentation, Adaptations Digestives, Nerveuses et Comportementales, F-35590 Saint Gilles, France*

[b]*INRA, UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, F-35590 Saint Gilles, France*

[c]*Agrocampus Ouest, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Élevage, F-35000 Rennes, France*

Abstract

The present study investigated the acquisition of conditioned flavour preferences in pigs using the caloric value and/or sweet taste of sucrose. Nine water-deprived juvenile pigs were given four three-day conditioning sessions during which they received flavoured solutions as conditioned stimuli (CS). The CS solutions were paired with three treatments that generated a gustatory and/or a caloric reinforcement (US). The CS++ solution was added with 16% sucrose and paired with an intraduodenal (ID) infusion of water, the CS+ solution was paired with an ID infusion of 16% sucrose and the CS– solution was paired with an ID infusion of water. One and two weeks after conditioning, the water-deprived pigs were subjected to two-choice preference tests with the unreinforced CS solutions. Solutions intake, behavioural activity and some drinking parameters were measured. Despite no difference in CS intake during conditioning, the animals spent less time inactive and more time standing during CS++ than CS+ conditioning. When receiving CS++, the pigs explored the drinking trough more than when receiving CS–. Compared to the CS– condition, the numbers of drinking episodes and intra-drinking episode (IDE) pauses were also 36% and 49% lesser in the CS++ condition, but these differences were not significant. During the two-choice tests, the pigs did not show significant preferences. Nevertheless, during the first session, the pigs seemed to show a slight preference for the CS++ (57% of total intake) compared to CS+. The duration of CS++ drinking episodes represented 64% of the total duration compared to CS+ and CS–. The total time spent drinking the CS++ also represented 57% of the total time in the CS++ vs. CS– test. To conclude, although no clear-cut preferences were found during two-choice tests, the oral perception of 16% sucrose during conditioning induced changes in behavioural activities, motivational responses and microstructure of CS intake, suggesting the importance of oral food perception for food selection processes in pigs. Further studies are needed to investigate the impact of water deprivation on the expression of flavour preferences in pigs.

Keywords: sucrose, preference, calories, sweet taste, learning, cephalic phase.

Highlights

- Conditioned flavour preference induced by oral or visceral sucrose is studied.
- Oral perception of sucrose induced increased motivational responses for the flavour.
- No clear-cut preferences were found during two-choice preference tests.
- Sweet taste seemed more crucial than calories for food selection processes in pigs.
- Water-deprivation may impair the preference expression during preference tests.

1. Introduction

Rodents, primates and pigs have the ability to associate the flavour of food with its appetibility and/or the consequences of its ingestion to modulate further food intake. When food intake generates positive appetitive (e.g. pleasant taste) and/or postingestive consequences (e.g. satiety), the organism learns to preferentially consume this particular food during subsequent exposures, as referred as conditioned food preference [1, 2]. Although the oral hedonic reinforcement and the postingestive caloric reinforcement are frequently confounded during the ingestion of a particular food (e.g. highly palatable food is often paired with a substantial caloric load) [2], two types of independent learning have been described and widely studied in experimental dissociation studies, especially in rodents [3-11] and humans [12-18]. On the one hand, the flavour-taste conditioning consists in the association between an arbitrary flavour (conditioned stimulus, CS) and a flavour/taste (unconditioned stimulus, US) that already has a high hedonic value (e.g. sweet taste). On the other hand, the flavour-nutrient preference learning is induced by pairing the CS flavour with a US that triggers positive postingestive consequences (e.g. caloric load).

Although flavour-taste and flavour-nutrient preference conditionings both modify further food preferences, intake and acceptance, some authors suggested that preferences induced by a gustatory or a caloric reinforcement were mediated by different physiological mechanisms [19] and affected different components of feeding behaviour (i.e. appetitive vs. consummatory responses) [8, 9]. Moreover, it has been proposed that the behavioural responses triggered by these two types of learning responded differently to extinction and temporal parameters (for review, see [2]).

Some studies indicated that oral and postoral reinforcements are not as efficient for the establishment of food preferences, depending on the species. In humans, a caloric reinforcement alone could be efficient to induce a strong flavour preference even in the absence of oral rein-

forcement, whereas an oral reinforcement alone seems not sufficient (e.g. sweet taste) [17]. Similarly, Capaldi and Privitera [15] reported that subjects receiving high-fat flavoured food subsequently rated the flavour more pleasant than subjects that tasted the same flavour in a low-fat food (even if the two versions of the flavoured food were not palatable, e.g. bitter taste). In rats, however, both oral or postoral reinforcements may be sufficient in themselves to result in successful flavour preference acquisition [4, 6, 9, 20, 21]. Some studies have also shown that in a situation where animals received flavoured solutions having equal caloric values but different palatability, the animals subsequently preferred the flavour previously associated with the more palatable taste [6, 9]. These findings suggest that in a situation of caloric stimulation, a palatability advantage induced stronger preferences in rats.

As several mammal species, including rodents, pigs and primates are spontaneously attracted by the sweet taste of sugars (for reviews, see [22, 23]), carbohydrates are frequently used as USs in preference conditioning studies. Sucrose, a sweet high caloric (4 kcal/g) carbohydrate, has been described as a particularly efficient US to condition flavour preference in that it induced enhanced food intake and preference in rodents [7, 24-26] and/or increased liking ratings in humans [16]. Recently, we found similar results in pigs in that adding 10% sucrose in a flavoured solution during training induced short-term preference for the flavour [27]. However, as the sucrose added in the solution was orally consumed, we were not able to determine whether the caloric value of sucrose would have been sufficient to induce a preference, even in the absence of sweet taste.

The present study investigated the development of conditioned flavour preference in pigs using the caloric value and/or sweet taste of sucrose as reinforcements. As previous studies reported that caloric reinforcement alone was sufficient to induce strong preferences in rodents and humans, we aimed at testing whether calories were sufficient to condition a strong flavour preference in the absence of sweet taste of sucrose in pigs. The second aim was to

determinate whether, in the context of stimulation by calories, a palatability advantage (i.e. sweet taste) induced a stronger flavour preference. We expected that the calories supplied by intraduodenal (ID) infusions of sucrose during conditioning would be sufficient to condition a flavour preference, but that pairing the caloric load with the perception of sweet taste would condition a more robust flavour preference, characterised by a higher resistance to short-term extinction.

2. Methods

The experiments presented in this paper were conducted in accordance with the current ethical standards of the European Community (Directive 86/609/EEC), Agreement No. A35-622 and Authorizations No. 01894 and No. 35-88. The Regional Ethics Committee in Animal Experiment of Brittany has validated the entire procedure described in this paper (R-2011-CC-01).

2.1. Animals

A total of nine Large White/Landrace × Piétrain female pigs of 27.7 ± 0.8 kg at the beginning of the study were used. The animals were fed daily at 1330 h with 1 kg of a pelleted meal composed of 23.2% wheat, 25% corn, 22.8% barley, 24.3% soybean meal, 1.11% bi-calcic phosphate, 1% carbonate, 0.5% vitamin complement, 0.4% vegetal oil and 0.4% salt. Before surgery and during the recovery week, the animals had free access to water. During the habituation, conditioning and testing periods, the animals had access to water or experimental solutions from 0930 h to 1030 h. They aslo received 300 mL of tap water right after the meal, in order to improve the animals' comfort after ingestion of the meal, which was composed of dry and solid pellets.

2.2. Apparatus

The pigs were housed in individual pens (150 × 60 × 80 cm) modified for the implementation of two-choice drinking tests. A chain was suspended in each pen to enrich the environment of the animals and to fulfil their natural disposition to play. Two drinking troughs were installed on both sides of the pen to distribute tap water or the CS solutions. The lateral drinking troughs were connected with silicone pipes to plastic tanks equipped with a valve to turn on/off the water flow. These tanks were removable and were placed high up, above the front eating trough. The room was maintained at approximately 24°C with a 13:11 h light:dark cycle.

2.3. Surgery

The pigs were implanted with a duodenal catheter for further infusions of water or sucrose solution, as previously described [28, 29], and a venous catheter for further blood sampling. Briefly, after a 24-h fasting period, the pigs were preanaesthetised with an intramuscular injection of ketamine (15–20 mg/kg, Mérial, Lyon, France). The animals were then put on isoflurane (3–5% v/v, Isoflurane Belamont, Nicholas Piramal, London, UK) anaesthesia and subjected to tracheal intubation. Analgesia was obtained by intravenous injection of a morphinic agent (Fentanyl 4 mL, 1.4 mL/min, Renaudin, Paris, France). A midline laparotomy was performed and a catheter was inserted into the proximal duodenum, tunnelled under the skin and exteriorized between the shoulders. An incision was done in the left side of the animal's neck and a catheter was inserted into the jugular vein, tunnelled under the skin and exteriorized on the top of the neck. The animals were allowed one week to recover from surgery before the beginning of the experiments. During the recovery week, the animals had free access to water and were fed at 1330 h with the standard pelleted diet.

2.4. Test solutions and treatments

The CS solutions were beverages composed of tap water flavoured with 0.025% essential oils of thyme, cinnamon or orange provided by Laboratoires Phodé (Terssac, France). At this concentration, the effect of neophobia toward the novel flavours is minimized but flavours are perceptible by pigs and there is no spontaneous preference for one of the flavours over the others (unpublished preliminary study).

The CS solutions were paired with three treatments that generated a gustatory and/or a caloric reinforcement (US). The CS++ solution (oral perception of sweet taste and calories) was added with 16% sucrose (384 kcal in 600 mL) and paired with an ID infusion of 600 mL of distilled water. The CS+ solution (600 mL, no sweet taste but calories) was paired with an ID infusion of 600 mL of 16% sucrose (384 kcal). The CS– solution (600 mL, control, no sweet taste and no calorie) was paired with an ID infusion of 600 mL of distilled water. Since we demonstrated that 10% sucrose induced only short-term preference in pigs [27], 16% sucrose was used as reinforcement in the present study, which is in accordance with literature in rodents (e.g. [30, 31]). The amount of calories provided by 16% sucrose was approximately 0.014 kcal per g of live weight, which is in accordance with the values found in studies on rats (e.g. 0.0028 kcal/g [9] ; 0.017 kcal/g [6]). The ID infusions were performed with a peristaltic pump connected to the duodenal catheter and the injection rate was 10 mL/min for 1 hour.

2.5. Procedure

2.5.1. Habituation sessions

The week following recovery from surgery, the pigs were trained to drink during limited periods of time. Each day, the animals had access to tap water via the two lateral drinking troughs connected to the tanks from 0930 h to 1030 h. Each tank contained 5 L of tap water.

At 1030 h the tanks were removed and the animals received 300 mL of tap water after the meal at 1400 h. Habituation sessions enabled to establish the 1-h water consumption baseline at 2,279 ± 126 mL per animal.

2.5.2. Conditioning sessions

During two weeks, the pigs were given four three-day conditioning sessions (from 0930 h to 1030 h) with the CS solutions. For each conditioning session, they received the CS++, CS+ and CS– solutions paired with the appropriate experimental treatments on three consecutive days, so that the associations between the CS flavoured solution (i.e. orange-, thyme- or cinnamon-flavoured solutions) and the treatment were counterbalanced between animals. At 1030 h, the tanks were removed and refusals were weighted. After tank weighing and if the pigs did not drink the 600 mL of the CS++ solution at the end of the 1-h conditioning session, the tank containing the CS++ was kept available to the pig until it drank the remaining solution to ensure that the calories amount supplied by oral (CS++) or visceral (CS+) sucrose was identical. The right/left positions of the CS solutions were counterbalanced over days and between animals to avoid any methodological bias. During the conditioning period, the animals were fed at 1330 h and received 300 mL of tap water after the meal.

2.5.3. Preference tests

The week following conditioning, the animals were subjected to three two-choice drinking tests to assess their preferences for the different CS solutions. On day 1, the pigs were given the choice between the thyme-flavoured solution and the cinnamon-flavoured solution. On day 2, the pigs were given the choice between the thyme-flavoured solution and the orange-flavoured solution. On day 3, the pigs were given the choice between the cinnamon-flavoured solution and the orange-flavoured solution. During these tests, the pigs had access to the CS solutions from 0930 h to 1030 h. At 1030 h, the tanks were removed and water refusals were

weighted. As the pigs were thirsty due to water-deprivation, as soon as one out of the two tanks was empty, the two tanks were removed to prevent the pigs from drinking the whole amount of the two CS solutions. No sucrose was added to the flavoured solutions and no ID infusion was given during these tests. The right/left positions of the CS solutions in the tanks were counterbalanced over days and between animals to avoid any laterality bias. During the testing period, the animals were fed at 1330 h and received 300 mL of tap water after the meal. The same three two-choice tests were repeated the next week to ensure that the conditioned learning was robust and did not extinct rapidly. The right/left positions of the CS solutions were alternated from the first to the second test session.

2.6. Behavioural analyses

During the 1-h conditioning sessions and the 1-h two-choice tests, behaviours were recorded using the scan-sampling method (1 observation every 1 min) and the Pocket Observer® software (Noldus, Wageningen, Netherland) installed in a pocket PC (iPAQ 214, Hewlett-Packard, Palo Alto CA, USA). The behavioural repertoire was the following: drinks, explores the drinking troughs (bites or licks the drinking trough but does not drink), explores the eating trough (bites or licks the trough although there is no food in it), explores the ground (licks, paws, rubs the ground), explores the bars (bites or licks the pen's bars), explores the chain (chews or plays with the chain), performs self-centred activity (scratches or licks its own body), urinates/defecates, and remains inactive. The right/left positions of the drinking trough used by the animal when the items "drinks" or "explores the drinking trough" were recorded were systematically specified. Four postures were also recorded: standing, sitting, kneeling down, and lying. Additionally, the behavioural tests were recorded and video-observations were carried out. Using the focal-sampling method, the following data were recorded to characterise the microstructure of CS intake: the total time spent drinking (s/h), the mean duration of the drinking episodes (s), the number of drinking episodes and the num-

ber of intra-drinking episode (IDE) pauses (number/h). A drinking episode started as soon as the animal began to drink and took end when the animal stopped drinking for at least 2 min. The 2 min interval criterion was determined using the focal-sampling method on video-observations performed during conditioning sessions. All the drinking pauses occurring during the 1-h sessions and their duration (s) were recorded. A drinking pause started as soon as the animal removed its mouth from the drinking trough. The frequency of drinking pauses according to their duration (ranging from 1 to 1490 s) was determined. The choice of the interval criterion was based on the analysis of the curve *f*(duration of the pauses) = frequency. As the inflexion point of the curve was found at 120 s, the interval criterion was set at 2 min (unpublished results). If the animal resumed drinking before the end of the 2 min pause, it was considered as part of the same drinking episode. Pauses lasting least than 2 min were thus considered as IDE pauses.

2.7. Plasma osmolarity determination

Each day of conditioning and tests, blood samples were collected from the jugular vein 15 min prior and 15 min after the 1-h access to the CS solutions in order to assess the effect of water deprivation on plasma osmolarity. Blood samples (5 mL) were collected in tubes containing 50 µL EDTA diluted in distilled water (0.8 M) and centrifuged (4000 g) at 4°C during 10 min. Plasma samples were stored at -20°C until assaying. Osmolarity was measured on the blood plasma by using an osmometer (Roebling AUTOCAL Typ13DR, Berlin, Germany).

2.8. Statistical analyses

Statistical analyses were performed with the R 2.14.1 software (The R Foundation for Statistical Computing, Vienna, Austria). Consumption data (± SEM) were expressed in mL, as means and standard errors. Solution intakes during two-choice tests were also expressed as

the mean percent intake relative to total intake (e.g. CS++ intake/total intake × 100). According to the scale of Goatcher & Church described in Kennedy and Baldwin [32], a percent solution intake from 60 to 80% indicated a moderate preference and from 80% to 100%, a strong preference for the solution. During two-choice tests, total time spent drinking, duration of the drinking episodes, number of IDE pauses and number of drinking episodes were also expressed as the mean percent value relative to total (e.g. total time spent drinking the CS++ solution/total time spent drinking × 100). Data presenting a Gaussian distribution were analysed using 2-way repeated measures analysis of variance (ANOVA) followed by pairwise comparisons where appropriate, or using paired *t*-tests. Otherwise, data were analysed using non-parametric Friedman and Wilcoxon tests. When multiple comparisons were performed, a Bonferroni correction was applied. Otherwise, the significant level for all analyses was set as $p < 0.05$.

3. Results

3.1. Conditioning sessions

During the conditioning sessions, the water-deprived animals often drank almost the whole amount of the CS solutions, i.e. 600 mL. Consequently, the average consumptions of the CS++, CS+ and CS– solutions did not significantly differ (CS++: 547 ± 22 mL, CS+: 486 ± 30 mL, CS–: 475 ± 31 mL, $F(2,16) = 1.22$, $p = 0.32$). The 2-way within subjects ANOVA showed no global effect of the conditioning session (session 1: 513 ± 25 mL, session 2: 512 ± 36 mL, session 3: 497 ± 35 mL, session 4: 489 ± 35 mL, $F(3,24) = 0.32$, $p = 0.81$), and no significant session × treatment interaction ($F(6,48) = 1.32$, $p = 0.27$). Although the mean time taken to finish the CS++, CS+ and CS– solutions during conditioning did not significantly differ (CS++: 17 ± 6 min, CS+: 31 ± 9 min, CS–: 26 ± 8 min,

F(2,16) = 2.20, p = 0.32), it worth noticing that the time taken to finish the CS++ solution was 34% shorter than that taken to finish the CS– solution.

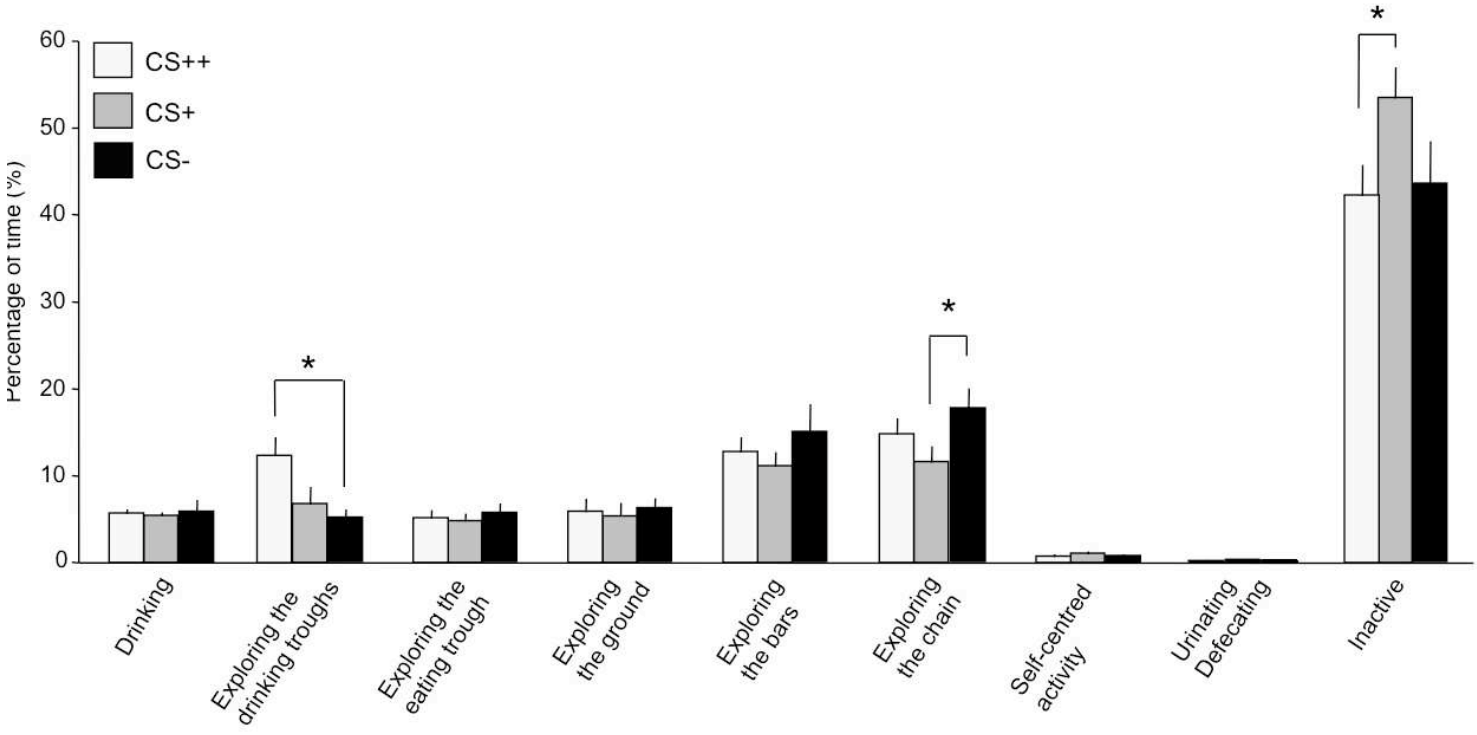

Fig. 1. Mean percentage (%) of time spent in each behavioural activity during the conditioning sessions. Data (± SEM) were an average of the four conditioning sessions. During the 1-h conditioning sessions, the animals received the CS++, CS+ or CS– solutions. The CS++ solution was added with 16% sucrose and paired with ID infusion of distilled water, the CS+ was paired with ID infusion of 16% sucrose and the CS– was paired with ID infusion of distilled water. The asterisks indicate significant differences after Bonferroni correction for multiple comparisons ($p < 0.016$).

General activity exhibited by the pigs during conditioning is shown in **Fig. 1**. The animals spent less time inactive ($z = 2.66$, $p < 0.016$) when they received the CS++ than when they received the CS+. The pigs also spent less time exploring the chain ($z = 2.52$, $p < 0.016$) and tended to spend more time inactive ($z = 1.79$, $p < 0.1$) when they received the CS+ than when they received the CS–, but this last tendency did not resist the Bonferroni correction. The

animals spent significantly more time standing during the CS++ than CS+ conditioning (CS++: 60 ± 6%, CS+: 50 ± 6%, $z = 2.45$, $p < 0.016$), and less time lying during the CS++ than CS+ conditioning (CS++: 25 ± 6%, CS+: 34 ± 7%, $z = 2.26$, $p < 0.05$). They also spent less time standing (CS+: 50 ± 6%, CS–: 57 ± 7%, $z = 2.06$, $p < 0.05$) and more time lying (CS+: 34 ± 7%, CS–: 26 ± 8%, $z = 2.34$, $p < 0.05$) during the CS+ than CS– conditioning. Those three last results did not resist the Bonferroni correction.

Although the time spent drinking the CS++, CS+ and CS– solutions did not differ ($\chi^2 = 0.17$, $p = 0.92$), the time spent exploring the drinking troughs (i.e. the trough that contained the CS or the opposite empty trough) differed between treatments ($\chi^2 = 9.31$, $p < 0.01$). More precisely, no difference was found for the time spent exploring the opposite empty trough ($\chi^2 = 0.45$, $p = 0.80$; **Fig. 2**), while the time spent exploring the trough that contained the CS+, CS+ and CS– solutions differed ($\chi^2 = 8.4$, $p < 0.016$). The pigs that received the CS++ spent more time exploring the CS trough than the pigs that received the CS– (CS: 39 ± 1%, CS++: 11 ± 3%, $z = 2.45$, $p < 0.016$), and tended to spend more time exploring the CS trough than that receiving the CS+ ($z = 1.92$, $p < 0.1$). Though, this last tendency did not resist the Bonferroni correction. Overall, the pigs also spent more time exploring the trough that contained the CS than the opposite trough, regardless of the treatment ($z = 3.82$, $p < 0.001$; Fig. 2). More precisely, the pigs that received the CS++ spent more time exploring the trough that contained the CS than the opposite trough ($z = 2.66$, $p < 0.01$), while only a tendency was observed for the CS+ ($z = 1.79$, $p < 0.1$) and CS– ($z = 1.92$, $p < 0.1$).

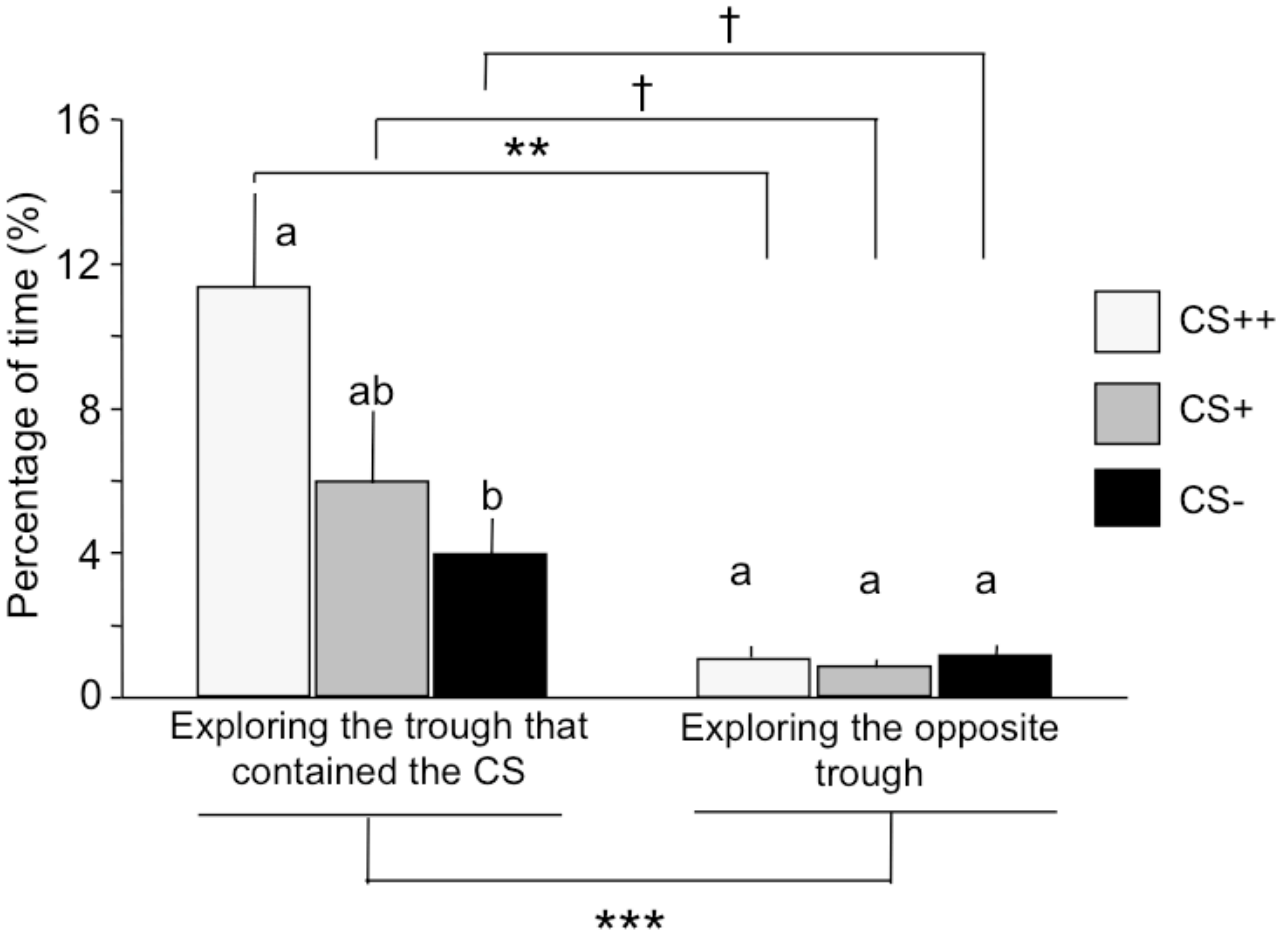

Fig. 2. Mean percentage (%) of time spent exploring the drinking troughs during conditioning sessions. Data (± SEM) were an average of the four conditioning sessions. During conditioning, only one out of the two troughs were filled with the CS, while the other trough was empty. The symbols indicate significant intra-treatment differences † $0.05 < p < 0.1$, ** $p < 0.01$, *** $p < 0.001$. Two different letters indicate a significant difference between treatments for a single behavioural item (after Bonferroni correction for multiple comparisons, $p < 0.016$).

Although no significant effect of the treatment was found for the variables characterising the microstructure of the CS intake (**Table 1**), data described a number of drinking episodes 36% lesser in the CS++ than in the CS– condition. Compared to the CS– condition, the number of IDE pauses was also 49% and 44% lesser in the CS++ and CS+ conditions, respectively. A global effect of the session was found for the mean duration of drinking episodes ($F(3,24) = 4.25$, $p < 0.05$) and the number of IDE pauses ($F(3,24) = 4.50$, $p < 0.016$). The global duration during the first session was lower than that during the second and third ses-

sions ($p < 0.05$; **Fig. 3**) and there were more pauses during the second than during the fourth session ($p < 0.05$). The 2-way within subjects ANOVA also showed a weak global effect of the session on the total time spent drinking ($F(3,24) = 2.92$, $p < 0.1$), in that the animals spent less time drinking during the first than the second and third sessions ($p < 0.05$). Except for the number of IDE pauses, all post-hoc comparisons, however, did not resist the Bonferroni correction.

Table 1. Variables characterising the microstructure of CS intake during conditioning. Data (± SEM) were averaged on the four conditioning sessions. During the 1-h conditioning sessions, the animals received the CS++, CS+ or CS− solutions. The CS++ solution was added with 16% sucrose and paired with ID infusion of distilled water, the CS+ was paired with ID infusion of 16% sucrose and the CS− was paired with ID infusion of distilled water. A drinking episode started as soon as the animal began to drink, and took end when the animal stopped drinking for at least 2 min. If the animal resumed drinking before the end of the 2 min pause, it was considered as part of the same drinking episode. A pause that lasted less than 2 min was considered as an intra-drinking episode (IDE) pause.

Variables	Conditioned stimuli			Statistical analyses	
	CS++	CS+	CS−		
Total time spent drinking (sec)	142 ± 21	120 ± 18	130 ± 23	$F(2,16) = 0.29$	$p = 0.76$
Duration of the drinking episodes (sec)	112 ± 16	91 ± 24	95 ± 21	$F(2,16) = 0.45$	$p = 0.65$
Number of drinking episodes	1.95 ± 0.30	2.78 ± 0.55	3.06 ± 0.89	$\chi^2 = 4.27$	$p = 0.12$
Number of IDE pauses	8.78 ± 2.18	9.53 ± 1.26	17.07 ± 5.39	$F(2,16) = 1.77$	$p = 0.20$

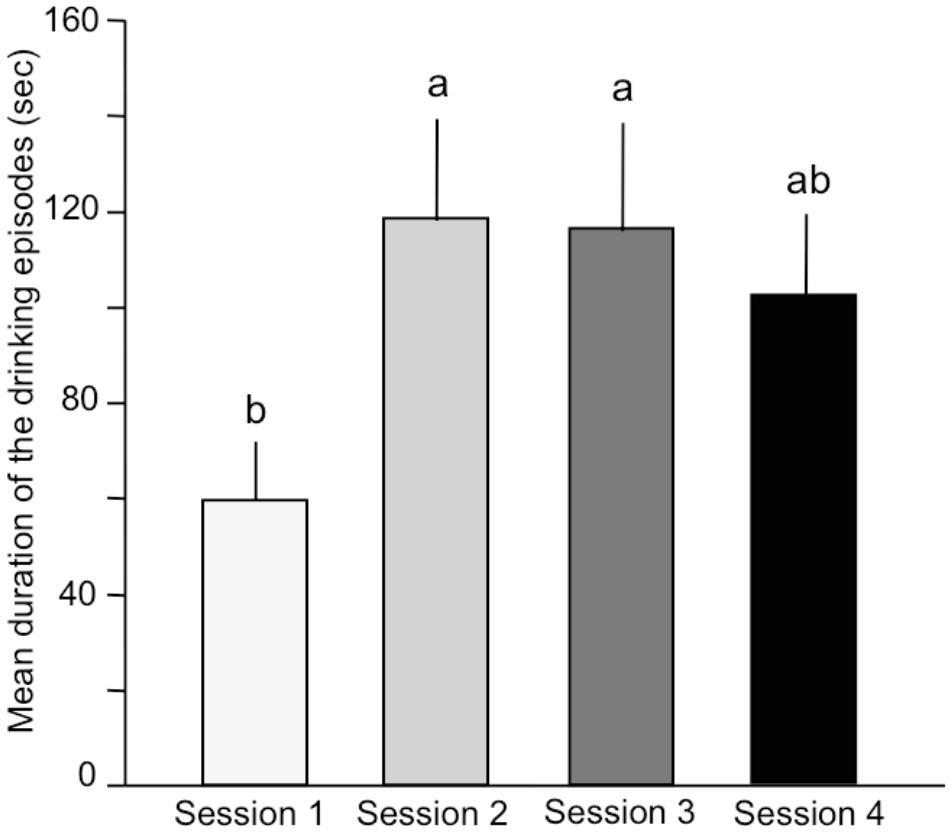

Fig. 3. Mean duration (sec) of the drinking episodes during the four conditioning sessions. Data (± SEM) are averaged on the three experimental treatments, that is CS++, CS+ and CS−. The CS++ solution was added with 16% sucrose and paired with ID infusion of distilled water, the CS+ was paired with ID infusion of 16% sucrose and the CS− was paired with ID infusion of distilled water. Two different letters indicate significant difference between conditioning sessions ($p < 0.05$ before Bonferroni correction).

3.2. Preference tests

Since the second session of tests only aimed at investigating the extinction of the learning and since no significant preference has been found during the first session, analyses of the consumption and behavioural data resulting from the second tests session were not presented in the present paper.

Overall, consumption of the CS solutions presented during the first session of two-choice tests did not significantly differ (**Table 2**). The mean percent CS intake relative to total in-

take, however, showed a slight preference for the CS++ compared to the CS+ (57% vs. 43%) during the first session of tests.

Table 2. Mean (± SEM) consumption (mL) of the CS solutions during the first session of two-way preference tests. During conditioning only, the CS++ was paired with 16% sucrose and with ID infusion of distilled water, the CS+ solution was paired with ID infusion of 16% sucrose and the CS– solution was paired with ID infusion of distilled water. The CS solutions were not reinforced during two-way preference tests though. The percentages under the consumption values indicate the mean percent consumption for the CS solution relative to total intake.

	Preference tests					
	CS++ vs. CS+		CS++ vs. CS–		CS+ vs. CS–	
	CS++	CS+	CS++	CS–	CS+	CS–
Consumption (mL)	1052 ± 255	810 ± 284	898 ± 258	918 ± 263	1019 ± 265	827 ± 282
% consumption	57	43	49	51	55	45
Paired *t* test	$t(8) = 0.53$	$p = 0.61$	$t(8) = 0.04$	$p = 0.97$	$t(8) = 0.44$	$p = 0.67$

No overall difference was found for the behavioural activity and the postures exhibited during the first session of preference tests ($p > 0.05$). Overall, there was no significant difference either between the two CS solutions for the four parameters characterising the microstructure of CS intake ($p > 0.1$; **Fig. 4**). It worth noticing, however, that, in the CS++ vs. CS– test, the mean duration of the CS++ drinking episodes represented 64% of the mean total duration of drinking episodes, and the time spent drinking the CS++ represented 57% of the total time spent drinking. In the CS++ vs. CS+ test, the mean duration of the CS++ drinking episodes represented 64% of the mean total duration of drinking episodes. In the CS+ vs. CS– test, the mean percentage data showed that the mean duration of the CS+ drinking episodes repre-

sented 71% of the mean total duration of drinking episodes, while the number of IDE pauses at the CS+ through represented 60% of the total number.

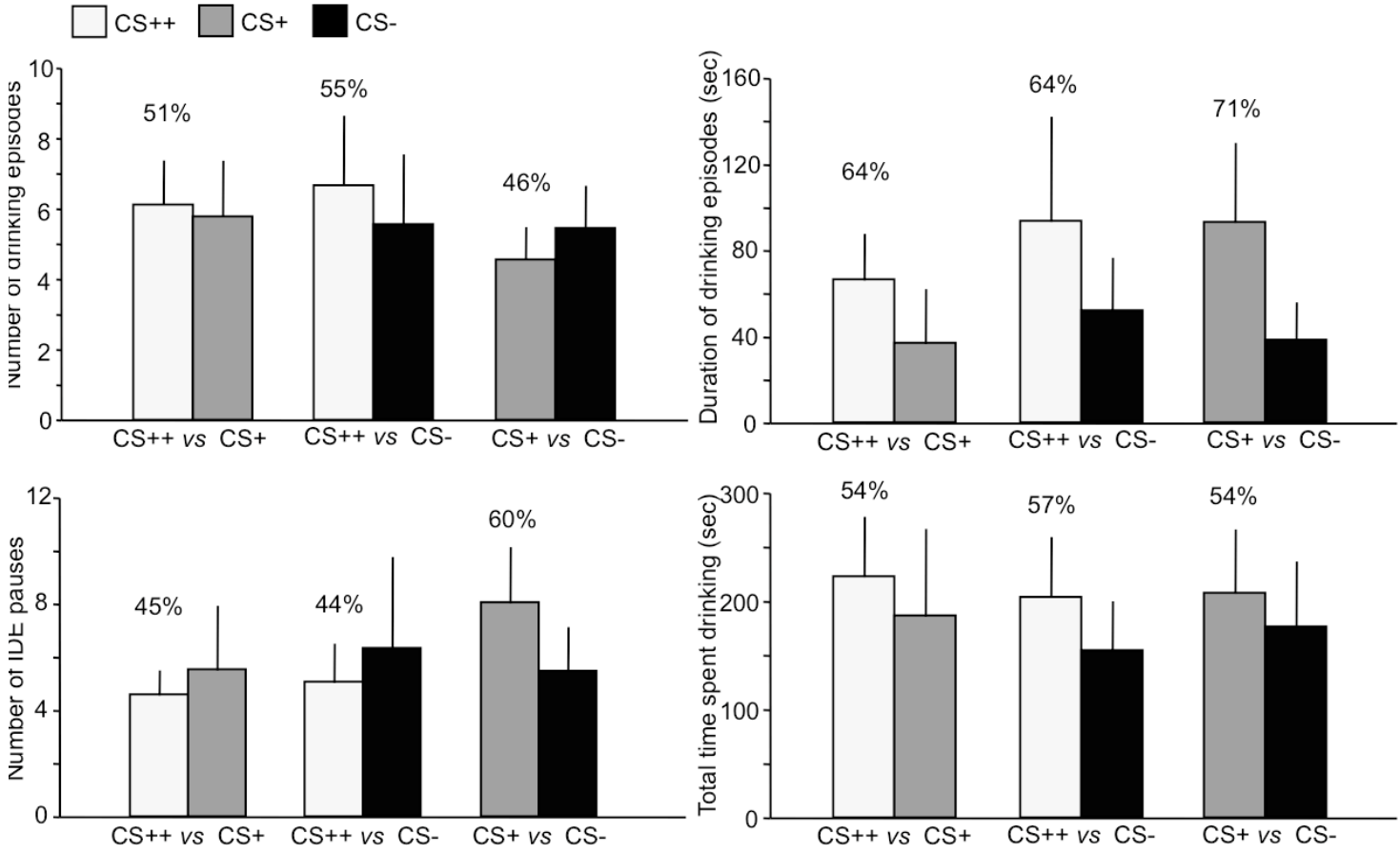

Fig. 4. Variables characterising the microstructure of CS intake during the first two-choice tests session. Data are presented as mean ± standard error. The CS++ solution was paired with 16% sucrose and with ID infusion of distilled water during conditioning, the CS+ solution was paired with ID infusion of 16% sucrose during conditioning, and the CS– solution was paired with ID infusion of distilled water during conditioning. However, the CS solutions were not reinforced during two-choice tests. A drinking episode started when the animal began to drink and took end when the animal stopped drinking for at least 2 min. If the animal resumed drinking before the end of the 2 min pause, it was considered as part of the same drinking episode, a pause of least than 2 min being considered as an intra-drinking episode (IDE) pause. The percentages above bars indicate the mean percent data for the CS solution relative to the total data for the two solutions presented during the test.

3.3. Plasma osmolarity

During conditioning, no significant effect of the moment, i.e. before or after the drinking session ($F(2,16) = 3.35$, $p = 0.10$), treatment ($F(2,16) = 3.38$, $p = 0.06$) or session × treatment interaction ($F(2,16) = 1.29$, $p = 0.30$) on plasma osmolarity were found. Overall, during preferences tests, plasma osmolarity was higher 15 min before than 15 min after the 1-h drinking test (before: 331.19 ± 6.45 mOsm/l vs. after: 306.39 ± 5.11 mOsm/l, $t(8) = 2.51$, $p < 0.05$).

4. Discussion

The present study investigated the development of conditioned flavour preference induced by oral (CS++, sweet taste and calories) or visceral (CS+, calories) 16% sucrose in pigs. Although the pigs did not show clear-cut preferences for the CS solutions during two-choice preference tests, the administration of oral 16% sucrose (CS++) during conditioning induced changes in the pigs' behavioural responses and might be responsible for slight modifications of CS intake microstructure compared to the control CS– solution, while the administration of visceral 16% sucrose (CS+) did not.

No difference in intakes of the CS++, CS+ and CS– solutions was found during conditioning and the animals most often drank the total amount of the CS. That the animals were highly motivated to drink the solutions was predictable given that they were water-deprived and the amount provided (600 mL) only represented 26% of their 1-h water consumption baseline. This assumption is supported by behavioural indicators, which showed that, overall, the animals explored more the trough that contained the solution than the opposite empty trough, suggesting a high motivation to obtain the solution, regardless of the reinforcement. In their study, Sclafani and Glendinning [30] compared the ability of two strains of mice to acquire conditioned flavour preference for an unsweetened solution paired with IG infusions of 16% sucrose (CS+; Experiment 1A). While the C57BL/6J mice consumed the CS+ more than CS–

solutions, no difference in the consumption of the CS+ and CS– solutions was found for the 129P3/J mice during both one-bottle training tests and subsequent two-bottle reinforced preference tests. These findings indicate that the absence of preference during conditioning might be predictive of the absence of clear-cut preference during subsequent two-bottle tests, which would be consecutive to learning failure.

Although no difference in CS++, CS+ and CS– intake was found during conditioning, the administration of oral or visceral 16% sucrose induced changes in pigs' behavioural profiles. The animals explored the drinking trough more when it contained the CS++ than CS–, and tended to explore the drinking trough more when it contained the CS++ than CS+. As the time taken to finish the CS++ was 34% shorter than that taken to finish the CS– solution, we can assume that the increased exploration of the drinking trough that provided the CS++ reflected an increased motivation for the solution, rather than a reluctance to drink it. Altogether, these findings indicate that pigs exhibited a preference for a sweet and caloric solution compared to an unsweetened solution, whether or not the unsweetened solution was paired with ID infusions of calories. This finding was quite expected considering that pigs are highly attracted by the sweet taste of sugar and show spontaneous preferences for sweet solutions over water [32, 33]. Besides, it is in accordance with previous studies demonstrating that, in a situation where food- and water-deprived rats received sweetened or unsweetened flavoured solutions having equal caloric values, weaning and/or adult animals showed increased olfactory (i.e. orienting, sniffing towards the solution) and oral (i.e. mouthing elicited by oral infusions of solution) responsiveness for the sweetened flavoured solution [9]. Conversely, we did not show any difference between the time spent exploring the troughs containing the unsweetened caloric CS+ or non caloric CS– solutions in pigs, whereas Myers and Hall [9] found that adult rats exhibited increased oral responsiveness to an unsweetened CS solution having high caloric value compared to an unsweetened CS solution having low caloric value.

Altogether these findings suggest that sweet taste might be the major factor responsible for the increase in pigs' motivation to obtain the solution, while both calories and sweet taste can modulate drinking motivation in rats – highlighting inter-species differences in the identification of the main food features for feeding motivation and selection processes.

Administration of oral or intestinal 16% sucrose during conditioning also seemed to induce slight modifications in CS intake microstructure. Although the total time spent drinking the CS++ and the CS– did not differ, the number of drinking episodes and the number of IDE pauses during CS– intake were slightly higher than those observed during CS++ intake. Similar results have been found in water-deprived rats, as Spector and St John [34] demonstrated that unpleasant taste (quinine) induced an increase in drinking episodes (i.e. bursts) and intra-burst pause numbers. Spector and St John [34] suggested that the aversive taste of quinine promoted burst termination, but as rats were water-deprived, they resumed drinking to rehydrate, which resulted in an increased number of bursts and intra-burst pauses. Smith [35] also demonstrated that sucrose drinking bouts number decreased as sucrose concentration (i.e. palatability) increased in rats. Based on these previous findings, and since CS++ solution exposure resulted in a decreased number of drinking episodes and IDE pauses in our study, we assumed that the sweet and caloric CS++ solution was perceived as more palatable than the CS– solution. Conversely, no difference was found between the CS+ and CS– conditioning concerning those same variables. As shown previously, this highlights the predominant role of sweet taste compared to calories for the motivation of pigs to consume a flavoured solution. Nevertheless, our results should be taken cautiously as we did not succeed in highlighting statistically significant differences between CS++, CS+ and CS– intake microstructure. Further studies with more animals are needed to enhance statistical power and confirm these trends.

It also worth noticing that the CS intake microstructure differed between the first and further conditioning sessions, in that the duration of the drinking episodes and the total time spent drinking during the first session were lower than during the further sessions, while there was more IDE pauses. These results might illustrate the neophobic response showed by animals, including humans [18] or pigs [36], towards novel food or solution, which may lead to modified feeding or drinking activities and impaired intakes. Besides, the high number of IDE pauses during the first conditioning might be related to that neophobic response, which would support the aforementioned assumption that the more IDE pauses there are, the less palatable the solution is. These findings also indicate that the first conditioning session is not necessarily a good predictor of the events occurring in later sessions in preferential conditioning studies.

Although the perception of oral and visceral 16% sucrose during conditioning induced changes in behavioural profiles and, in a lesser extent, in CS intake microstructure, the pigs exhibited no significant preference during subsequent two-choice tests with unreinforced CS solutions. Nevertheless, during the first session of the CS++ vs. CS+ test, the duration of CS++ drinking episodes represented 64% of total duration. Similarly, in the CS++ vs. CS–, the duration of CS++ drinking episodes represented 64% of total duration, while the mean total time spent drinking the CS++ represented 57% of total time. Despite the results did not reach statistical significance, these data indicate that the animals were somehow more motivated to obtain the solution that was previously paired with sweet taste and calories than the solution that was paired with calories only or no reinforcement. This is in accordance with the assumptions resulting from conditioning data, and with studies in rodents reporting that, in a situation where animals received equally caloric flavoured solutions, the animals preferred the flavour previously associated with the more palatable taste, that is the sweetened

solution [6, 9]. Furthermore, D'Aquila [37] and Genn et al. [38] reported that the administration of dopamine receptor antagonists (i.e. SCH 23390 and raclopride) had a reducing effect on bout duration during the intake of 10% sucrose in rats, and proposed that bout durations represented good and reliable indicators of reward and stimulus palatability evaluation, which supports the hypothesis that CS++ might be perceived as more palatable than CS+ and CS– by the pigs. It worth noticing that the mean duration of the CS+ drinking episodes also represented 71% of the mean total duration of drinking episodes in the CS+ vs. CS– test, which might suggest a slight positive effect of calories on further feeding choices, and required further investigation.

Different phases can be described during food intake and digestion, including cephalic, gastric and intestinal phases. According to Zafra et al. [39], "the cephalic phase of digestion refers to a set of physiological, endocrine and autonomic responses of the digestive system that result from stimulation of sensory systems at the cephalic level, especially in the oropharyngeal cavity". The cephalic phase – and the passage of foods through the oral cavity – plays a major role in food digestion, as it regulates several digestive events mediated by the vagus nerve at different levels of the digestive system. Consequently, the absence of cephalic phase (e.g. when food is directly administrated into the intestine) decreases salivary and gastric secretions, which, in turn, affects the normal sequence of digestion [39]. According to Zafra et al. [39], some studies also demonstrated that intestinal infusions of nutrients induced impairments of the intestinal mucosa, and might be responsible for subsequent decreased food intakes. These findings emphasize the predominant role of the cephalic phase during food intake and might explain numerous findings of the present study, including the higher motivation for the CS++ during conditioning, as well as the lack of preference for the CS+ in two-choice tests. During CS+ conditioning, the infusion of a large amount of calories directly into the duodenum may have modified the course of further digestive events and decreased subse-

quent CS+ intake. That the pigs spent more time lying and inactive when they received the CS+ solution also supports this hypothesis, given that an increased time spent lying or inactive may indicate discomfort or visceral malaise in pigs [28, 40-42].

Ackroff et al. [43] suggested that the osmotic factors might induce changes in preference through gastrointestinal distension and gastric emptying and absorption during intake of sucrose solutions. In the present study, plasmatic osmolarity was higher before than after solution intake during preference tests, which is in accordance with the previous finding that water access decreased plasma osmolality (mOsm/kg H_2O) in water-deprived pigs [44]. According to Otsuki et al. [45], however, plasma osmolarity in non water-deprived pigs varied from 290 to 300 mOsm/l, meaning that the rates found in our study matched standard values. Furthermore, plasmatic osmolarity did not vary as a function of reinforcement type during conditioning, and cannot account for impairment of preference acquisition. Altogether, these physiological data suggest that osmotic factors are likely not involved in the acquisition or expression of preferences induced by sweet caloric compounds, as previously suggested in rats [46, 47]. Since some studies managed to condition strong preference for flavoured solution paired with intragastric (IG) 16% and 32% sucrose administrated at slower rates, that is 0.5 mL/min [30] and 1.3 mL/min [19], a lower sucrose infusion rate might enable to verify these assumptions.

As previously stated, some studies did find significant preferences for solutions previously paired with visceral [19, 26, 30] and/or oral sucrose infusions [26] in rats and humans [17]. Besides, we already managed to induce a short-term preference for a CS solution previously added with 10% sucrose in pigs [27], which led us to think that methodological factors might be responsible for these inter-studies discrepancies. Although pigs appear to have good cognitive abilities (e.g. spatial learning) [48, 49], the learning of three concomitant CS/US associations might represent a too difficult task for this species. In previous studies, we used simi-

lar paradigms in which animals had to pair flavours with sweet taste and/or calories induced by ID glucose [28], *per os* maltodextrin, saccharin or sucrose [27]. When the pigs were required to simultaneously acquire three associative learning, no clear-cut preference emerged during subsequent two-choice tests, whereas significant preferences appeared when the pigs only had to learn two flavour/stimuli associations, e.g. CS+ solution paired with 10% sucrose vs. CS– control solution [27]. Conversely, Azzara and Sclafani [19] managed to induce flavour-nutrient preferences in a paradigm in which rats were required to learn three CS/US associations, i.e. CS+M solution paired with IG 32% sucrose, CS+S solution paired with 32% maltose and a unreinforced CS– control solution. This finding suggests that rats could acquire more complex food learning, and might represent a more pertinent model than pigs for the investigation of conditioned flavour preference. These data also suggest that the learning task complexity may greatly complicate the acquisition of food preference, and that an appropriate match between learning complexity and species cognitive abilities is a crucial factor to be taken in consideration in conditioning studies.

Several studies also reported the impact of food or water deprivation on the acquisition and expression of flavour-nutrient and flavour-taste preference conditioning in rats [20, 50] and humans [13, 14]. Drucker et al. [20] reported that food deprivation during conditioning increased preference for a CS+ solution paired with IG polycose infusions in rats, while Yiin et al. [50] reported that rats showed a preference for a CS+ solution previously paired with IG 16% glucose, whether or not they were food deprived during conditioning. In humans, Brunstrom and Fletcher [14] demonstrated that hungry participants showed a preference for a tea added with a non-caloric sweetener during training compared to two unsweetened teas, whereas satiated participants did not. Altogether, these studies indicate that food deprivation rather enhances the preference for a flavoured solution and is likely not the factor that impaired the expression of CS++ or CS+ preference in our study.

Drucker et al. [20] also reported that water deprivation increased both CS– and CS+ intakes during two-choice tests, CS+ being a solution that was paired with IG polycose during conditioning. Therefore, we assume that water deprivation minimised the expression of the preference for CS++ and/or CS+ compared to CS– in the present study, the pigs' motivation to drink being rather driven by thirst than hedonic considerations, which resulted in balanced CS++, CS+ and CS– intakes. In a previous study, non water-deprived pigs that were given 7 h/day access to the CS solutions during conditioning and preference tests, expressed a short-term preference for a solution paired with 10% sucrose [27], indicating that water-deprivation is a determinant factor for the expression of conditioned preference given that thirsty pigs are highly motivated to drink, whether the solution is preferred or not.

5. Conclusions

To conclude, given that the oral perception of 16% sucrose during conditioning induced increased motivational responses and changes in the CS intake microstructure, the present study suggests that oral sucrose administration (i.e. the association of sweet taste and calories) might be more efficient than visceral sucrose infusions (i.e. calories in the absence of sweet taste) in inducing flavour preference in pigs. This assumption is broadly based on the analysis of conditioning data but conclusions must be drawn carefully given the absence of statistically significant preference during two-choice tests. Further investigations using more animals are needed to confirm these trends and minimize the effect of inter-individual variability. A further attempt should also consider using non water-deprived animals and simpler associative learning to overcome the limiting effect of these methodological factors on flavour preference acquisition and expression in pigs.

Acknowledgements

The study was supported by a grant from the Brittany Region (Région Bretagne) and INRA (Department of Human Alimentation; Department of Animal Physiology and Livestock Sys-

tems). The authors wish to thank Laboratoires Phodé (Terssac, France) for providing the essential oils used in this study. The authors gratefully acknowledge the efforts and cooperation of the scientific and technical staff, and especially Sylvie Guérin for participating in this study and Alain Chauvin for surgery and caring for the animals.

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Partie 2. Préférences spontanées pour le saccharose : réponses motivationnelles et cérébrales induites par la perception orale et/ou duodénale de saccharose (article n°5)

1. Contexte et objectifs

Chez de nombreuses espèces comme les rongeurs, le porc ou l'Homme, la perception orale et/ou viscérale du saccharose suffit à induire des réponses ingestives et motivationnelles positives et des préférences alimentaires marquées. Par ailleurs, des études d'imagerie cérébrale chez l'Homme mais également chez le porc ont démontré que la perception orale et/ou duodénale de sucres induisait des changements d'activation dans des régions cérébrales connues pour être impliquées dans la régulation hédonique de la prise alimentaire et dans le traitement des récompenses, comme le striatum ou encore l'amygdale. L'objectif de notre étude était de comparer les réponses motivationnelles et cérébrales provoquées par la perception orale et/ou duodénale de saccharose chez le porc à jeun *via* l'utilisation de technique de conditionnement opérant (ratio progressif) et de l'imagerie cérébrale SPECT.

2. Méthodes

Au total, 12 porcs juvéniles femelles en situation de privation alimentaire et implantés d'un cathéter duodénal ont été utilisés au cours de l'étude. Dans un premier temps, les animaux ont été soumis à deux répétitions de trois jours de tests de ratio progressif (PR) pendant lesquels ils devaient appuyer sur un bouton pour obtenir une récompense alimentaire. La récompense alimentaire était soit un aliment standard (OS–), soit un aliment standard additionné de saccharose 5% (OS+). Pendant les 30 premières minutes des tests, les animaux recevaient une infusion intra-duodénale (i.d.) de sérum physiologique (DS–) ou de saccharose 16% (DS+). Ainsi, les animaux étaient exposés à trois traitements constitués d'un couple stimulation orale (OS) sucrée ou non et stimulation duodénale (DS) calorique ou non (OS+DS+ ; OS–DS+ ; OS–DS–). La semaine suivant les tests, les animaux anesthésiés ont été soumis à quatre séances d'imagerie cérébrale SPECT *via* le ^{99m}Tc-HMPAO à la suite de stimulation orale avec de la salive artificielle neutre (OS–) ou additionnée de saccharose 5% (OS+) couplée à une stimulation duodénale avec du sérum physiologique (DS–) ou du saccharose 16% (DS+). Les animaux ont donc été exposés à quatre types de stimulations : OS+DS+ ; OS–DS+ ; OS+DS– ; OS–DS–. Les images cérébrales obtenues après chaque stimulation ont été comparées entre elles pour déterminer les différences d'activité dans des régions d'intérêt (ROI) choisies sur des hypothèses *a priori*.

3. Résultats

Concernant les performances pendant les tests de PR, aucun effet significatif du traitement sur les différentes variables des tests de PR n'a été mis en évidence, que ce soit sur le nombre total de récompenses obtenues ($F(2,20) = 0.09$, $P = 0.91$), le nombre total d'appuis ($F(2,20) = 0.07$, $P = 0.94$), le taux d'appuis ($F(2,20) = 0.99$, $P = 0.39$), le « breakpoint » ($F(2,20) = 0.09$, $P = 0.92$) et la pause post-renforcement ($F(2,20) = 0.81$, $P = 0.46$).

Comparée au traitement contrôle, la perception orale et duodénale combinée de saccharose (OS+DS+ *vs* OS–DS–) a induit des différences d'activation dans l'APFC, l'OB, le PreC, le PiC, le PeC, l'hippocampe, l'AMY, le PHC et le striatum dorsal (PUT et CAU). Comparées au contrôle, la perception orale (OS+DS– *vs* OS–DS–) ou duodénale (OS–DS+ *vs* OS–DS–) de saccharose ont induit des différences d'activation dans l'OB. Comparée à la perception duodénale de saccharose, la perception orale de saccharose (OS+DS– *vs* OS–DS+) a induit des différences d'activité dans le PUT. Comparée à une situation dans laquelle aucune stimulation orale sucrée n'a été réalisée, la perception duodénale de saccharose couplée à la perception orale de saccharose a induit des différences d'activité dans le PeC, le PHC, le cortex entorhinal, le PreC et l'hippocampe (OS+DS+ *vs* OS–DS+). La présence ou l'absence de stimulation duodénale au saccharose lors de la perception orale de saccharose (OS+DS+ *vs* OS+DS–) n'a induit aucune différence significative d'activation dans les ROI.

4. Conclusions

Le principal résultat de ces travaux réside dans le fait que la perception combinée de saccharose au niveau oral et duodénal a induit des changements de l'activité cérébrale dans les régions impliquées dans la mémoire (hippocampe, PHC, PeC), le traitement de la récompense (AMY, APFC, CAU, PUT), et dans l'identification des stimuli sensoriels (PiC, PreC). Au contraire, la perception orale ou duodénale de saccharose n'a pas suffi à induire de telles différences d'activation. Malgré ces différences d'activité, aucune différence dans les réponses motivationnelles exprimées par les animaux pendant les tests de PR pour une récompense sucrée ou non n'a été mise en évidence. Ces données suggèrent que, bien que les réponses motivationnelles pour un aliment sucré chez des animaux en état de privation alimentaire semblent plutôt contrôlées par des facteurs homéostatiques que hédoniques, au niveau central, le traitement des signaux oraux et duodénaux *via* le saccharose entraîne bel et bien un traitement hédonique des informations.

Article n°5

Motivational and brain responses triggered by oral and/or duodenal sucrose sensing in pigs

C. Clouard[1,2,3], M.C. Meunier-Salaün[2,3], C.H. Malbert[1], P. Meurice[1] and D. Val-Laillet[1]

(En préparation pour soumission à *Chemical Senses*)

[1]INRA, UMR UR1341 Alimentation & Adaptations Digestives, Nerveuses et Comportementales, F-35590 Saint Gilles, France

[2]INRA, UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, F-35590 Saint Gilles, France

[3]Agrocampus Ouest, UMR1348 Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, F-35000 Rennes, France

Abstract

In rodents, pigs and humans, both oral and duodenal sucrose sensing are able to induce food preferences and increase food intake. Brain imaging studies revealed that oral and postoral sugar sensing induce changes in activation in regions known to participate in the hedonic regulation of food intake and reward processing. The present study aimed at comparing the motivational and cerebral responses triggered by oral and/or duodenal sucrose sensing in pigs. Twelve food-deprived female pigs were subjected to progressive ratio (PR) operant-conditioning tests. The food reward was either a control standard diet or a 5% sucrose diet and was paired with intraduodenal (ID) infusion of saline or 16% sucrose. The week following the PR tests, the anaesthetised animals underwent single photon emission computed tomography (SPECT) further to oral stimulation with neutral or 5% sucrose artificial saliva paired with ID infusions of saline or 16% sucrose. During PR tests, in a situation of duodenal stimulation with sucrose, no difference was found between the motivational responses to earn the sweetened and unsweetened food reward, suggesting that, in hungry pigs, motivational responses for food are driven by homeostatic rather than hedonic factors. Only paired oral and duodenal sucrose sensing was able to modify the activation of brain regions known to be involved in reward processing, memory and hedonic evaluation of sensory stimuli, including the anterior prefrontal cortex, amygdala, dorsal striatum (caudate and putamen), hippocampus, prepiriform, piriform, parahippocampal and perirhinal cortices, whereas independent oral or duodenal sucrose sensing did not. The perception of duodenal sucrose in the presence vs. in the absence of oral sucrose stimulation triggered differential CBF in memory processing regions, including the hippocampus, the parahippocampal, perirhinal and entorhinal cortices, suggesting that the oral signals might be more determinant than visceral signals for memory processes during sugar sensing. Further studies should consider investigating the

effect of the internal state (i.e. hunger or satiety) on brain responses triggered by oral and duodenal sugar sensing in pigs.

Key words: sweet taste, reward, motivation, memory, SPECT, progressive ratio.

Introduction

The sweet taste of sucrose is known to be very attractive in several species. In rats, humans and non-human primates, newborn infants and pups spontaneously respond to sweet taste stimulation with sucrose by specific positive facial expressions (for reviews, see Berridge 2000; Steiner et al. 2001). Capaldi & Privitera (2007) also reported that mixing sour or bitter food with the sweet taste of sucrose increased liking for these foods in infant and adult humans, while pigs and rats showed spontaneous preferences for sucrose solutions over water (Ackroff and Sclafani 2011; Glaser et al. 2000). Given the spontaneous preferences exhibited by these species for sucrose, it has been frequently used as a positive reinforcement in operant and pavlovian conditioning procedures. Rats showed high motivational responses for sucrose food reward during progressive ratio (PR) operant-conditioning (Dickson et al. 2012; Figlewicz et al. 2011), and the adjunction of sucrose in a flavoured solution induced strong conditioned flavour preferences in rats (Bonacchi et al. 2008; Gilbert et al. 2003; Warwick and Weingarten 1996), humans (Mobini et al. 2007), and pigs (Clouard et al. 2012a). Some authors also reported clear-cut conditioned flavour preferences for unsweetened solutions paired with intragastric (IG) infusions of sucrose in rats (Azzara and Sclafani 1998; Sclafani and Glendinning 2005), suggesting that, like the sweet taste of sucrose, visceral sucrose sensing might be sufficient in itself to induce positive hedonic responses even in the absence of sweet taste.

Nevertheless, visceral infusion of sugar may also have negative consequences on food intake and preferences. Cabanac et al. (1973) reported that humans rated sucrose oral stimuli as less pleasant after IG glucose infusions than when no IG glucose infusion was performed, this phenomenon being known as negative alliesthesia. According to Cabanac (1971), alliesthesia is defined as a change in the perceived pleasantness for a taste stimulus or food further to modifications of the individual's internal state. When the perceived pleasantness decreased after visceral calories uptake, the alliesthesia is negative, while the alliesthesia is positive when the perceived pleasantness increased further to changes of the internal state. Alliesthesia plays a major role in food intake regulation and is a specific mechanism in that visceral sensing of sugars only affects the perceived pleasantness of oral sugar sensing, and has no effect on the perceived pleasantness of salty oral stimuli (Cabanac and Duclaux 1970).

Brain imaging studies in different species, including humans and pigs, identified brain regions that participate in the processing of sugar sensing. Using functional resonance imaging (fMRI), Smeets et al. (2005) reported metabolic changes in the hypothalamus, a region known to control the homeostatic regulation of food intake responses, after glucose ingestion. Though, Purnell et al. (2011) found that intravenous (IV) infusions of carbohydrates (i.e. glucose, fructose) induced activity changes in cortical structures, but not hypothalamic regions, suggesting that plasmatic sensing of sugars is likely not determinant to the homeostatic regulation of sugar intake, as previously suggested by Baldwin (1996) in pigs. Some brain imaging studies also investigated the extra-hypothalamic responses triggered by oral and/or postoral sugar sensing. Using fMRI, Frank et al. (2008) and Smeets et al. (2011) showed that the processing of oral sucrose signals involved the insular cortex, the striatum (caudate, nucleus accumbens, putamen), the globus pallidus, the amygdala, as well as the prefrontal and the anterior cingulate cortices. Recently, Boubaker et al. (2012) investigated the brain responses triggered by glucose duodenal sensing in pigs using the single photon emission computed

tomography (SPECT). They reported metabolic changes in the orbitofrontal and dorsolateral prefrontal cortices, the caudate and the putamen, but also in regions that participate in odour processing and memory, including the prepiriform area and the anterior entorhinal cortex. Altogether, these findings emphasised the fact that the processing of both oral and duodenal sugar signals are mediated by both homeostatic and nonhomeostatic factors and involve extra-hypothalamic regions that participate in the hedonic regulation of food intake.

Only a few studies, however, compared the brain responses triggered by sweet taste and/or calories. Using different sweet and/or caloric compounds (e.g. glucose, aspartame, maltodextrine), Chambers et al. (2009) reported that oral glucose sensing (combining sweet taste and calories) activated reward-related brain regions, including the anterior cingulate cortex and the striatum (caudate), while oral saccharin sensing (sweet taste but no calorie) did not. Oral glucose and maltodextrin (calories but no sweet taste) sensing induced similar brain responses in the insular and orbitofrontal cortices and the striatum. To date, however, we found no study comparing the brain metabolism triggered in the aforementioned extra-hypothalamic regions during oral and/or duodenal sensing of the same sugar (e.g. sucrose). Though, one might wonder to what extent combined oral and duodenal sucrose sensing, or independent oral vs. duodenal sugar sensing modify brain metabolism in those specific regions.

Chronic consumption of high-sugar diets has been proved to induce metabolic and neurophysiological alterations leading to eating disorders and obesity in humans, pigs or rats (e.g. Val-Laillet et al. 2010; Zhao et al. 2005). Furthermore, motivation to eat is strongly influenced by nonhomeostatic factors, such as food palatability depending on its organoleptic properties. The regulation of food intake driven by hedonic factors can override the regulation driven by homeostatic factors and plays a major role in the global control of energy intake (Berthoud 2006; Zheng and Berthoud 2007). Hedonic regulation of feeding behaviour may result in an increased motivation to eat, leading to overeating and to the emergence of

eating disorders or obesity. Consequently, the characterisation of brain networks that contribute to the processing of oral and/or duodenal sugars signals in pigs, by leading to a better understanding of impaired feeding behaviours, like the emergence of exacerbated sugar preferences in humans, might fulfil the current needs in human nutrition and health research (Clouard et al. 2012b).

The first aim of the present study was to assess the motivational responses of food-deprived pigs to a sweetened vs. unsweetened food reward in the presence or the absence of duodenal sucrose sensing during PR operant-conditioning tests. At a central level, the aim of the study was to identify and compare the brain circuits involved in the processing of oral and/or duodenal sucrose signals with SPECT. First, our aim was to demonstrate that paired oral and duodenal sucrose sensing induce global changes in brain metabolism within the aforementioned regions, that is in regions involved in the reward processing and the hedonic evaluation of sensory stimuli. Second, we investigated whether oral or duodenal sucrose sensing were sufficient on their own to induce similar brain responses and compared the brain circuits involved during the processing of oral or duodenal sucrose signals. We focused our analyses on the regions of interest (ROIs) chosen upon *a priori* hypotheses based on previous studies that characterised brain structures involved in the processing of sugar signals in humans or pigs.

Materials and methods

The experiments presented in this paper were conducted in accordance with the current ethical standards of the European Community (Directive 86/609/EEC), Agreement No. A35-622 and Authorizations No. 01894 and No. 35-88.

Animals and Housing

A total of 12 Large White x Landrace female pigs of 28.5 ± 1.6 kg at the beginning of the study were used. The experiment was carried out in four successive batches with four animals each, from February to June 2012, at the INRA experimental research station of Saint Gilles, France (N48°8'39" W1°49'55''). The pigs were housed in individual pens (150 x 60 x 80 cm) and had free access to water. A chain was suspended in each pen to enrich the environment of the animals and fulfil their natural disposition to play. The room was maintained at approximately 24°C with a 13:11-h light-dark cycle. Apart during the behavioural tests when the animal received experimental diets, the animals were fed daily with a pelleted "pea diet" of which the organoleptic properties differed significantly from that of the experimental diets. The pea diet was composed of 40% pea, 15% corn, 14.46% barley, 13.92% wheat, 13.56% soybean meal, 0.68% calcium carbonate, 0.58% mono-calcic phosphate, 0.3% vegetable oil, 0.3% vitamin complement, 0.24% salt. They received 600 g and 1 kg of pea diet per day during training and imaging sessions, respectively (see *Experimental procedure* section).

Surgery

After a 24-h fasting period, the pigs were pre-anesthetised with an intramuscular injection of ketamine (15-20 mg/kg, Mérial, Lyon, France). Suppression of pharyngotracheal reflex was obtained by inhalation of isoflurane (3-5% v/v, Baxter SAS, Maurepas, France) immediately before tracheal intubation. A surgical level of anaesthesia was maintained by isoflurane (2-3% v/v) delivered by a mechanical ventilator and analgesia was obtained by IV injection of a morphinic agent (Fentanyl 4 ml, 1,4ml/min, Renaudin, Paris, France). Heart and respiratory rates were continuously monitored throughout surgery using a pulse oxymeter (Ohmeda oxymeter, GE Healthcare Clinical Systems, Limonest, France) and an IR capnometer (Amstrong capnometer, Gambo Engström, Bromma, Sweden). A midline laparotomy was per-

formed under aseptic conditions. A catheter was fixed into the proximal duodenum, tunnelled under the skin and exteriorized between the shoulders for intraduodenal (ID) infusions of saline (NaCl) or 16% sucrose during behavioural tests and brain imaging. After surgery, all the animals had one week of recovery before the beginning of the experiments. During the recovery week, the animals were exclusively fed with the pea diet.

Experimental treatments and diets

During progressive ratio (PR) operant-conditioning, the animals received different experimental treatments, combining an oral stimulation (OS) with a duodenal stimulation (DS). The OSs were obtained by the use of two experimental diets specifically formulated. The control OS (OS–) consisted in a standard unknown pelleted diet formulated with 25% corn, 24.1% barley, 23.2% wheat, 22.6% soybean meal, 1.13% calcium carbonate, 0.97% mono-calcic phosphate, 0.5% vegetable oil, 0.5% vitamin complement, 0.4% salt (3.24 Mcal/kg of food). The positive OS (OS+) consisted in a sweetened pelleted diet (95%: standard pelleted diet; 5%: sucrose; 3.29 Mcal/kg of food). As the OS+ diet was a bit more caloric compared to the OS– diet and to ensure that the animals received isocaloric rations of food during the behavioural tests, 1 kg of OS– and 950 g of OS+ were distributed, that is 3240 kcal in total. The DSs were obtained by ID infusions of experimental solutions. The positive reinforcement was induced by an ID infusion of 300 ml of 16% sucrose (DS+, 197 kcal). The choice to use 16% sucrose instead of 5% sucrose for ID infusion was based on the will to obtain a rapid and marked effect of ID sucrose on plasma glycaemia (**Figure 1A**). To obtain a sufficient caloric load with ID infusion of 5% sucrose (197 kcal), it would have required a longer infusion-time and a larger amount of solution infused, which may have resulted in undesirable supra-physiological conditions. As NaCl has no particular postingestive effect, the control treatment was induced by an ID infusion of 300 ml of 0.9% NaCl (DS–). The solutions were injected

with a peristaltic pump connected to the duodenal catheter, and the injection rate was 10 ml per min.

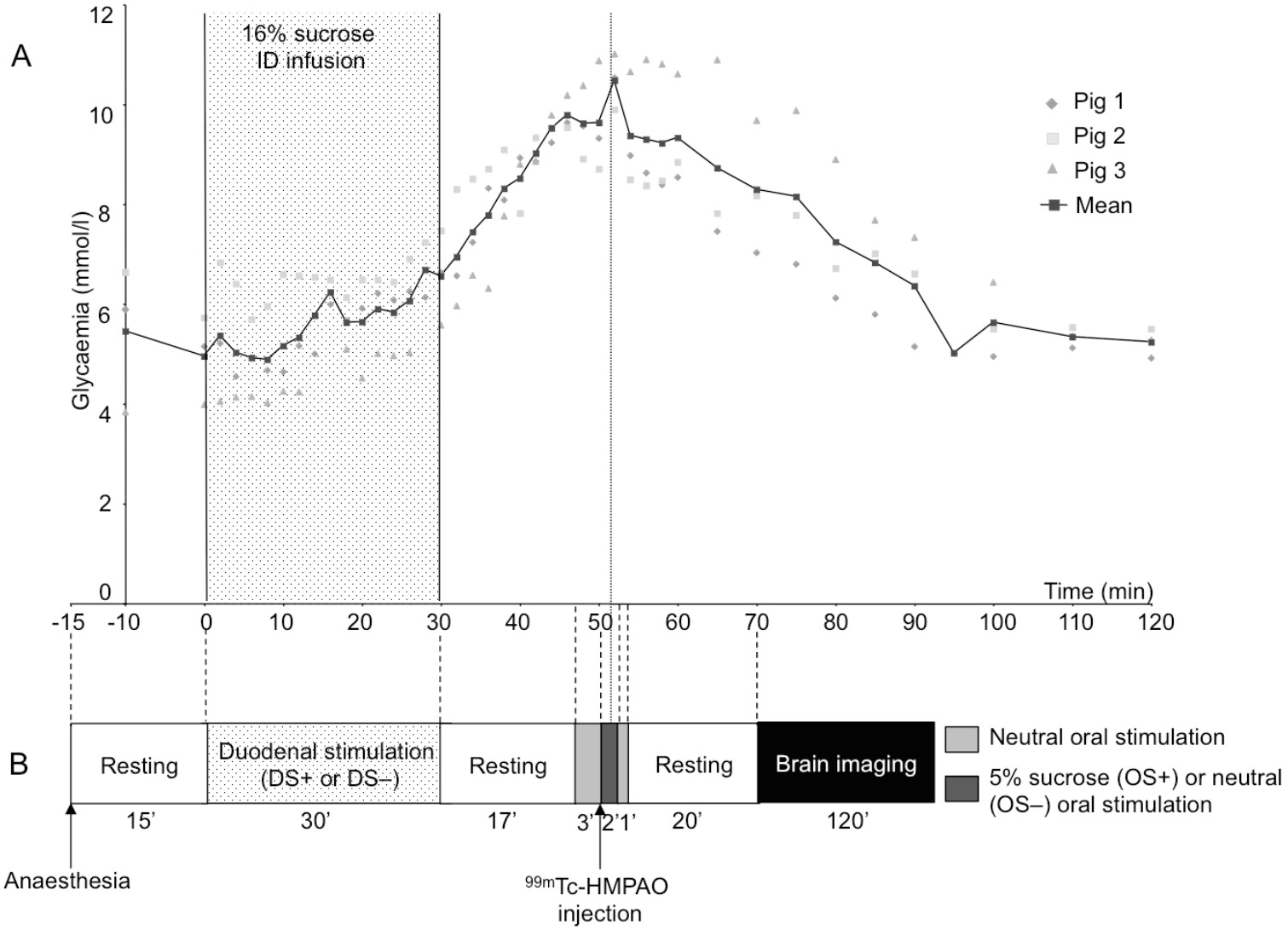

Figure 1 A) Plasma concentrations of glucose before and after a 30-min duodenal infusion of 16% sucrose (10ml/min, 300ml corresponding to 197 kcal). Blood sample were collected on three animals different from that used for behavioural tests and cerebral imaging. B) Schematic representation of the experimental paradigm used for the oral/duodenal stimulations before brain imaging. This experiment aimed at exploring the brain metabolism (single photon emission computed tomography of ^{99m}Tc-HMPAO further to duodenal and/or visceral sucrose infusions. As the ^{99m}Tc has a fast fixation time, the initial tracer uptake reflects cerebral blood flow (CBF) at a fast time window after injection (Kapucu et al. 2009). Consequently, the CBF recorded during imaging, that is 20' after the radiolabel

injection, corresponded to the CBF measured at the time of the radiolabel injection, i.e. during the oral sensing of artificial saliva added with 5% sucrose (OS+) or unsweetened artificial saliva (OS–, control), and during the peak of maximal glycaemia resulting from the ID infusion of 16% sucrose (DS+) or after ID infusion of 0.9% NaCl (DS–, control).

Experimental procedure

Preliminary determination of plasma glucose kinetic. Three 30-kg Large White x Landrace female pigs different from those used in the study were used to measure plasma levels of glucose further to a 30-min ID infusion of 16% sucrose (300 ml, 197 kcal). Briefly, the animals were implanted a duodenal and a jugular catheters (see *Surgery* section). The jugular catheter was fixed into a vein in the neck, tunnelled under the skin and exteriorized at the nape level. After a 12-h fasting period, the pigs were anaesthetised and subjected to a ID sucrose infusion using the same procedure as that used during the brain imaging sessions (see *Brain imaging procedure).* Blood samples were collected at -10 min, immediately before (0 min) the sucrose infusion, every 2 min from 2 to 60 min after the start of the infusion, every 5 min from 60 to 100 min after the start of the infusion and every 10 min from 100-120 min after the start of the infusion. Blood samples were collected in tubes containing 5 µl of ethyleediaminetetraacetic (EDTA 0.8 M; Sigma Aldrich, Saint Quentin, France). Blood samples were centrifugated at 4000 g during 10 min at 4°C and the resulting plasma sample were conserved at -20° C until assaying. Plasma glucose was measured in duplicate by an automated spectrophotometric method (Konélab 20i ; Thermo Fisher Scientific Inc., Waltham, USA) using the enzymatic assay Glucose RTU™ (BioMérieux® SA, Marcy l'Étoile, France). The intra-assay coefficient of variation was < 3%.

Training. During the recovery week after surgery, the food-deprived animals were trained to perform PR operant conditioning tests during three consecutive days. The apparatus used

during training and testing consisted of a blue button fixed near the pig's feed trough and an automated reward delivery device placed above the trough. The reward device and the button were connected to a computer (MacIntosh II vx, System software 7.5.3, Apple Computer) equipped with a software (LabVIEW, version 3.1.1) controlling reward delivery and recording the number of reward obtained and the corresponding time. Approximately 30 min before the beginning of the training test, the animals received a preload ration of 100 g pea diet in an attempt to minimize the motivational responses driven by hunger. On Day 1, the animals were trained on a fixed ratio schedule: they had to press the button once to obtain a 5-g food reward during the 2:30-h test to progressively habituate the animals to the experimental device. On Days 2 and 3, the animals were trained on the PR schedule in two steps to allow them to learn the task progressively. On Day 2, the number of times the pigs had to push the button to earn a reward was increased every 3 rewards by 3 pushes until the end of the 4-hour test (i.e. 1-1-1, 4-4-4, 7-7-7, 10-10-10, 13-13-13, etc., pushes for a 5-g reward). On Day 3, the number of times the pigs had to push the button to earn a reward was increased every 3 rewards by 5 pushes until the end of the 4-hour test (i.e. 1-1-1, 6-6-6, 11-11-11, 16-16-16, 21-21-21, etc., pushes for a 5-g reward). During training, the food reward was the pea diet. After the tests, the button was removed to avoid excessive non-reinforced button pushing, and the animals received a pea diet complement to reach 600 g of food per day if they had not consumed this amount already.

Progressive ratio testing. The week following the training sessions, the food-deprived animals were subjected to two three-day PR sessions on six consecutive days. Approximately 30 min before the test, the animal received 200 g of pea diet as preload. The animals were then tested on a 4-h PR schedule during which the number of times the animals had to push the button to earn a reward was increased every 3 rewards by 5 pushes until the end of the 4-h test (i.e. 1-1-1, 6-6-6, 11-11-11, 16-16-16, 21-21-21, etc., pushes for a 5-g reward). The food

reward was either the control diet (OS–) or the 5% sucrose diet (OS+). During the first 30 min of the PR test, the animals received a duodenal stimulation, that is an ID infusion of 0.9% NaCl (DS–) or an ID infusion of 16% sucrose (DS+). The DS moment and duration were chosen according to the results of the plasma glucose assay (see *Preliminary determination of plasma glucose kinetic* section). Plasma kinetic for glucose showed a peak of maximal glycaemia approximately 50 min after the start of the 16% ID sucrose infusion (DS+, **Figure 1A**). Performing the DS during the first 30 min of the tests enabled to reliably assess the impact of calories sensing on PR performance from the early period of the PR tests. During the three consecutive days of the two PR sessions, the animals received the OS+DS+ (i.e. sweet taste and additional caloric load), the OS–DS– (i.e. no sweet taste and no additional caloric load) and the OS–DS+ (i.e. no sweet taste but additional caloric load) treatment. Experimental treatments were counterbalanced across days and animals to avoid bias. The PR session ended when 2 h elapsed without a reward delivery, or 4 h after the beginning of the test. After the test, refusals were weighted and distributed to the animals to reach 1 kg (OS–) or 950 g (OS+) of food per day.

Brain imaging procedure

The week following the behavioural tests sessions, the nine animals from the three first batches underwent four brain imaging sessions each (one session per week and per animal) to investigate the brain metabolism following oral and duodenal stimulations. The brain imaging modality used to investigate cerebral blood flow (CBF) was the single photon emission computed tomography (SPECT) of technetium-99m (^{99m}Tc, CIS Bio International, France) coupled with hexamethyl-propylene-amine-oxime (HMPAO, Ceretec, GE Healthcare, Velizy, France).

Animal preparation and oral/duodenal stimulations. After a 12-h fasting period, the animals were anaesthetised in a quiet room and subjected to a tracheal intubation following the same

procedure as described above (see *Surgery* section). A venous catheter was inserted into their right ear in order to inject the radiolabel. Light and noise were set to a minimum at least 15 min before the start of oral and duodenal stimulations. Ears and eyes of the animals were sealed with cotton and surgical tape respectively, in order to minimize auditory and visual stimulations.

The animal underwent oral (OS) and duodenal (DS) stimulations before brain image acquisition. The experimental devices are illustrated in **Figure 2**. The OS was carried out by a computer-assisted automat designed in our laboratory (Gustautomat, INRA, St Gilles, France) and originally described in Gaultier et al. (2011). It consisted in irrigating the pig's tongue (24 mL/min) with an unsweetened (OS–) or a sweetened artificial saliva (OS+, 5% sucrose) (see Hellekant et al. 1997 for the saliva composition). A tube was positioned on the middle of the tongue and connected to the computer-operated automat developed in our laboratory (Gustautomat, INRA, St Gilles, France) and inspired by the Taste-o-Matic by Hellekant's group (Danilova et al. 2002). The DS was obtained by ID infusions of solutions. The DS+ corresponded to an ID infusion of 300 ml of 16% sucrose (197 kcal) and the control treatment corresponded to an ID infusion of 300 ml of 0.9% NaCl (DS–). The solutions were injected with a peristaltic pump connected to the duodenal catheter, and the injection rate was 10 ml per min.

The schematic representation of the experimental paradigm used for the oral/duodenal stimulations before brain imaging is described in **Figure 1B**. The delays between OS and DS were chosen in such a way that the oral sensing of sucrose (OS+) and the peak of maximum glycaemia after the ID sucrose infusion (DS+) were synchronised. Briefly, at least 15 min after the anaesthesia, the animals were subjected to the DS for 30 min. The OS was performed 47 min after the start of the DS. The OS+ consisted in a 3-min neutral oral stimulation (i.e. unsweetened saliva) to accommodate the mucosa thermoreceptors and mechanoreceptors to

the stimulation, preceding the diffusion of sweetened saliva for 2 min. The OS+ was ended by a 1-min neutral stimulation. The OS– consisted in a 6-min neutral stimulation with unsweetened saliva.

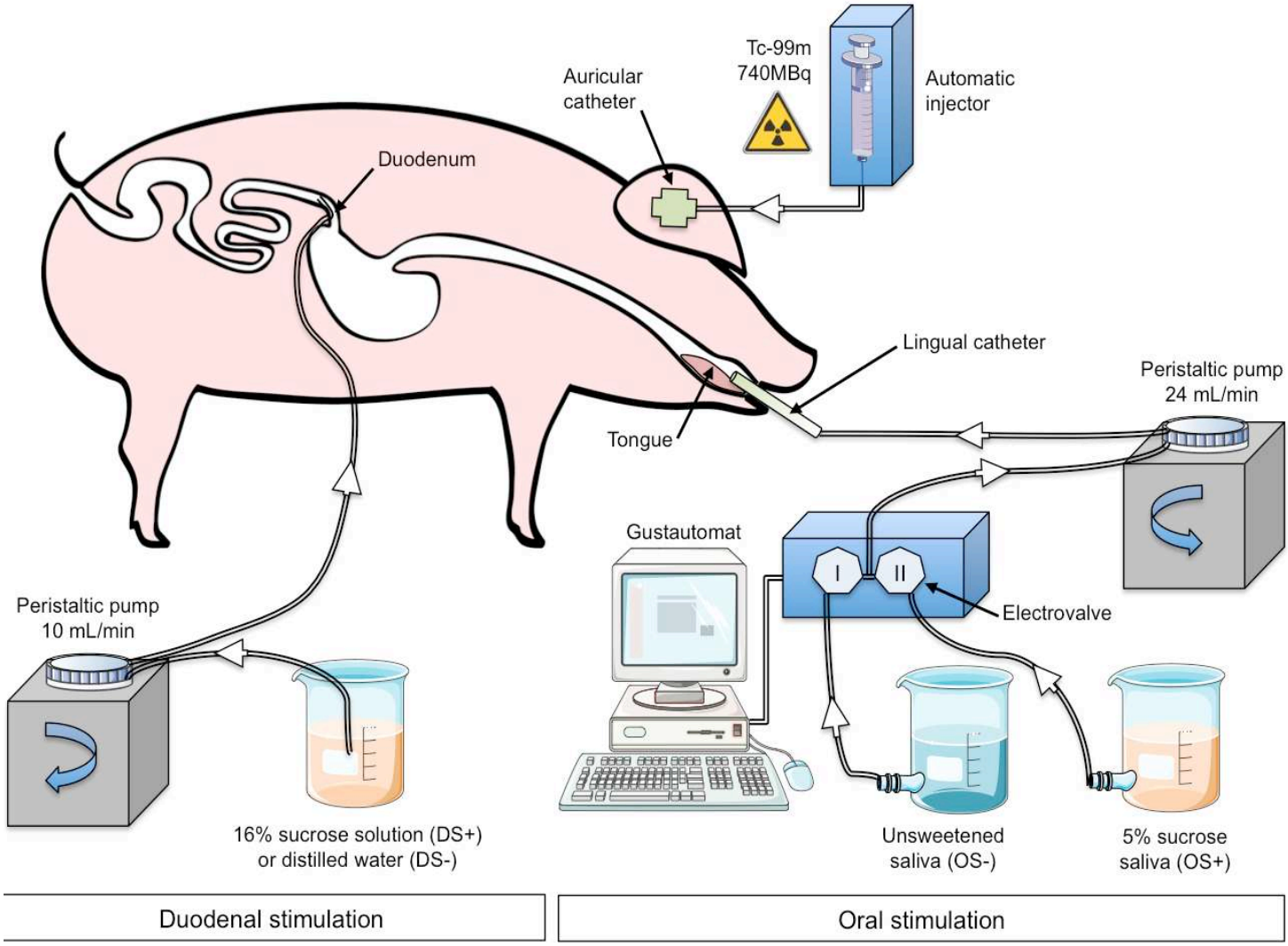

Figure 2. Experimental device and paradigm designed to perform oral (OS) and duodenal (DS) stimulations for brain imaging in anaesthetised pigs. The illustrations used to make this figure were obtained from the "Servier Medical Art" website, http://www.servier.fr/servier-medical-art. OS-: oral infusion of a neutral artificial saliva OS+: oral infusion of artificial saliva added with 5% sucrose DS-: duodenal infusion of distilled water DS+: duodenal infusion of 16% sucrose solution. Injection of ^{99m}Tc-HMPAO was performed 50 min after the start of the DS.

Radiolabel administration and image acquisition. As the plasma glucose peak occurs approximately 50 min after the start of the DS+ (**Figure 1A**), the radiolabel (^{99m}Tc-HMPAO, 740 MBq) was injected 50 min after the start of the DS, i.e. 3 min after the start of the OS.

At least 15 min after the radiolabel injection, the anaesthetised animals were transferred and placed in a Head First Prone position on the bed of a gamma-camera (APEX SP-6, Elscint, Tel-Aviv, Israel) fitted with a fan-beam collimator (50-cm focus). Brain image acquisitions were performed at least 20 min after the radiolabel injection, when complete brain-blood equilibrium is reached (Thomsen et al. 2008). SPECT data were acquired on a gamma-camera fitted with a fan-beam collimator (50-cm focus). Sixty projections with a 120-s exposition were acquired at different projection angles (6° per step). Transaxial images were reconstructed using the filtered back projection method (FBP) applying a Metz filter (power parameter q = 3). An acquisition matrix size of 128 x 128 was used and spatial resolution of the final images was 0.6 mm per pixel for x- and y-axis and 1.47 mm per pixel in z-axis.

Image processing. The images were processed with statistical parametric mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB 7.9 R2009B (The Mathworks Inc., Natick, MA, USA). SPM8 software was adapted to the characteristics of the pig brain.

MRI and SPECT template images were used as reference images for the processing of the SPECT images acquired in the present study. Sixteen female pigs of the same age and breed of those used in this study were used to build those MRI and SPECT template reference images. SPECT images were acquired as described above. MRI images were used as an anatomical reference for the identification and localisation of brain structures and clusters of differential activation. The procedure of the MRI images acquisition has been described in previous papers of our laboratory (Boubaker et al. 2012; Gaultier et al. 2011).

In the present study, the actual original DICOM files were converted in NIfTI files with the IMAGEJ software (Wayne Rasband NIH, Besthesda, MA, USA) and the images were manually reoriented (pitch = -1.57077, roll = 3.14159, z = 2.946 mm). The spatial coordinates were centred compared to a reference point (x_0, y_0, z_0) set at CP (*commissura posterior*) in the CA-CP plane (*commissura anterior-commissura posterior*) according to the stereotactic reference defined by Saikali et al. (2010). The images were then co-registered using the inter-modal registration function of SPM8. After co-registration, the images were spatially normalized using the SPECT template image. Normalisation was restricted to 16-parameter affine transformations in order to minimize deformations of the original images. Following normalisation, tri-linear interpolation was performed with a final voxel size of 2 x 2 x 2 mm. Finally, the spatially normalized images were masked using a brain mask image to remove the extracerebral matter, and smoothed using a Gaussian smoothing kernel with the full width at half maximum (FWHM) set at 5 x 5 x 5 mm. The brain mask image was obtained by thresholding the MRI template, and smoothing this image with a Gaussian kernel filter with FWHM set at 4 x 4 x 4 mm.

Statistical Analysis

Statistical behaviour analysis. During the PR sessions, total number of rewards earned, total number of button pushes, pushes rate (pushes/min), breakpoint (the number of pushes required for the last reward earned) and the post-reward pause (the average time elapsed between the delivery of a reward and the next push) were measured. To examine the effect of treatment on pigs' performance, data were averaged for each treatment over the two PR sessions and analysed using one-way within subjects ANOVA with the R 2.14.1 software (the R Foundation for Statistical Computing, Vienna, Austria).

Statistical image analysis. The regional ^{99m}Tc-HMPAO uptake was standardized to the mean global uptake using proportional scaling. The SPECT images acquired after oral and/or post-

oral stimulations were analysed with SPM8 implemented in MATLAB 7.9 R2009B. A full factorial analysis followed by paired *t*-tests was performed to compare the effects of oral and/or duodenal sucrose sensing on brain metabolism. A total of six contrasts were analysed: 1) oral and duodenal sucrose sensing vs. control (OS+DS+ / OS–DS–); 2) oral sucrose sensing vs. control (OS+DS– / OS–DS–); 3) duodenal sucrose sensing vs. control (OS–DS+ / OS–DS–); 4) oral vs. duodenal sucrose sensing (OS+DS– / OS–DS+); 5) oral sucrose sensing in the presence vs. in the absence of duodenal sucrose sensing (OS+DS+ / OS+DS–); and (6) duodenal sucrose sensing in the presence vs. in the absence of oral sucrose sensing (OS+DS+ / OS–DS+). Variances were considered unequal. The dependency and heteroscedasticy induced different error covariance components that were estimated using REML (Restricted Maximum Likelihood) and used to adjust the statistics and degrees of freedom during inference. By default, SPM uses weighted least squares to produce Gauss-Markov or Maximum likelihood estimators using the nonsphericity structure specified at this stage (cf. SPM8 User Manual). A Small Volume Correction (SVC) analysis was performed with SPM8. This analysis allowed for voxel to voxel comparisons within restricted Regions Of Interest (ROIs) selected upon the *a priori* hypotheses presented in the introduction. With this analysis, we managed to identify within specific ROIs the voxels for which the activity was statistically different between treatments. An uncorrected value of $P = 0.05$ was set as the threshold (extent threshold of five voxels).

The statistical analysis with SPM8 produced a listing of voxels that corresponded to peaks of maximum intensity for which the CBF differed between treatments in each ROI. Each voxel/peak was associated with a set of coordinates (x y z) corresponding to its spatial location in the CA-CP (*commissura anterior-commissura posterior*) plane with CP set as the origin. The ROIs chosen for the SVC analysis were anatomically identified on the basis of a 3D digitized pig brain atlas developed in our laboratory (Saikali et al. 2010), and representa-

tion of the clusters with different metabolism was performed using 3DSlicer (http://www.slicer.org/). *A priori* ROIs were the orbitofrontal, dorsolateral and anterior prefrontal cortices and the cingulate cortex, the hippocampus, the amygdala, the dorsal striatum (caudate and putamen), the nucleus accumbens and the globus pallidus. These regions have been known to be involved in the hedonic evaluation of sensory stimuli in humans and pigs. Additionally, the SVC analyses were performed in *a priori* ROIs that corresponded to brain networks known to participate in the primary recognition of gustatory stimuli (insular cortex and perirhinal cortex) and olfactory stimuli (olfactory bulb, piriform cortex, prepiriform area, anterior entorhinal cortex and parahippocampal cortex).

Results

One animal out of the 12 fell ill during PR operant conditioning and has been excluded from behavioural and brain imaging analyses. Another animal died during brain imaging and has been excluded from brain imaging analyses. Consequently, 11 animals were used for PR data analyses and 7 animals were used for brain imaging analyses.

Progressive ratio performance

The animals' performances averaged over the two PR sessions for each treatment are shown in **Table 1**. The one-way within subjects ANOVA showed no effect of the treatment on the total number of reward earned ($F(2,20) = 0.09$, $P = 0.91$), the total number of pushes ($F(2,20) = 0.07$, $P = 0.94$), the pushes rate ($F(2,20) = 0.99$, $P = 0.39$), the breakpoint ($F(2,20) = 0.09$, $P = 0.92$), and the post-reinforcement pause ($F(2,20) = 0.81$, $P = 0.46$).

Table 1 Pigs' performance during progressive ratio (PR) operant-conditioning. Variables were recorded during the 4-h PR tests. All data are expressed as the average data (± s.e.) of the two PR sessions for each treatment and each animal. OS+DS+: 5% sucrose diet paired with duodenal infusion of 16% sucrose, OS–DS+: unsweetened standard diet paired with duodenal infusion of 16% sucrose, OS–DS–: unsweetened standard diet paired with duodenal saline infusion (control).

Variables	Treatments			One-way ANOVA		
	OS+DS+	OS–DS+	OS–DS–	F-value	*P*-value	
Total number of reward earned	46 ± 4	44 ± 4	45 ± 4	0.09	0.91	n.s.
Total number of pushes	1837 ± 301	1765 ± 328	1789 ± 331	0.07	0.94	n.s.
Pushes rate (pushes/min)	10.3 ± 2.4	9.3 ± 1.9	8.6 ± 1.5	0.99	0.39	n.s.
Breakpoint (pushes)	74 ± 7	72 ± 6	73 ± 6	0.09	0.92	n.s.
Post-reward pause (s)	66 ± 14	73 ± 19	80 ± 19	0.81	0.46	n.s.

Brain imaging

The regions of differential CBF values obtained with the SVC analyses on the contrasts are summarized in **Table 2**.

Table 2 Peaks of differential cerebral blood flow identified during the SVC analyses in different regions of interest chosen upon *a priori* hypotheses for each type of contrasts. The threshold for significance was set at $P < 0.05$ (uncorrected). Peak *t* indicates the *t*-value of the peak of maximal intensity for each cluster, and x y z indicate the stereotaxic coordinates (in mm) of the peak in the CA-CP (*commissura anterior-commissura posterior*) plane with CP set as the origin. OS+: oral 5% sucrose stimulation. L: left, R: right, OS–: oral non-sweet stimulation, DS+: duodenal 16% sucrose stimulation, DS–: duodenal saline stimulation.

		Oral and duodenal sucrose vs. control		Oral sucrose vs. control		Duodenal sucrose vs. control		Oral sucrose vs. duodenal sucrose		Duodenal sucrose with vs. without oral sucrose	
		OS+DS+ vs. OS–DS–		OS+DS– vs. OS–DS–		OS–DS+ vs. OS–DS–		OS+DS– vs. OS–DS+		OS+DS+ vs. OS–DS+	
		Peak *t*	x y z	Peak *t*	x y z	Peak *t*	x y z	Peak *t*	x y z	Peak *t*	x y z
Amygdala	L	1.97	-16 7 -4								
Amygdala	R										
Anterior entorhinal cortex	L										
Anterior entorhinal cortex	R									1.80	12 -1 -5
Anterior prefrontal cortex	L	1.75	-4 29 -8								
Anterior prefrontal cortex	R	1.84	4 33 -8								
Caudate nucleus	L										
Caudate nucleus	R	1.75	4 27 -4								
Hippocampus	L	1.76	-12 6 -6								
Hippocampus	R									1.72	14 0 -4
Olfactory bulb	L	2.92	-6 50 -4	2.36	-6 50 -4	1.91	-6 49 -4				
Olfactory bulb	R	2.49	2 50 -5	2.05	2 50 -4						
Parahippocampal cortex	L	1.94	-18 7 -4								
Parahippocampal cortex	R									1.72	16 -2 -5
Perirhinal cortex	L	1.81	-14 6 -8								
Perirhinal cortex	R	1.88	12 6 -9							1.80	12 -2 -5
Piriform cortex	L	2.01	-16 8 -6								
Piriform cortex	R	1.85	14 7 -8								
Prepiriform area	L	1.85	-6 30 -9							1.75	-10 29 -3
Prepiriform area	R	1.81	6 32 -10								
Putamen	L							1.71	-6 25 -3		
Putamen	R	1.73	6 26 -4								

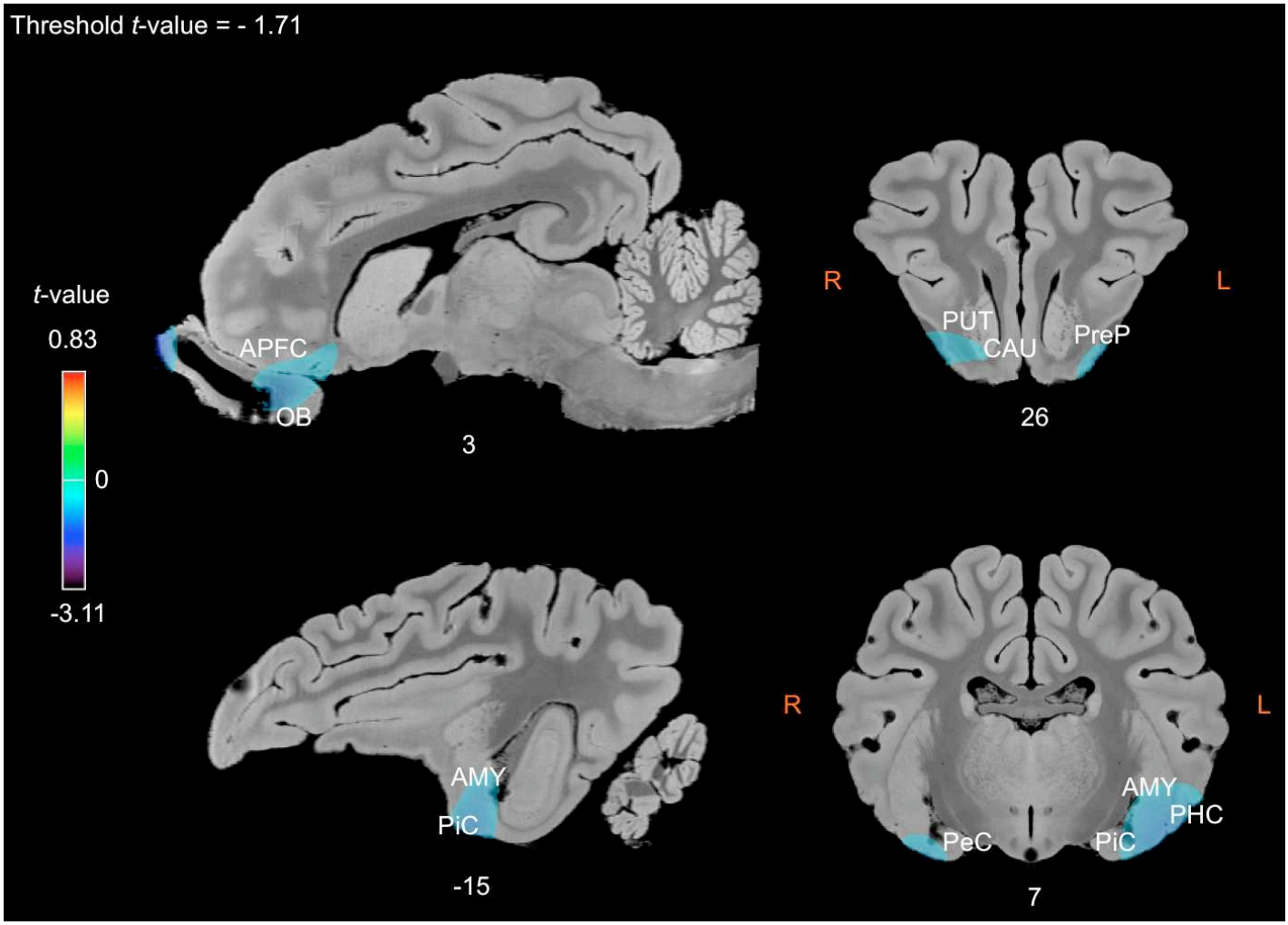

Figure 3 Sagittal and coronal MRI section showing clusters of differential cerebral blood flow identified during the SVC analyses in different regions of interest chosen upon *a priori* hypotheses for the OS+DS+/OS–DS– contrast, that is the comparison between the combined sensing of oral and duodenal sucrose and the control. The x or y coordinates are indicated below the images. The threshold for significance was set at $P < 0.05$ (uncorrected). L: left, R: right, APFC: anterior prefrontal cortex, AMY: amygdala, CAU: caudate, OB: olfactory bulb, PeC: perirhinal cortex, PHC: parahippocampal cortex, PiC: piriform cortex, PreP: prepyriform area, PUT: putamen.

Oral and duodenal values *sucrose sensing vs. control (i.e. OS+DS+/OS–DS– contrast).* The regions of differential CBF obtained with the SVC analysis on the OS+DS+/OS–DS– contrast are presented in **Figure 3**. Compared to control, the combined perception of oral and duodenal sucrose induced differential activation in clusters located bilaterally in the anterior

prefrontal cortex, as well as in structures of the olfactory system, including the olfactory bulb, the prepiriform area, the piriform and the perirhinal cortices. In the left hemisphere, clusters of differential CBF were found in the hippocampus, the amygdala and the parahippocampal cortex, while in the right hemisphere, clusters of differential CBF were found in the dorsal striatum, i.e. the caudate and the putamen.

Oral (i.e. OS+DS–/OS–DS– contrast) or duodenal sucrose sensing (i.e. OS–DS+/OS–DS– contrast) vs. control. Compared to control, oral sucrose sensing or duodenal sucrose sensing did not induce significant differences in brain metabolism, except for the OB in which clusters of differential activation were found both during oral and during duodenal sucrose sensing compared to control.

Oral vs. duodenal sucrose sensing (i.e. OS+DS–/OS–DS+ contrast). Compared to each other, oral sucrose sensing induced differential CBF in the left PUT compared to duodenal sucrose sensing (**Figure 4A**).

Duodenal sucrose sensing in the presence vs. in the absence of oral sucrose stimulation (i.e. OS+DS+/OS–DS+ contrast). Compared to a situation where no oral sucrose stimulation was performed, the perception of duodenal sucrose in the presence of oral sucrose stimulation triggered differential CBF in structures of the olfactory system, including the right perirhinal cortex, the right parahippocampal cortex, the right anterior entorhinal cortex and the left prepiriform area. Clusters of differential activation were also found in the right hippocampus (**Figure 4B**).

Oral sucrose sensing in the presence vs. in the absence of duodenal sucrose stimulation (i.e. OS+DS+/OS+DS– contrast). The presence or the absence of duodenal sucrose stimulation during the perception of oral sucrose did not induce any significant changes in CBF.

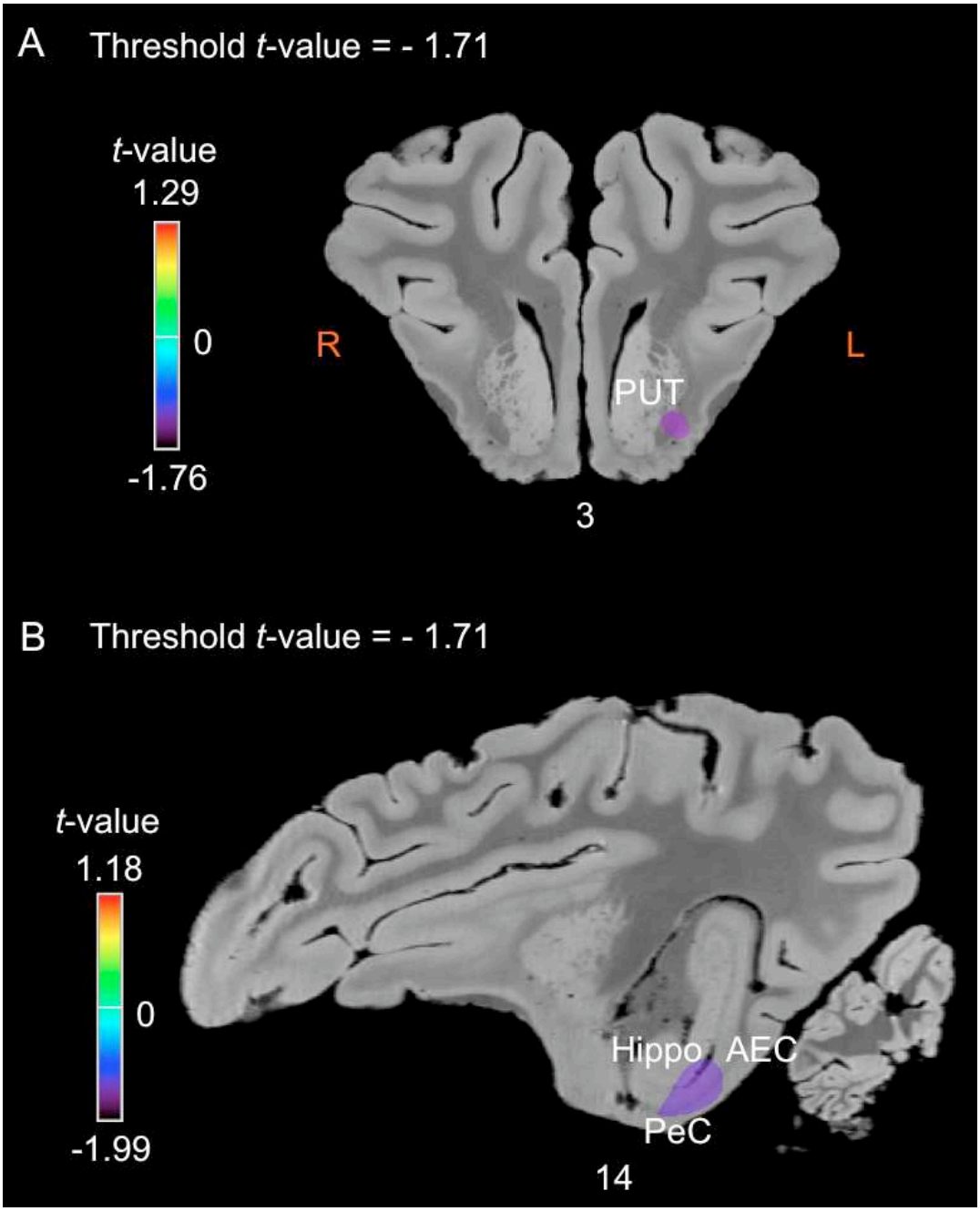

Figure 4 Sagittal and coronal MRI section showing clusters of differential cerebral blood flow identified during the SVC analyses in different regions of interest chosen upon *a priori* hypotheses for the OS+DS–/OS–DS+ contrast, that is the comparison between oral and duodenal sucrose sensing (A), and the OS+DS+/OS–DS+ contrast, that is the comparison between duodenal sucrose sensing in the presence vs. in the absence of oral sucrose stimulation (B). The x or y coordinates are indicated below the images. The threshold for significance was set at $P < 0.05$ (uncorrected). L: left, R: right, Hippo: hippocampus, PeC: perirhinal cortex, PreP: prepiriform area, PUT: putamen.

Discussion

The major finding of the present study was that combined oral and duodenal sensing of sucrose induced changes in CBF in brain regions known to be involved in memory (hippocampus, parahippocampal and perirhinal cortices) and reward processes (amygdala, anterior prefrontal cortex, caudate and putamen) and in the identification of sensory stimuli (piriform and prepiriform cortices), whereas oral or duodenal sucrose sensing taken separately did not. These findings are in accordance with previous studies in both fasted and sated humans that revealed changes in activation in these regions further to sugar ingestion (glucose, Chambers et al. 2009) or during oral taste stimulation paired with caloric load (sucrose, Haase et al. 2009; Haase et al. 2011; Smeets et al. 2011).

Compared to control, combined oral and duodenal sucrose sensing induced differential activation in the amygdala, the dorsal striatum (caudate and putamen) and the anterior prefrontal cortex, regions that are known to be involved in reward processing (Schultz 2000). The anterior prefrontal cortex, which closely interacts with the amygdala and the striatum, is also known to be involved in the processing of food-related stimulations (Ramnani and Owen 2004), and to participate in motivation, memory and cognitive functions in humans (Cardinal et al. 2002; Kouneiher et al. 2009). Simons et al. (2005) reported that the anterior prefrontal cortex might participate in memory processes, and more specifically in 'the recollection of context details', that is in the association between a past event (e.g. food intake) and the contextual information relating to that event (e.g. internal state, subjective feelings, etc.). In the present study, the changes in brain activation in these regions might be due to the retrieval of contextual information arising from the PR tests, that is the recall of oral sensing of sucrose in the food reward paired with the duodenal sucrose sensing further to ID injection of sucrose.

The anterior prefrontal cortex also shares connexions with the dorsolateral prefrontal cortex, which is known to play a major role in the regulation of food intake, notably by mediating the inhibitory inputs send to the orexigenic network (Del Parigi et al. 2002; Gautier et al. 2000; Le et al. 2006). Consequently, oral sensing of the sweet taste of sucrose in the mouth paired with the calories uptake in the duodenum might have induced metabolic changes in the prefrontal cortex to mediate the termination of food intake. Conversely, action on the orexigenic network is likely not required during oral sucrose sensing in a situation where the animal did not perceive calories in its duodenum, or after a caloric uptake when no sweet taste stimulation is performed, which might explain the lack of differential activation of these structures further to independent oral or duodenal sucrose sensing. This assumption is supported by the changes in activation found in the hippocampus and the parahippocampal cortex, structures that are thought to be implicated in the physiological state of hunger (Tataranni et al. 1999) and in the integration of subjective internal states with relevant sensory cues (LaBar et al. 2001). Altogether, these findings suggested that the changes in activation found in these regions during the processing of congruent oral and duodenal sucrose signals might be mediated by the concordance between the sweet signals perceived in the mouth and the internal clues (calories) perceived in the duodenum, as suggested by Haase et al. (2009).

In addition to changes in activation in the aforementioned reward and memory regions, we managed to highlight differential CBF in the olfactory bulb, the prepiriform area, the piriform cortex, the parahippocampal cortex and the perirhinal cortex further to oral and duodenal sucrose sensing. The prepiriform area and the piriform cortex receive a large amount of afferent fibres from the olfactory bulb and are thus recognized as major regions of the primary olfactory cortex. The primary olfactory cortex is connected to several cortical regions, including the parahippocampal cortex, which is part of the secondary olfactory cortex (Gottfried 2010; Soudry et al. 2011). In the present study, the changes in activation found in olfactory

regions was quite unexpected given the absence of odour sensing during imaging, although, Boubaker et al. (2012) found similar results in pigs and revealed activation in the prepiriform area during duodenal glucose infusions in the absence of odour or oral sensing.

Unlike the other sensory areas (e.g. taste, vision, etc.), the piriform cortex had numerous reciprocal projections in cortical structures known to be involved in cognition, emotion and memory, including the amygdala, the prefrontal cortex, and the perirhinal cortex (Johnson et al. 2000), in which we managed to highlight significant differences in activation during oral paired with duodenal sucrose sensing. Based on this fact, Johnson et al. (2000) assumed that, unlike the other primary sensory areas, the piriform cortex was rather involved in associative processes than primary sensory processes. In the present study, changes in activation in the piriform cortex, in association with that in the perirhinal cortex, the amygdala and the anterior prefrontal cortex, might result rather from associative processes between duodenal and oral signals than from olfactory information processing, which might explain the lack of differential response in these regions when the oral and duodenal stimulations are performed separately.

The parahippocampal and perirhinal cortex also share close connexions with the hippocampus, a structure widely known to be involved in high cognitive functions. The hippocampus supports memory and cognitive functions and also participates in emotional processes (for a review, see Fanselow and Dong 2010). In rodents, the perirhinal cortex, which is part of the parahippocampal cortex, send sensory information to the hippocampus, directly or via the entorhinal cortex (Furtak et al. 2007; Kerr et al. 2007), suggesting the involvement of those structures in learning and memory processes. Besides, as reported beforehand, the piriform cortex has important connexions with the prefrontal cortex, the amygdala, as well as the perirhinal and entorhinal cortices, which are involved in sensory stimuli associative processes, motivation, cognition and memory (Johnson et al. 2000). Consequently, changes in CBF

in the hippocampus, parahippocampal and perirhinal cortices during oral and duodenal sucrose sensing suggest that the processing of duodenal and oral sucrose signals during imaging were mediated by regions involved in memory and emotion. One might assume that these changes in activation were related to the recall of oral and duodenal sucrose sensing during the previous PR tests.

In the present study, differential activation in structures known to be involved in memory and emotion processing (for a review, see Fanselow and Dong 2010) were also found during duodenal sucrose sensing depending on whether oral sucrose stimulation was performed or not. That included the parahippocampal, perirhinal and anterior entorhinal cortices and the prepiriform area. Clusters of differential activation were also found in the right hippocampus. Contrarily, no difference was found between oral sucrose sensing in the presence or in the absence of duodenal sucrose stimulation. These findings suggest that, although independent oral or duodenal sucrose sensing was not sufficient on their own to induce metabolic changes in these regions, oral signals seems to be more determinant for recall and memory processes than visceral signals during the processing of sugar sensing. One might assume that oral sucrose sensing during brain imaging triggered recall processes relative to the oral sensing of sucrose in food reward during PR tests, and that this recall was independent of duodenal signals.

Although the combined sensing of oral and postoral sucrose induced changes in CBF in various brain regions, we failed to highlight differential CBF further to oral or duodenal sucrose sensing compared to a control situation. These findings might suggest that: (1) the combination and the concordance between oral and visceral signals during sugar sensing is necessary for the onset of brain responses in structures involved in the hedonic evaluation of sensory stimuli, or (2) the synergy between oral and visceral signals during sugar sensing is necessary to obtain a signal that is sufficiently substantial and relevant to trigger brain responses in the-

se structures. Though, some authors managed to highlight differential brain responses in the aforementioned structures further to separated oral or visceral sugar sensing. In a recent study, Boubaker et al. (2012) found that duodenal infusions of glucose in the absence of oral glucose sensing induced differential CBF responses compared to a control stimulation in pigs. They reported clusters of differential CBF in the prepiriform area, the anterior entorhinal cortex, the dorsolateral and anterior prefrontal cortices, the orbitofrontal cortex, the hippocampus, as well as in the dorsal striatum. In terms of oral sugar sensing, Frank et al. (2008) also reported specific changes in brain metabolism further to sucrose oral sensing in sated women. Compared to a control situation, the oral sensing of sucrose triggered differential activation in the insular and the anterior cingulate cortices, the nucleus accumbens and the caudate nucleus. In fasted humans, ingestion of saccharine, an artificial noncaloric sweetener, also induced changes in brain metabolism in the insular cortex and the dorsolateral prefrontal cortex, while ingestion of maltodextrin, a caloric non sweet compound, triggered activation in the insular, the orbitofrontal and dorsolateral prefontal cortices and in the caudate nucleus (Chambers et al. 2009).

Contrasted findings might be attributed to discrepancies between studies' experimental paradigms. Numerous studies reported that brain activation was modified by the internal state of the subjects, i.e. hunger or satiety, at the time of imaging. Using PET, Tataranni et al. (1999) found an increased CBF in regions involved in the homeostatic regulation of food intake (hypothalamus), in taste recognition and hedonic evaluation (insular cortex, orbitofrontal cortex), in emotion and memory processing (anterior cingulate cortex, caudate and putamen parahippocampal cortex, hippocampus) in a hunger state compared to a situation where the subjects had ingested a caloric meal before imaging. Using fMRI, Haase et al. (2009) reported that, in the hunger and sated state, sucrose ingestion induced changes in activation in similar regions, including the cingulate and parahippocampal cortices and the amygdala but also in different

regions, including the insular and orbitofrontal cortex and the hippocampus. Haase et al. (2009; 2011) also reported that there was significantly greater responses in the hunger than in the sated condition during sucrose stimulation in taste regions (orbitofrontal cortex, insular cortex), in regions that participate in emotion processing (amygdala, caudate, cingulate cortex) and memory (hippocampus, parahippocampal cortex). Altogether, these findings suggest that internal state play a major role on brain activation during food-related stimulations, although most studies seem to support the fact that higher brain responses emerged in the hunger state compared to the sated state.

In the present study, the absence of differential activation between a situation where pigs received duodenal sucrose infusions and a control situation might also be due to the fact that the caloric load supplied by sucrose infusions, that is 197 kcal, was too low. In the studies of Haase et al. (2009; 2011), participants were subjected to fMRI before or after the ingestion of a 700 kcal liquid meal, to investigate the effect of hunger and satiety on brain activation triggered by oral sucrose sweet taste sensing. In the study of Smeets et al. (2005), the participants were asked to ingest 300 mL of a 75 g-glucose solution (300 kcal), 300 mL of aspartame solution or 300 mL of maltodextrin solution (300 kcal) to investigate the hypothalamic responses to sweet taste and calories. Chambers et al. (2009) used 90 g- (360 kcal) and 180 g-glucose (720 kcal) solutions in fasted humans to investigate the effect of carbohydrates oral sensing on brain activity. Consequently, the large majority of imaging studies in humans used higher energy content than that used in the present study, and we cannot rule out the possibility that a higher energy content might have resulted in more clusters of significant differential activation.

Although we highlighted differential activation in brain regions involved in reward processing, motivation, memory processes, and hedonic regulation of food intake, we did not manage to demonstrate any increase in the motivational responses for a food reward sweet-

ened with 5% sucrose compared to an unsweetened food reward, in a situation of duodenal sucrose administration during PR tests. We assumed that this result was attributable to the fact that the pigs were food-deprived and hungry at the start of the PR tests, the caloric load corresponding to the duodenal 16% sucrose (197 kcal) being too low to induce significant satiety effects. Given that the animals were hungry, the motivational responses driven by hunger may have overridden the motivational responses that were driven by pleasure, the pigs being highly motivated to earn the reward, whether the food was sweetened or not. These results suggest that in food-deprived animals, homeostatic factors might be more determinant for the regulation of feeding behaviour than hedonic factors. However, it worth noticing that, although the behavioural motivational responses of hungry pigs for sweet reward paired with ID infusion of sucrose were rather mediated by homeostatic than hedonic factors during PR tests, at the central level, we managed to highlight changes of activation in the regions known to be involved in reward processing and in the hedonic evaluation of food stimuli further to oral and duodenal sugar sensing. These findings suggest that sugar signals processing is not only driven by homeostatic factors, but that hedonic factors are involved as well. Further studies on sated animals, or using a more caloric duodenal infusion of sucrose might enable to highlight the impact of hedonic factors on the behavioural motivational responses of pigs during PR tests, and demonstrate the competing effects between homeostatic (negative alliesthesia) and hedonic (reward palatability) regulation of food intake.

In conclusion, we revealed that paired, but not separate, oral and duodenal sensing of sucrose induced changes in activation in regions involved in reward processing, memory and associative processes and hedonic evaluation of sensory stimuli in hungry pigs. Though, no difference in motivational responses for a sweetened or unsweetened food reward was found during PR tests before brain imaging. Altogether, these data suggest that, although the behavioural motivational responses of food deprived pigs for a food reward during PR tests seemed

to be predominantly driven by homeostatic factors, at the central level, the processing of oral and duodenal sugar signals appeared to be also mediated by hedonic factors, and not only by homeostatic factors. Further studies should consider investigating whether changes in the internal state from hunger to satiety would induce differential motivational and brain responses in pigs so as to highlight correlates between the behavioural and cerebral processing of oral and duodenal sucrose sensing.

Acknowledgments

This study was supported by a grant from INRA (Department of Human Alimentation; Department of Animal Physiology and Livestock Systems) and the Brittany Region (Région Bretagne), and notably by the CRITT Santé Bretagne (Centre Régional d'Innovation et de Transfert de Technologies). The authors wish to thank Servier Medical Art and Henri Flageul for providing the illustrations used in this paper. The authors gratefully acknowledge the efforts and cooperation of the scientific and technical staff, and especially Sylvie Guérin, Alain Chauvin, Régis Janvier, Francis Legouevec and Vincent Piedvache for participating in this study and/or caring for the animals.

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CHAPITRE IV

Utilisation d'Additifs Alimentaires pour le Développement de Préférences Spontanées chez le Porc

CHAPITRE IV : UTILISATION D'ADDITIFS ALIMENTAIRES POUR LE DÉVELOPPEMENT DE PRÉFÉRENCES SPONTANÉES CHEZ LE PORC

Grâce à des techniques expérimentales de conditionnement alimentaire, nous avons démontré dans les chapitres précédents qu'il était possible de modifier les choix alimentaires *via* l'association entre des aliments (ou eau de boisson) et les conséquences de leur ingestion (goût plaisant, apport calorique, malaise gastrique). Ces modifications du comportement et des choix alimentaires sont par ailleurs accompagnées de changements du métabolisme cérébral dans des structures extra-hypothalamiques qui, chez l'Homme, sont impliquées dans la reconnaissance primaire et dans le traitement hédonique des informations olfacto-gustatives, dans les processus de récompense et de motivation et, de manière plus générale, dans la régulation hédonique de la prise alimentaire.

Cependant, outre les phénomènes d'association entre l'aliment et les conséquences passées de sa mise en bouche et/ou de son ingestion, existe-t-il d'autres moyens pour modifier le comportement alimentaire ? Chez l'Homme, certaines odeurs spécifiques peuvent améliorer l'appétit *via* une modification de la sensitivité olfactive aux aliments. Chez les animaux d'élevage, certains additifs alimentaires, flaveurs, huiles essentielles ou facteurs d'appétence peuvent modifier la palatabilité et les propriétés organoleptiques des aliments. Ainsi, dans le dernier chapitre, nous nous pencherons sur l'impact d'additifs alimentaires supposés stimuler l'appétit, sur l'évolution de la consommation et des préférences alimentaires chez le porc. Nous nous intéresserons ici à des ingrédients fonctionnels sensoriels spécifiques qui pourront soit entraîner une modification des signaux de satiété (sphère viscérale), soit modifier la palatabilité de l'aliment *via* un changement de ces propriétés organoleptiques (sphère sensorielle). Deux études ont donc été menées afin d'évaluer les effets des noyaux fonctionnels (*Partie 1*) et l'impact de leur concentration sur les effets observés (*Partie 2*).

Partie 1. Effets de noyaux fonctionnels sur les préférences, la prise alimentaire et la croissance chez le porc juvénile (article n°6)

1. Contexte et objectifs

Les additifs sensoriels sont des composés supposés améliorer la palatabilité des aliments et induire des effets positifs sur l'appétit et/ou les performances des animaux. Dans les élevages porcins, lors de l'exposition à un aliment nouveau lors des transitions alimentaires, les animaux montrent fréquemment une diminution de leur prise alimentaire qui peut conduire à une croissance perturbée. La caractérisation d'ingrédients sensoriels fonctionnels modulant la prise alimentaire pourrait donc s'avérer utile en élevage porcin pour le maintien de la consommation et de la prise de poids pendant ces phases sensibles de transition alimentaire.

L'objectif de cette étude était de tester la palatabilité de huit additifs alimentaires (mélanges d'huiles essentielles et d'extraits végétaux) et d'identifier les additifs qui pourraient améliorer la prise alimentaire et le gain de poids chez des porcs sevrés par le biais de l'expression de préférences alimentaires spontanées. L'hypothèse de ce travail est que l'ajout des additifs dans l'aliment favorisera la consommation vis-à-vis de l'aliment *via* une action des ingrédients sensoriels fonctionnels sur la sphère viscérale (satiété) ou gustative (plaisir à la mise en bouche).

2. Méthodes

Dans l'expérience 1, douze femelles juvéniles ont été soumises à des tests d'aliment unique ou à des tests de double choix alimentaire pour évaluer leurs préférences spontanées entre un aliment standard et les neuf aliments expérimentaux composés de l'aliment standard additionnés des additifs alimentaires (N1–N8) aux concentrations choisies. Dans l'expérience 2, douze femelles juvéniles ont été soumises à des tests de double choix alimentaires pour évaluer leurs préférences relatives pour les quatre additifs sélectionnés à l'issue de l'expérience 1. Dans l'expérience 3, la prise alimentaire spontanée de 24 femelles juvéniles sevrées a été évaluée pendant une phase de transition alimentaire pour évaluer l'impact des additifs sélectionnés (N1 et N3) sur la prise alimentaire. Après neuf jours d'aliment 1er âge standard, les animaux ont été divisés en trois groupes expérimentaux et nourris pendant 19 jours avec trois régimes différents composés d'un aliment 2ème âge inconnu additionné de l'additif N1 (groupe N1), de l'additif N3 (groupe N3) ou sans additif (contrôle).

3. Résultats

Dans l'expérience 1, les animaux ont consommé moins d'aliment N2A, N5, N6 et N7 et N8 que de l'aliment contrôle standard ($P < 0,05$) pendant les tests d'aliment unique et de double choix alimentaire, suggérant ainsi que ces additifs diminuent la palatabilité de l'aliment et la prise alimentaire des animaux, à ces concentrations. Aucune différence de consommation n'a été mise en évidence pour les additifs N1, N2B, N3 et N4, ces ingrédients ont donc été sélectionnés pour l'expérience 2. Dans l'expérience 2, les aliments N3 ($P < 0,01$) et N4 ($P < 0,05$) étaient significativement préférés à l'aliment N2B. Comparé aux autres aliments, l'aliment N1 était globalement plus consommé pendant la première présentation et sa consommation restait élevée pendant les présentations successives, alors que la consommation globale de l'aliment N2B restait basse pendant l'ensemble des présentations. Dans l'expérience 3, aucune différence de consommation n'a été observée après la transition alimentaire entre les trois groupes expérimentaux ($P > 0,1$). Cependant, le jour de la transition, la consommation de l'aliment contrôle était 10,31% inférieure comparée à la consommation de l'aliment 1er âge le jour précédent la transition, alors que les consommations des aliments N1 et N3 étaient 5,46% inférieure et 3,77% supérieure, respectivement.

4. Conclusions

L'analyse des quantités d'aliment ingéré et des préférences individuelles et collectives obtenues dans les expériences 1 et 2 suggère que certains additifs sont plus palatables que d'autres et donc plus susceptibles de modifier les préférences alimentaires. L'établissement de préférences alimentaires semble cependant être soumis à une importante variabilité interindividuelle. La concentration des additifs dans l'aliment pourrait être un facteur important pour le développement de ces préférences. Les résultats de l'expérience 3 suggèrent que l'ajout des ingrédients fonctionnels dans un aliment nouveau n'améliore pas la prise alimentaire et la prise pondérale après la transition alimentaire, dans les conditions expérimentales de cette étude. Cependant, il semblerait que l'ajout de l'additif N1, et dans une moindre mesure N3, parvient à maintenir une consommation normale le jour de la transition, contrairement à l'aliment standard contrôle, suggérant une diminution de la réponse néophobique pour le nouvel aliment. Cependant, ces résultats ne sont pas significatifs et sont associés à une importante variabilité interindividuelle ; aussi des études supplémentaires sont nécessaires pour appuyer cette hypothèse.

Article 6

The effects of sensory functional ingredients on food preferences, intake and weight gain in juvenile pigs.

Caroline Clouard[1,2], Marie-Christine Meunier-Salaün[1,2], David Val-Laillet[1,2,*]

Applied Animal Behaviour Science, 2012, 138(1): 36-46

(Accepted 22 January 2012)

[1]*INRA, UMR Systèmes d'Élevage, Nutrition Animale et Humaine, F-35590 Saint Gilles, France*

[2]*Agrocampus Ouest, Systèmes d'Élevage, Nutrition Animale et Humaine, F-35000 Rennes, France*

*Corresponding author: david.val-laillet@rennes.inra.fr

Abstract

When exposed to a novel diet during food transition, pigs often decrease voluntarily their food intake, which can lead to impaired growth. The aim of the present study was to test the palatability of eight unknown food additives (mixture of various essential oils and other plant extracts) and to identify among them the additives that were likely to improve food preferences, intake and weight gain in juvenile pigs. In Experiment 1, 12 juvenile female pigs were subjected to one- and two-way choice tests to investigate their spontaneous preferences between a standard grower diet and nine experimental diets composed of the familiar grower diet added with the sensory functional ingredients (N1-N8) at predefined concentrations. As the control diet was preferred over the N5, N6, N7 and N8 diets, only the N1, N2, N3 and N4 additives were selected for Experiment 2. In Experiment 2, 12 juvenile female pigs were subjected to two-choice feeding tests to investigate their relative preferences between the four remaining experimental diets. Overall, the analysis of individual and collective consumption and preferences suggests that some additives might be more palatable than others, at the tested concentrations, and that preferences are subjected to a great inter-individual variability. Experiment 2 enabled to identify the N1 and N3 additives as the best candidates for Experiment 3. In Experiment 3, the spontaneous food intake of 24 juvenile female pigs was assessed during a food transition. After nine days during which they were fed a familiar starter diet, the pigs were divided into three experimental groups and fed an unfamiliar grower diet added with either the N1 additive, the N3 additive, or with no additive (control). Albeit the adjunction of the functional ingredients in the novel diet did not increase food intake or weight gain in these particular experimental conditions, it might enable to maintain a normal consumption the day of the food transition, contrary to the control diet, although the results were not significant. Further studies using complementary tests where a unique food is provided in controlled conditions, in addition to two-choice tests, might enable to investigate more accurately

the efficiency of the sensory additives to improve food palatability, preferences and intake in pigs.

Keywords: Essential oils; Food additive; Palatability; Pig; Stevia; Saponin

1. Introduction

Sensory additives are compounds aimed at improving the palatability and organoleptic properties of food, and inducing positive effects on appetite and/or performance of the animals (Regulation EU No 1831/2003). Recently, the use of plants and their extracts, such as essential oils, as feed additives for livestock production has gained great interest. Indeed, the ban of antibiotics in animal husbandry has led the scientific community to find alternative compounds to improve or maintain food intake and animal performances, including growth rate and meat production of pigs (Janz et al., 2007), cattle (Yang et al., 2010) or broilers chicken (Hernandez et al., 2004), reproductive performance of sows (e.g. see Meriden Animal Health reports) or milk yield of dairy cows (Kung et al., 2008; Alemu and Doepel, 2011). Numerous studies investigated the effects of feed additives based on essential oils on food intake and weight gain in various farm species. For instance, Javandel et al. (2008) reported that a garlic-flavoured meal (*Allium sativum*) does not affect daily food intake of broiler chickens, but that chicken fed with low garlic-concentrated diet have a higher weight gain than chicken fed with the same unflavoured diet. In cattle, the diet supplementation with a small dose of cinnamaldehyde, the main active component of cinnamon oil (*Cinnamomum sp.*), leads to a short-term increase of food intake (Yang et al., 2010). The effectiveness of feed additives has not been consistently observed in scientific literature yet (Jacela et al., 2010). This inconsistency arises in a large extent from a great variability between studies in terms of experimental design (e.g. one- *vs.* two-way choice tests), environmental context (e.g. individual

pens *vs.* group-housed animals), species (e.g. cattle, pigs, chicken) or individual characteristic (e.g. age, physiological stage). This variability made it difficult for us to compare the relative efficiency of different feed additives to modulate food intake and animals' performances.

In livestock production, pigs are frequently exposed to stressors during sensitive periods during which their feeding activity is strongly affected by unfamiliar feeding and environmental conditions (Meunier-Salaün and Picard, 1996). During the growth period for instance, different diets are formulated in order to satisfy the animals' energy and nutritive requirements, and piglets constantly have to face these diet changes paired with important modifications in their physical and social environment (e.g. early disrupted mother-litter bond, unfamiliar pens and conspecifics). Consequently, during these food transitions, a decreased voluntary food intake is often reported and causes impaired growth, until pigs adapt to and accept the novel feeding and environmental conditions (Campbell, 1976; Dong and Pluske, 2007). Improving palatability of the novel food by the use of food additives might enable to increase food intake during food transition in juvenile pigs. Only a few studies, however, investigated the effects of feed additives based on essential oils on food intake and performances in pigs. Although some oils, including fennel (*Foeniculum vulgare*) or caraway (*Carum carvi*), oregano- or ginger-oils, did not improve food palatability and subsequent food intake (Jugl-Chizzola et al., 2006; Schöne et al., 2006; Janz et al., 2007; Windisch et al., 2008), garlic oil has been found to increase food intake of pigs (Janz et al., 2007). Based on the assumption that a preferred food is consumed in greater quantity, this last finding suggests that food additives based on essential oils might improve food palatability and the use of such additives may turn out to be a useful method to modulate appetite and food intake and preferences in pigs.

The aim of the present study was to assess the effects of eight sensory functional ingredients composed of various essential oils mixtures, other plant extracts and flavouring compounds

on the food intake, preferences and weight gain of juvenile pigs. The aim of Experiments 1 and 2 was twofold: (1) to test the palatability of eight unknown additives, as well as to assess the existence of spontaneous preferences; and (2) to select the most efficient sensory functional ingredients on the basis of the positive feeding and behavioural responses induced by their adjunction in food. In Experiment 3, we aimed at investigating the mid-term influence of the selected sensory functional ingredients on food intake and weight gain of pigs during a food transition period.

2. Experiment 1

The aim of Experiment 1 was to characterise the behavioural and feeding responses of pigs in the situation of one-way and two-way choice tests during the presentation of food added with the sensory functional ingredients *vs.* standard food. This may enable to assess the palatability of each compound and select the best candidates for being tested in a food transition situation.

2.1. Materials and methods

2.1.1. Animals and housing. Experiment 1 was carried out from September to October 2010 in the INRA experimental research station of Saint Gilles (France; 48° 09' 13'' N, 01° 49' 34'' W). A total of twelve 75 ± 2 day-old Large White/Landrace x Large White female pigs of 26.42 ± 0.18 kg at the beginning of the study were used. All the pigs were weaned at 28 ± 2 days of age and housed in groups from weaning. Before the experiments, i.e. 47 days post-weaning, the animals were moved into individual pens (132 x 122 cm; 12 pens per room) equipped with a removable two-part trough. The room was maintained at approximately 24°C with a natural day/night cycle. The animals were fed daily at 9:00h with a pelleted grower diet composed of 25% corn, 24.3% soybean meal, 23.2% wheat, 22.8% barley, 1.11% bi-calcic phosphate, 1% carbonate, 0.5% vitamin complement, 0.4% vegetal oil and 0.4% salt

(3.6% crude fiber, 2.31 Mcal/kg net energy). The food ration was 1 kilogram per day and per animal during the first week and then increased of 100 grams per week. The animals had free access to water.

2.1.2. Experimental meals. The present study was conducted using eight sensory functional ingredients (i.e. phytogenic products) provided by a commercial company specialised in functional sensory feed formulation (Phodé Laboratories, Terssac, France). The products were composed of various essential oils mixtures plus other defined plant extracts or flavouring compounds (Table 1). The sensory functional ingredients were labelled: N1, N2, N3, N4, N5, N6, N7 and N8. A total of 11 experimental pelleted diets were elaborated during the study. Two control diets were elaborated by the adjunction of 10 ml of water (C1) or vegetal oil (C2; Phodé Laboratories, Tessac, France) per kilogram of pelleted grower diet. Nine experimental diets were elaborated by the adjunction of the sensory functional ingredients diluted in water (N1 and N4) or in vegetal oil (N2A, N2B, N3, N5, N6, N7 and N8) at predefined concentrations and according to the solubility properties of each ingredient, with 10 ml of product per kilogram of pelleted grower diet. The choice of the concentrations tested for each additive was done by our industrial partner (Phodé Laboratories) and was justified by previous unpublished findings obtained in their laboratory on human panels and farm species, such as poultry. After a preliminary study, we decided to test the N2 additive at high (A) and low (B) concentrations. The concentrations tested for each additive, as well as a brief description of the ingredients composition are indicated in Table 1.

Table 1. Brief description of the sensory functional ingredients composition and concentration tested (ml/kg of food).

Nucleus	Composition	Concentration
N1	Extract of *Stevia rebaudiana* (stevia) and high-saponin plant	3.7 ml/kg
N2A	Extract of *Citrus sinensis* (orange)	0.12 ml/kg
N2B	Extract of *Citrus sinensis* (orange)	0.03 ml/kg
N3	Extract of hot-flavoured spices	0.045 ml/kg
N4	Molecules of the family of aromatic aldehydes and short- to medium-chain fatty acids	0.58 ml/kg
N5	Extract of *Cinnamomum camphora L.* (camphor)	0.23 ml/kg
N6	Extract of *Cinnamomum aromaticum Nees* (cinnamon)	0.2 ml/kg
N7	Extract of *Illicium verum* (star anise)	0.11 ml/kg
N8	Molecules of the pyrazine family	0.02 ml/kg

2.1.3. Procedure. After one week of habituation to the pens and the experimental troughs, the animals were subjected to four weeks of tests: two experimental diets were tested per week and for each experimental diet, a session of two consecutive days of tests was necessary, that is four days of test per week for four weeks. On Day 1, the animals were subjected to two successive one-way feeding tests to minimize the neophobic response of the pigs towards the experimental unfamiliar diets and ensure that pigs tasted both the N and C diets before the subsequent two-choice preference tests. A control diet (C) and an experimental diet (N) were subsequently presented during 15 min to the animal. The C1 diet was paired with the N1 and N4 diets, and the C2 diet was paired with the N2A, N2B, N3, N5, N6, N7 and N8 diets, according to the diluent used (water or vegetable oil, respectively). During these tests, the first diet was presented during 15 min in one part of the trough. After then refusals were removed

and weighed and the second diet was presented during 15 min in the other part of the trough. After 15 min, the trough was removed and refusals were weighed. During these one-way tests, 550 g of each diet was presented during the first week of tests and then increased by 50 g per week. On Day 2, the animals were subjected to a two-choice feeding test to investigate their food preference between the experimental and control diets. The animals had the choice between the control and the experimental diets presented on Day 1. During these two-choice tests, the two-part trough contained 1.5 kg of each diet and was presented during 30 min. After then, the two-part trough was removed and refusals were weighed. In both one-way and two-choice tests, meal distribution in the trough was interchanged over days and animals to avoid any bias. One day was left free of experimentation between each session of tests.

2.1.4. Behavioural analysis. Tests were video-recorded during the meals and behavioural video-observations were carried out. Using the focal-sampling method, the latency to reach the trough during the one-way tests was recorded.

2.1.5. Statistical analysis. The mean amount of each diet (± SEM) consumed during the tests sessions, as well as the latency to access the trough during the one-way tests were compared using Wilcoxon signed-rank tests thanks to the StatView software 4.57 (Abacus Concepts Inc., USA). The significance level for all analyses was set as $P < 0.05$.

2.2. Results

During the one-way feeding tests, the pigs consumed the control C diet more than the N5 (C: 598 ± 19 g, N5: 559 ± 16 g, z = 2.05, $P = 0.04$), and N8 diets (C: 693 ± 6 g, N8: 686 ± 8 g, z = 2.02, $P = 0.04$). The pigs tended to prefer the C diet over the N2A (C: 435 ± 16 g, N2A: 322 ± 51 g, z = 1.73, $P = 0.08$) and N6 diets (C: 630 ± 9 g, N6: 605 ± 21 g, z = 1.89, $P = 0.06$). Moreover, the latency to access the trough containing the C diet was shorter than that

to access the trough containing the N6 diet (C: 0.86 ± 0.11 s, N6: 0.48 ± 0.10 s, z = 1.96, *P* = 0.04). During the two-way choice tests, the C diet was preferred over the N2A, N6 and N7 diets and tended to be preferred over the N5 and N2B diets (Figure 1).

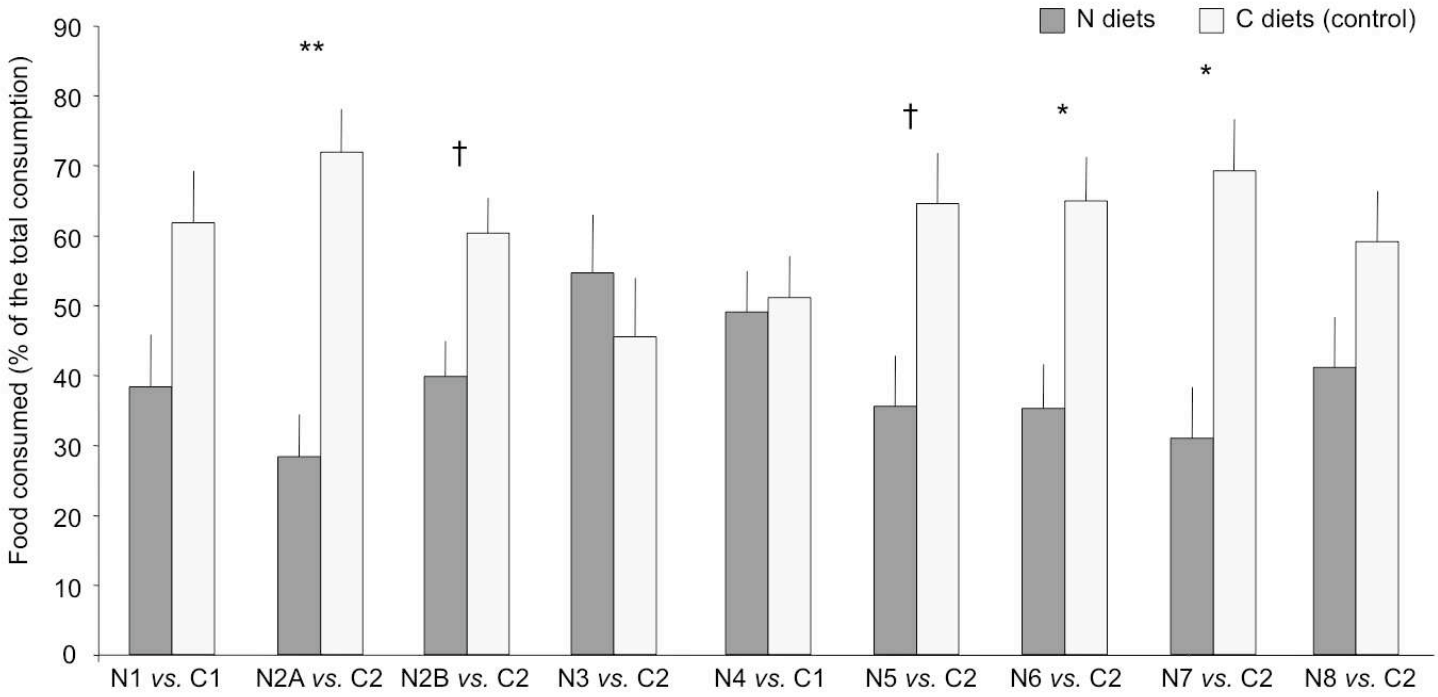

Figure 1. Quantity of food (% of the total food consumption) consumed during the two-choice feeding tests in Experiment 1. During 30-min meals, the animals had the choice between an experimental diet (N diets) and a control diet (C diets). The C1 diet was paired with the N1 and N4 diets, and the C2 diet was paired with the N2A, N2B, N3, N5, N6, N7 and N8 diets, according to the diluent used (water or glycerol, respectively). Data are presented with means and standard errors.

According to the results, the pigs consumed less the N2A, N5, N6, N7 and N8 meals than the control standard diet during one-way and/or two-way choice tests. These findings suggest that the adjunction of these functional ingredients in food, at these concentrations, did not improve food palatability and did not increase food intake. Consequently, these diets were excluded from the study and the N1, N2B, N3 and N4 diets were selected for Experiment 2.

3. Experiment 2

The aim of this experiment was to compare and rank the N1, N2B, N3 and N4 sensory functional ingredients on the basis of the relative preferences exhibited by pigs during two-choice tests with two different experimental diets with additives, and in the absence of alternative familiar food.

3.1. Materials and methods

3.1.1. Animals and housing. Experiment 2 was carried out in November 2010 in the INRA experimental research station of Saint Gilles (France; 48° 09' 13'' N, 01° 49' 34'' W). A total of twelve 71 ± 2 day-old Large White x Landrace female pigs of 25.40 ± 0.36 kg at the beginning of the study were used. All the pigs were weaned at 27 ± 2 days of age and housed in groups from weaning. Before the experiments, i.e. 44 days post-weaning, the animals were moved into individual pens with similar housing conditions than in Experiment 1.

3.1.2. Procedure. After one week of habituation to the pens and the experimental troughs, the animals were subjected to two-choice feeding tests to investigate their food preferences between the four experimental diets (N1, N2B, N3, N4). The animals were subjected to two two-choice feeding tests per week for three weeks, with at least one day free of experimentation between the tests. The combination of tests followed a latin square design across the three weeks, so that the animals received the four experimental diets once a week but not in the same order for each animal. Consequently, at the end of the three weeks, each animal had been subjected to six two-choice tests and had received each diet three times. During these tests, the two-part trough contained 1.5 kg of two different diets and was presented during 30 min. After then, the two-part trough was removed and refusals were weighed. Meal distribution in the trough was interchanged over days and animals to avoid bias.

3.1.3. Behavioural analysis. During the meals, tests were video-recorded and behavioural video-observations were carried out. Using the focal-sampling method, the number of trough-switching was recorded during the first 15 min of the meal.

3.1.4. Statistical analysis. The mean amount of each diet (± SEM) consumed, as well as the number of trough-switching recorded during the tests sessions were compared using Wilcoxon signed-rank tests thanks to the StatView software 4.57 (Abacus Concepts Inc., USA). The significance level for all analyses was set as $P < 0.05$. Moreover, for each animal, an index (I) was calculated in order to evaluate individual preferences in each testing condition: I = (amount of X diet consumed – amount of Y diet consumed)/amount of total food consumed from both diets. We considered that for $1 > I \geq 0.33$, the animal preferred the X diet, for $0.33 > I > -0.33$, the animal exhibited no preference, and for $-0.33 \geq I > -1$, the animal preferred the Y diet.

3.2. Results

During the two-choice feeding tests, the N3 and N4 diets were significantly preferred over the N2B diet, and the N1 diet tended to be preferred over the N3 diet (Figure 2). As for the individual preference profiles, the majority of animals showed clear-cut preference for the N1, N3 or N4 diets over the N2B diet (Figure 3). The N1 diet was also preferred over the N3 diet by the majority of the animals. In the N1 *vs.* N4 and N3 *vs*. N4 modalities, we noticed that the preference patterns were influenced by a high inter-individual variability. Either the animals showed clear-cut preference for the N4 diet or they showed clear-cut preference for the N1 or N3 diet, but only a few animals exhibited no clear-cut preference in the N1 *vs*. N4 modality.

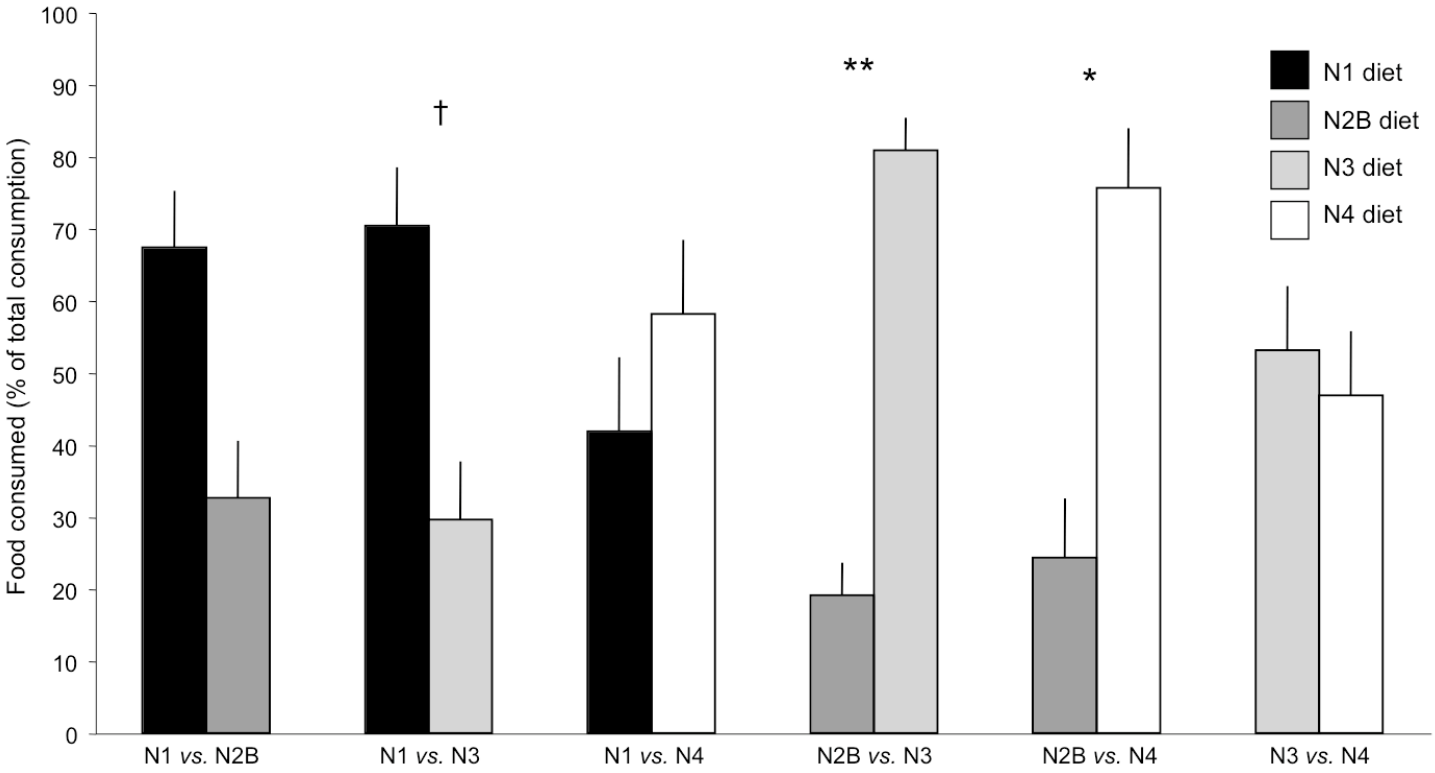

Figure 2. Quantity of food (% of the total food consumption) consumed during the two-choice feeding tests in Experiment 2. During 30-min meals, the animals had the choice between two out of the four experimental diets (N1, N2B, N3, N4). Data are presented with means and standard errors.

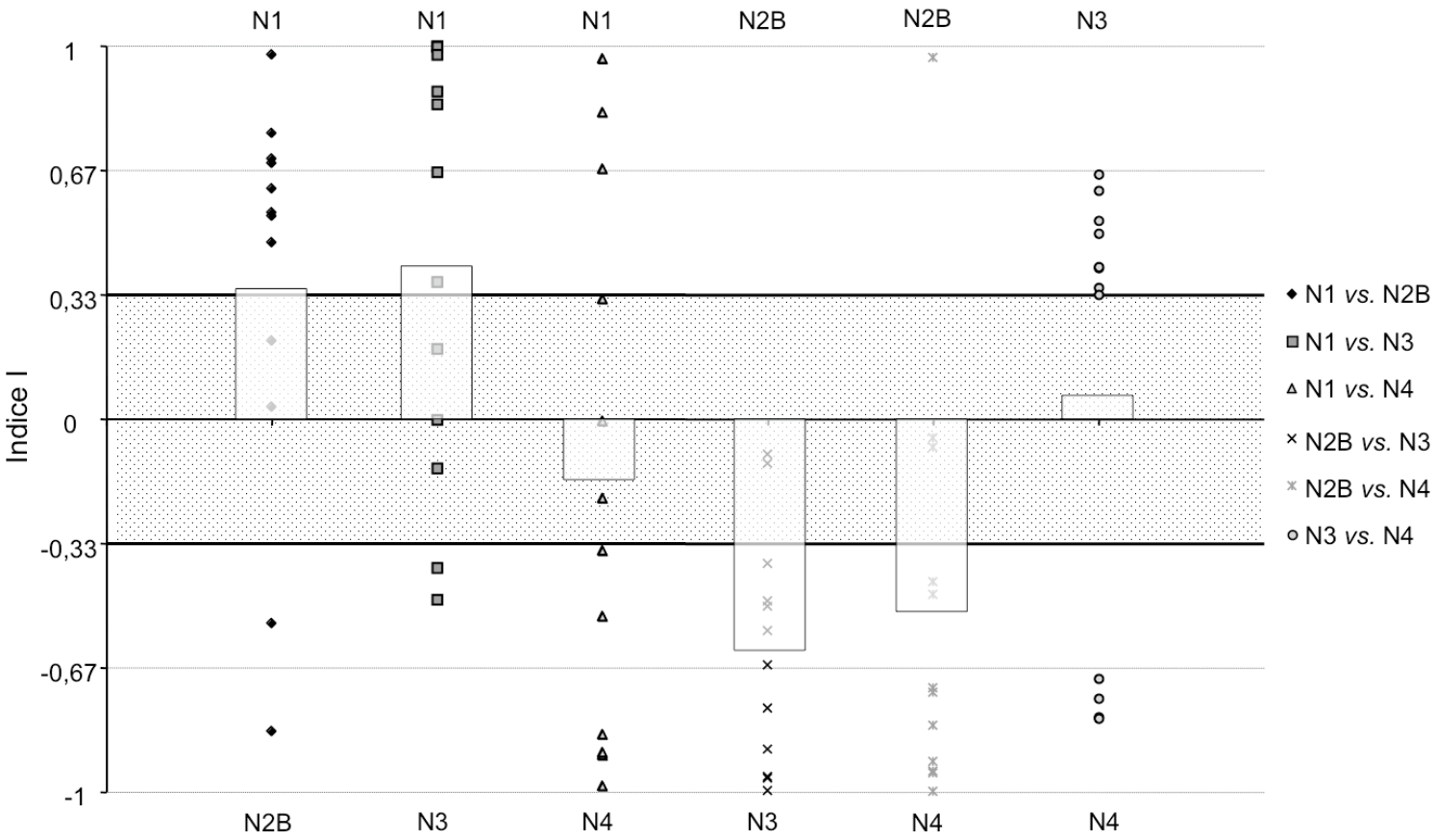

Figure 3. Individual and mean preferences exhibited during the two-choice feeding tests in Experiment 2 and illustrated by an index (I) with: I = (amount of X diet consumed – amount of Y diet consumed)/amount of total food consumed from both diets. We considered that for $1 > I \geq 0.33$, the animal preferred the X diet, for $0.33 > I > -0.33$, the animal exhibited no preference, and for $-0.33 \geq I > -1$, the animal preferred the Y diet. The diets indicated at the top of the figure represent the X diets, and the diets indicated at the bottom of the figure represent the Y diets for each testing condition. The symbols distributed in lines and the histograms illustrate individual and mean index (i.e. preference) respectively, for each testing condition. The symbols located in the dotted area represent the animals that exhibited no preference.

Compared to the other experimental diets, the N1 diet was more consumed during the first presentation (Figure 4) and the amount of food consumed during the subsequent presentations remained relatively high. On the contrary, according to the persistent low level of the N2B diet consumption, we concluded that the animals developed a spontaneous aversion for this food that persisted after at least three successive presentations. A slight avoidance of the N3 and N4 diets was noticed during the first presentation of these diets, but the aversion response tended to decrease quickly during subsequent exposures to reach high levels of consumption.

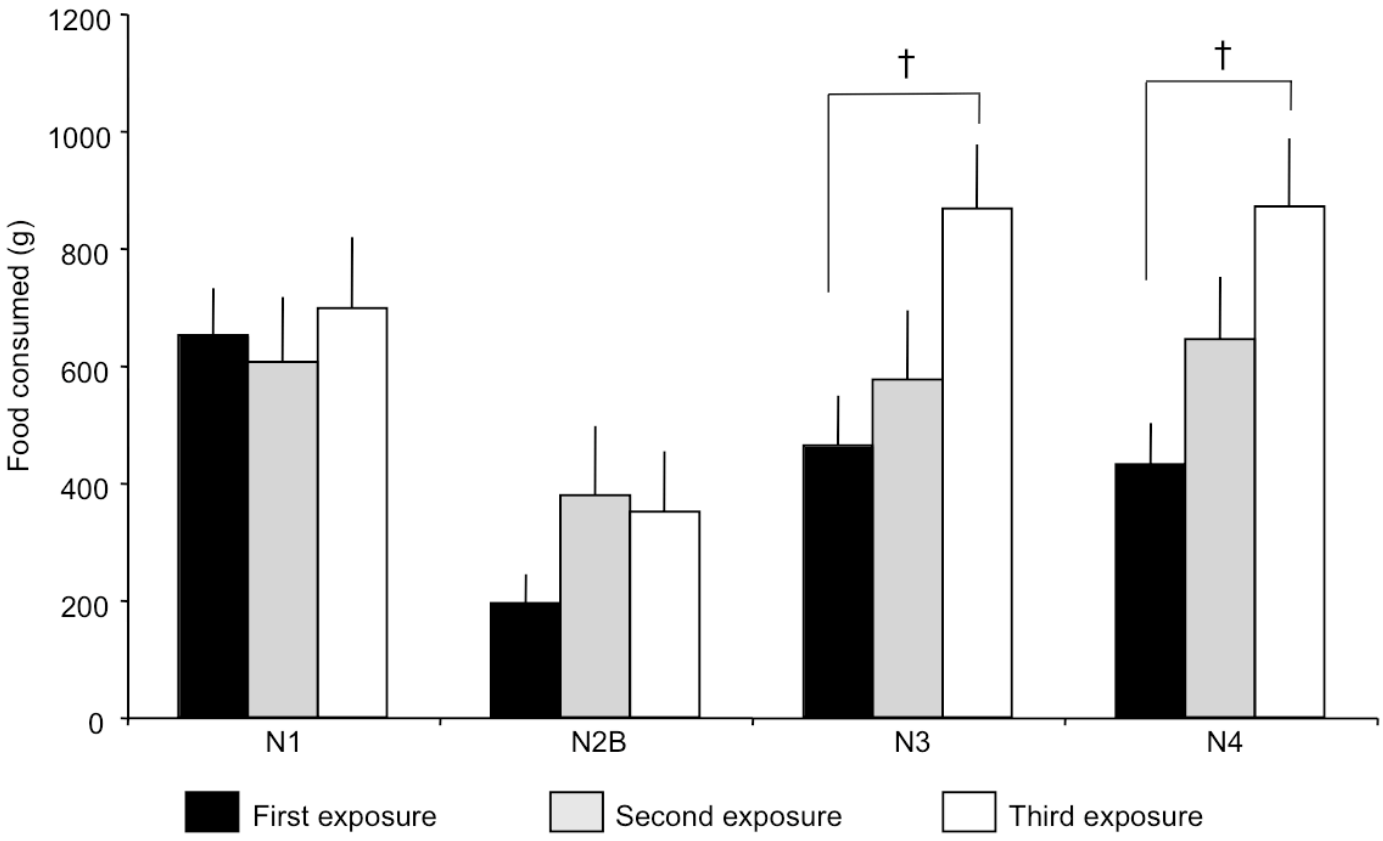

Figure 4. Quantity of food consumed (g) during the three successive presentations of each experimental diet during the two-choice feeding tests in Experiment 2. Data are represented with means and standard errors. The symbol indicates significant statistical difference before Bonferroni correction ($P < 0.1$).

Lastly, the animals switched trough significantly more in the N3 *vs*. N4 condition (13.08 ± 2.15 switches in 15 min) than in the N1 *vs.* N3 (6.17 ± 1.55 switches, z = 2.82, $P = 0.005$) and N2B *vs*. N4 conditions (6.42 ± 1.23 switches, z = 2.98, $P = 0.003$).

The N2B diet appears to be systematically avoided by animals during two-choice tests, while the N1 diet triggered no particular avoidance response. The N3 and N4 additives appear to induce very similar feeding responses in pigs, i.e. a quick increase of consumption during the first exposures, and preferences showing a high inter-individual variability. For practical and economical reasons, we could not test more than two sensory functional ingredients in Experiment 3. Consequently, we decided to run subsequent tests on the N1 and N3 diets only, because the pigs did not demonstrate a strong aversion to them, as they did for N2, and because these additives induced two different patterns of responses along time (high and stable consumption with N1; increasing consumption with N3). As N3 and N4 elicited relatively similar responses, the choice between N3 and N4 was done arbitrarily.

4. Experiment 3

The aim of the present experiment was to investigate the mid-term influence of two sensory functional ingredients on food intake and weight gain of pigs during a food transition period.

4.1. Materials and methods

4.1.1. Animals and housing. Experiment 3 was carried out from January to February 2011 in the INRA experimental research station of Saint Gilles (France; 48° 09’ 13’’ N, 01° 49’ 34’’ W). A total of twenty-four 30-day old Large White/Landrace x Piétrain female pigs of 8.86 ± 0.14 kg at the beginning of the study were used. At weaning, i.e. at 27 ± 2 days of age, all the piglets were removed from the mother and housed in group of 10 individuals according to sex and weight. On the second day post-weaning, the piglets were housed in individual pens

(132 x 122 cm) distributed in two similar rooms with 12 pens per rooms and with a natural day/night cycle. To fulfil the physiological requirements of the weaned pigs, the temperature of the rooms was decreased from 1°C per week during the course of the study, i.e. from 28°C the first week to 23°C the last week. Before the beginning of the experiment, the pigs were fed daily with 200 g of a pelleted starter diet adapted to the energy and nutrients requirements of the pigs and composed of 45.5% barley, 20% mild lactoserum, 17.5% soybean meal, 8% fattened milk, 2.5% soybean proteins, 2.3% vegetal oil, 1.48% carbonate, 0.78% bi-calcic phosphate and 0.5% vitamin complement (2.54 Mcal/kg net energy). The animals had free access to water.

4.1.2. Procedure. The paradigm was divided into two phases: the pre-transition and the post-transition phases. The pre-transition phase lasted from Day 1 (i.e. the 5th day post-weaning) to Day 9. During this phase, all the animals received daily rations of starter diet at 10:00h. On Day 10, i.e. the transition day, the animals were divided into three experimental groups of equivalent mean weight (N1 group: 12.04 ± 0.58 kg, N3 group: 11.98 ± 0.43 kg, C group: 11.93 ± 0.51 kg). The post-transition phase lasted from Day 10 to Day 29. During this phase, the animals received one of the three experimental grower diets: the N1 group received the N1 diet (standard grower diet + N1 additive), the N3 group received the N3 diet (standard grower diet + N3 additive) and the C group received the control standard grower diet with no additive. All along the experiment, the amount of food distributed per day was adjusted so as to ensure that no trough was emptied before the next food provision. Daily refusals were weighed at 09:00h the following day, i.e. 1 hour before the distribution of the new daily food ration. Moreover, the pigs were weighed every Tuesdays at 09:00h, i.e. before the distribution of the daily food ration.

4.1.3. Statistical analyses. The mean amount of each diet (± SEM) consumed, as well as the mean weight of the animals, were compared between treatments using Kruskal-Wallis tests thanks to the StatView software 4.57 (Abacus Concepts Inc., USA). The significance level for the analyses was set as $P < 0.05$. If the Kruskal-Wallis test was significant, a Wilcoxon signed-rank test was performed. In this case of multiple comparisons, the Bonferroni correction was applied.

4.2. Results

On Day 1, the mean amount of starter diet consumed was 202 ± 27 g. On Day 29, the mean amount of grower diets consumed, regardless of the experimental treatment (N1, N3 or C), was 1 352 ± 52 g. After the transition day, no difference in the mean daily amount of food consumed by the animals (Figure 5) and in the mean body weight per week (Table 2) was observed between the experimental groups.

Table 2. Mean weight (SEM) of the animals the day before the transition (Day 9) and during three successive weeks after the transition (Days 16, 23 and 30).

Days	N1 group	N3 group	T group	H	*P*-value[1]
Day 9	12.04 ± 0.58	11.98 ± 0.43	11.93 ± 0.51	0.035	0.98
Day 16	15.43 ± 0.79	15.41 ± 0.58	15.44 ± 0.81	0.125	0.94
Day 23	19.69 ± 1.13	19.79 ± 0.97	19.44 ± 0.98	0.315	0.85
Day 30	26.99 ± 1.27	24.60 ± 1.16	25.69 ± 1.16	1.715	0.42

[1] Kruskal-Wallis test among experimental groups

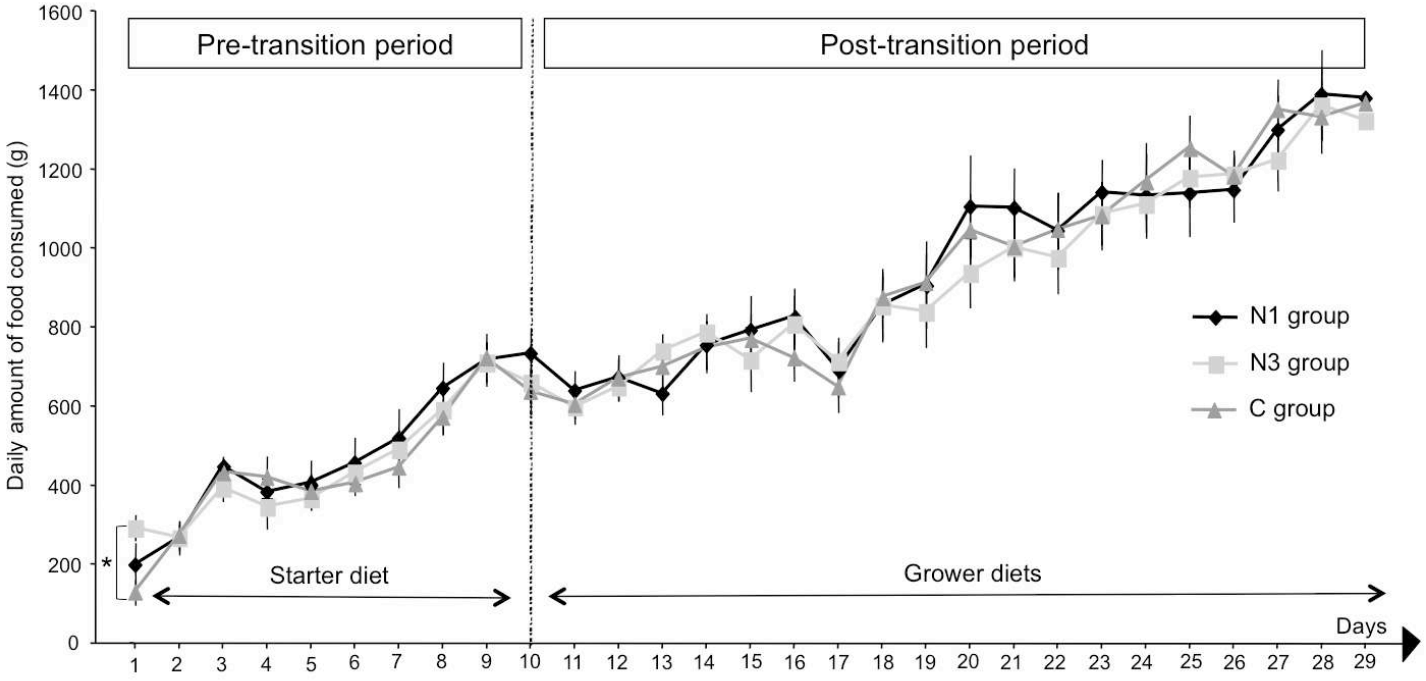

Figure 5. Daily quantity of food (g) consumed in each experimental group (N1, N3 and C groups) during Experiment 3. Data were presented with means and standard errors. The dotted line represents the day of the food transition. The asterisk indicates significant statistical difference after Bonferroni correction ($P < 0.017$).

On Day 10 (i.e. the transition day), compared to the consumption of the starter diet on Day 9, the consumption of the C diet was 10.31% lower, while the consumption of the N3 diet was only 5.46% lower and the consumption of the N1 diet was 3.77% higher (Figure 6). However, the difference of consumption between Day 9 and Day 10 did not reach the significant threshold due to high inter-individual variability.

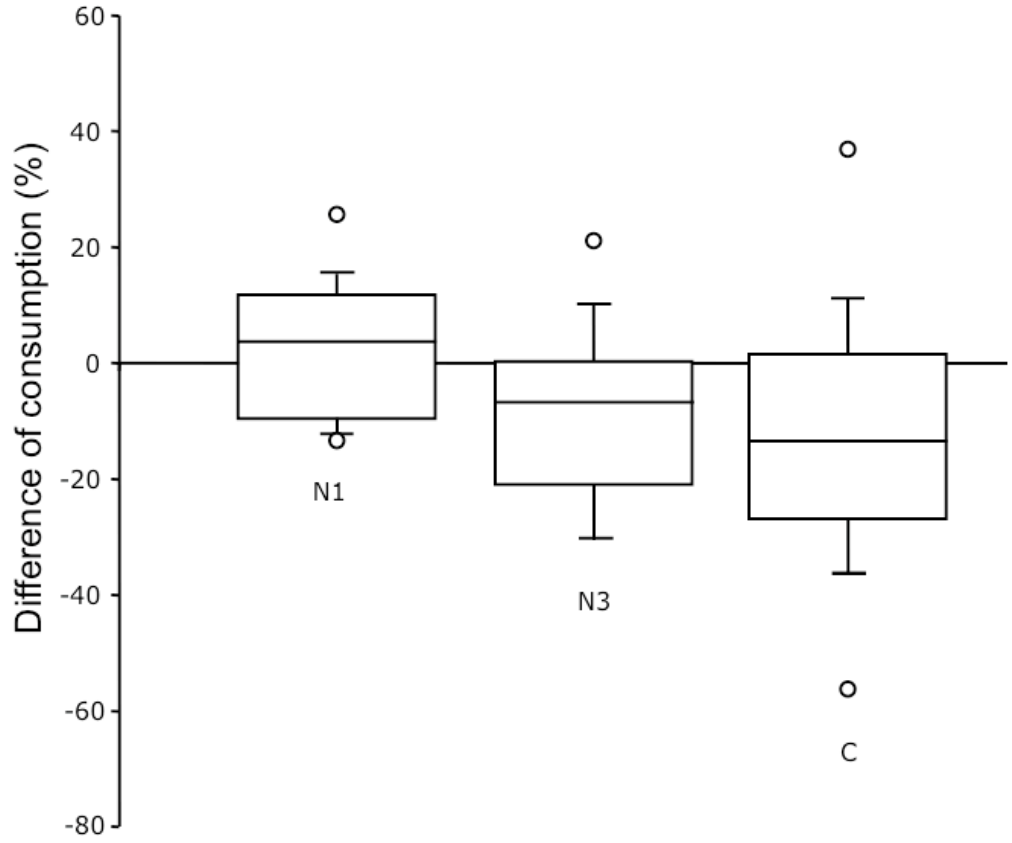

Figure 6. Difference of consumption (%) between the last day of the starter diet period (Day 9) and the day of the food transition, i.e. the first day of the grower diet period (Day 10) according to the experimental group (N1, N3 and C group) in Experiment 3. The whiskers represent the lowest datum within 1.5 interquartile range of the lower quartile and the highest datum within 1.5 interquartile of the upper quartile. Dots represent the data that are not included between the whiskers.

5. Discussion

In Experiment 1, we found that the control standard diet was preferred over several experimental diets, including the N5, N6 or N7 diets, suggesting that those functional additives did not enhance food palatability. In Experiment 2, some clear-cut preferences emerged when two experimental diets were offered to the animals, since some ingredients, like the N2 additive, were systematically avoided, while others were preferred, such as the N1, N3 and N4 additives. All together, these findings highlight the existence of spontaneous preferences for some functional ingredients, suggesting that some additives might be more palatable than others. Some compounds are likely to be inefficient to promote food intake in pigs, such as orange (*Citrus sinensis*, N2 additive), camphor (*Cinnamomum camphora L.,* N5 additive) or cinnamon (*Cinnamomum aromaticum Nees,* N6 additive) extracts. However, Yang et al. (2010) reported that supplementing cattle diet with cinnamaldehyde, the main active compound of cinnamon oil, did improve food intake of the animals, although it had a reduced impact on weight gain or carcass traits. Other studies, however, reported no effect of cinnamaldehyde supplementation on milk production, feeding behaviour or rumen fermentation in dairy cows (Tager and Krause, 2011). As for orange (*Citrus sinensis*), to our knowledge, no study investigated the influence of orange flavour on food intake, while various authors reported beneficial effect of orange aroma in stressful situations. In humans, Lehrner et al. (2000, 2005) found that stimulation with ambient odour of orange reduced anxiety and improved mood and calmness in patients waiting for dental care. Similarly, Faturi et al. (2010) reported that exposition to the orange aroma immediately before behavioural tests exerted an anxiolytic effect in rats. These findings suggest that the essential oil of orange would rather have an action on stress regulation than on food behaviour, which may explain the inability of N2 additive, at low (N2B) or high (N2A) concentration, to improve food intake in our study.

The overall absence of clear-cut preferences for some diets, e.g. in the N3 *vs*. N4 and N1 *vs*. N4 conditions, can be explained by two different patterns of individual preferences: (1) the majority of the animals exhibited no clear-cut preference and did not make a real choice between the two meals, e.g. in the N1 *vs*. N4 condition, or (2) all the animals exhibited clear-cut preferences but not for the same diets, e.g. in the N3 *vs.* N4 condition. These results suggest that preferences are subjected to a great inter-individual variability and raise a question about the factor that would have been responsible for such inter-individual differences in a controlled environment. One might argue that these specific patterns of preference are due to the existence of divergent spontaneous preferences, suggesting that the individuals allocate different hedonic value/palatability to the sensory functional ingredients. Palatability cannot be only considered as an intrinsic and invariant feature of food, since the hedonic value of a specific food also depends on the past experiences with this food, including habituation (Delumeau and Meunier-Salaün, 1995), maternal and social influences (Schaal et al., 2000; Prescott et al., 2005), as well as postingestive consequences (Bernstein, 1999). Moreover, sensory abilities may vary a lot between individuals and have an impact on the perception of food sensory characteristics, as well as on the expression of subsequent preferences (e.g. in humans, Burdach et al., 1985). Finally, for a given individual, palatability can also vary according to emotional, motivational and nutritional state (Bellisle, 2006), and food preferences in humans have been found to be unstable throughout life due to the numerous changes in metabolic responses to food or in the perception of food sensory characteristics (Bellisle, 1999). Consequently, according to the variety of factors that may influence food perception, different individuals can attribute a wide range of hedonic value to a specific food, a phenomenon that can be well illustrated in humans by looking at the considerable diversity in adult likings and dislikings for various food items. The use of an increased number of pigs would have

counterbalanced the effects of individual variability and would have been likely to result in more significant results in terms of food intake and preferences in our study.

Although the N1 and N3 sensory functional ingredients triggered positive food responses during the feeding preferences tests in Experiments 1 and 2, the results of Experiment 3 showed that these additives did not enhance middle-term food intake, pigs' appetite and weight gain during the starter/grower diet transition. Despite non-significant results, it is worth to notice, however, that the N1 additive (composed of extracts of stevia and high-saponin plants), and to a lesser extent the N3 additive (composed of hot-flavoured spices), might enable to maintain a normal consumption the day of the food transition. Although Experiment 3 failed to highlight positive effects of these additives on middle-term food intake, the preference responses exhibited by pigs during the feeding tests in Experiments 1 and 2 suggest that these additives might somehow improve diet palatability and raise the question about the compounds that would have been responsible for the modulation of hedonic reactions to food.

Stevia, saponins and spices have been found to have various effects on food intake in many species, although, to date, scientific data are quite contradictory. Stevia is a generic term that actually represents the plant *Stevia rebaudiana* (Bertoni) and its extracts, i.e. the steviol glycosides, including stevioside and rebaudioside A that are used since many years as low-calorie sweeteners in numerous countries (Carakostas et al., 2008). The effects of stevia on food intake and animal performances have been assessed in several farm species. In broilers, Wood et al. (1996) reported that stevia did not improve food consumption or weight gain. According to Geuns et al. (2003b), stevioside also appeared to be inefficient to improve food intake, body weight gain in chickens, as well as egg production in adult laying hens, whereas Atteh et al. (2008) showed that the supplementation of stevia leaves and stevioside in broiler

diets did improve food intake during growing period, but not their performances. In pigs, some studies reported that stevioside slightly reduced feed intake (Geuns et al., 2003a) and that there was no advantage of stevia compared to sucrose on feed intake, weight gain and feed/gain ratio of pigs (Munro et al., 2000). All together, these contradictory findings make it difficult to conclusively assume that stevia enhances food palatability in pigs, and determine whether stevia is responsible for the preferences responses exhibited by pigs toward the N1 diet during feeding choice tests. Nevertheless, we can assume that stevia is likely to be insufficient in itself to improve pigs' motivation to eat and increase food intake in a situation where a unique food is provided.

Saponins, the other main component of the N1 additive, are active compounds extracted from various plants and are also known to have many effects on food intake and appetite, but, to date, the findings have been quite controversial too. Some studies reported that saponins had antiobese effects as they reduce calorie intake, and consequently body weight. For instance, platycodi saponins, the primary constituents of *Platycodi radix* (Chinese Jiegeng) induced antiobese and hypolipidemic effects in obese rats, which showed weight reduction paired with calorie intake restriction (Han et al., 2000, 2002; Zhao et al., 2005). Similarly, saponins extracted from ginseng (*Panax ginseng*), reduced the body weight and food intake in high-fat diet fed rats (Karu et al., 2007; Kim et al., 2009). Fenugreek (*Trigonella foenum gaecum* L.) is another high-saponin plant that has been found to reduce spontaneous food intake in humans. Mathern et al. (2009) reported that fenugreek fibres increased ratings of satiety and reduced ratings of hunger, and consequently food consumption in obese humans, while Chevassus et al. (2009, 2010) reported that fenugreek seed extracts reduced spontaneous fat intake in overweight and healthy humans, but not carbohydrates or protein intake. Though, in some civilisations, fenugreek is used for years to stimulate appetite and promote weight gain, like for instance among the Moroccan Sahrawi women population (Rguibi and Belahsen,

2006). Besides, Petit et al. (1993, 1995) reported that the administration of fenugreek seed extract in normal rats increased food consumption and motivation to eat. Hence, saponins could be the active compound responsible for the preferences responses exhibited by pigs for the N1 diet during two-choice tests in Experiments 1 and 2, although further investigation is required to confirm this hypothesis.

The N3 functional ingredient is composed of hot-flavoured spices. Spices, including turmeric (*Curcuma longa*), cumin (*Cuminum cyminum*), paprika (*Capiscum annum*) or chilli pepper (*Capsicum sp.*), are important ingredients in food preparation, and particularly in some cultures and countries, such as India. Spices appear to be mainly used to enhance food palatability, but some studies reported that they might also be used as antimicrobial agents that contribute to health and promote a long lifespan (Billing and Sherman, 1998; De et al., 2009). However, some studies reported that hot-flavoured spices might also modulate food intake. For instance, intraperitoneal injections of curcumin, the main active substance of turmeric, were found to reduce appetite, and subsequent food intake in goldfish (Kang et al., 2011). Curcumin also resulted in a significant weight loss in obese ob/ob mice (Weisberg et al., 2008) and, therefore, Alappat and Awad (2010) argued that curcumin might be an efficient tool to decrease the incidence of obesity and associated risk factors. Piperine, which is the main active compound of pepper (*Capiscum spp.*) and is known to increase the bioavailability of curcumin (Suresh and Srinivasan, 2010), also reduced body weight, but not food intake in rats fed with a high-fat diet (Shah et al., 2011). Overall, these findings suggest that hot-flavoured spices appear to inhibit rather than improve food intake of humans and rats. Tough, spices are known to have a digestive stimulant action, mediated by an increase of salivary, gastric or bile secretions and a reduction in food transit time (Platel and Srinivasan, 2004). This digestive stimulant action might, in turn, result in a stimulation of appetite in humans.

It is quite difficult to compare our results with those of previous studies since many methodological discrepancies between studies, such as the compound dosage in food, are reported and might be responsible for the contradictory and inconsistent scientific data on the effects of these compounds on food intake and, consequently, on weight gain and animals performances. In the present study, the additive concentrations in the food may represent a decisive factor influencing the results, especially since subtle changes in additive concentration have been found to modify the additive effect on food intake or animal performances. For instance, Javandel et al. (2008) found that broiler chickens (1-21 days of age) fed with 0.125 and 0.25% garlic diet had a higher daily food intake than chickens fed with 0.5% garlic diet; and that chicken fed with 0.125 to 1% garlic diet have a higher daily weight gain than chicken fed with 2% garlic diet. However, in a preliminary study, we showed that no significant difference in spontaneous consumption was found for three different concentrations for the N1 (0.925, 3.7 and 14.8 ml/kg) and N3 (0.01125, 0.045 and 0.18 ml/kg) diets (unpublished data), suggesting that at those concentrations, we would have come to the same conclusions. But these results did not rule out the possibility that other concentrations might have induced greater effects during food transition.

In the present study, we excluded many of the eight initial sensory functional ingredients following preliminary two-choice feeding tests. According to our data, in the situation where two different experimental diets were available, the control standard diet was preferred over the N2 (A and B), N5, N6, N7 and, in a lesser extent over the N8 diet. Moreover, the N2B diet was clearly avoided by animals when presented simultaneously with the N1 or N3 diets. Two main issues emerge from our methodology. First, some authors reported that, in a situation where pig has a choice, the preferences exhibited by pigs do not predict the behaviour in a practical situation where a unique food is usually supplied (Meunier-Salaün and Picard, 1996), and do not necessarily mean that it would result in positive effect on food intake or

performances (Jacela et al., 2010). Secondly, as subtle changes in ingredients concentrations might have modified the effects of the N1 and N3 diets on food intake and palatability during food transition, it is likely that animals' preferences during the two-choice tests might have been quite different if other concentrations would have been tested for the eight additives. Jugl-Chizzola et al. (2006) tested various diets flavoured with thyme (1.15% or 0.12%), oregano (1.11% or 0.11%) or a mixture of the two essential oils (0.58% of thyme and 0.56% of oregano or 0.06% of thyme and 0.06% of oregano) in pigs. Using two-choice feeding tests, they found that the diets with lower concentrations were preferred to those with higher concentrations, and that all the experimental diets were less consumed than the control diet without additive, except from the 0.12% thyme diet, suggesting that when pigs have the choice between flavoured and unflavoured diets, they would almost systematically avoid flavoured meals of any kind and choose the unflavoured diet. These assumptions suggest that it is of fundamental importance to use caution in the interpretation of two-choice feeding tests performed under experimental controlled conditions. In the present study, it might be possible that some of the excluded additives would have been preferred to the N1 or N3 diets at different concentrations, and induced positive responses in terms of food intake and weight gain in a food transition period, where a unique food is presented to the animals. As revealed by our study, two-choice tests and tests during which a unique food is provided are two complementary tools that both aim at investigating food preferences in animals but are influenced by different parameters, e.g. the presence or not of another food. Further studies using preliminary tests where a unique food is provided in controlled conditions might enable to complement our study that used two-choice tests, and to select more accurately the most efficient additives for the improvement of food palatability and subsequent food intake in pigs in a practical situation.

6. Conclusions

In the present study, we highlighted that the establishment of preferences for various food additives composed of plant extracts in pigs was submitted to a great inter-individual variability, and that additive dosage in the food might be a decisive factor in the development of such preferences. Although some functional sensory ingredients appeared to be more palatable than others in two-choice tests, the adjunction of these additives in food in a practical situation of food transition did not influence mid-term food intake or weight gain, but might enable to maintain a normal consumption just after the transition.

Acknowledgements

The study was co-funded by INRA and Phodé Laboratories (Terssac, France), which also provided the sensory functional ingredients. We are grateful to the scientific and technical staff for their participation to the study and for care of the animals, and especially to Sylvie Guérin, Isabelle Nogret, Patrick Touanel and Fabien Guérin. We would like to thank our partners from Phodé Laboratories, who participated to the discussion of the experimental protocols and results: Virginie Noirot, Pierre Etienne and Daniel Eclache.

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Partie 2. Expérience complémentaire : effet de la concentration des additifs dans l'aliment sur les préférences alimentaires

1. Objectifs

Certaines études ont mis en évidence l'impact de la concentration des additifs alimentaires dans l'aliment sur la modulation de la consommation et des préférences alimentaires chez les animaux d'élevage (Javandel *et al.*, 2008). Ainsi, dans les travaux présentés dans la première partie de ce chapitre, l'aversion généralisée mise en évidence pour l'additif N2, et les préférences marquées pour les additifs N1 et N3 pourraient dépendre en grande partie de la concentration de ces additifs. Cette expérience a donc pour objectif de vérifier cette hypothèse et de tester, chez le porc, l'influence de la concentration de ces trois additifs sur les niveaux d'ingestion spontanée de l'aliment. Nous avons donc testé, pour chaque additif, trois concentrations différentes, choisies en accord avec les Laboratoires Phodé (Terssac, France) que nous avons également comparées à un aliment standard sans additif.

2. Matériels et méthodes

2.1. Animaux et logement

Au total, 32 femelles Large White × Landrace ou (Large White × Landrace) x Large White de 23 ± 3 kg au début de l'expérience ont été utilisées. Les animaux, répartis en trois lots expérimentaux (N1, N2 et N3) étaient logés en cages individuelles (122 × 123 cm pour les lots N1 et N2 et 75 × 126 cm pour le lot N3). Les animaux avaient accès à l'eau *ad libitum*. La température de la pièce était maintenue à 24 ± 0,7°C avec un cycle jour/nuit naturel. En dehors des jours d'expérimentation et à raison d'un repas unique par jour, les animaux étaient nourris avec un aliment « pois » composé de 60% d'aliment 2ème âge standard et de 40% de pois et dont les caractéristiques organoleptiques différaient de celles de l'aliment expérimental.

2.2. Protocole expérimental

Les animaux ont été répartis en trois lots expérimentaux, chaque lot visant à étudier les préférences vis-à-vis de différentes concentrations d'un seul noyau. Les 11 animaux du lot N1 ont reçu uniquement l'aliment additionné du noyau N1 (Optifeed), les 12 animaux du lot N2 ont reçu uniquement l'aliment additionné du noyau N2 (VéO) et les neuf animaux du lot N3 ont reçu uniquement l'aliment additionné du noyau N3 (Oléobiotec). Pour chaque noyau, quatre

concentrations différentes ont été testées (C0, C1, C2 et C3 ; **Tableau VI**), la concentration C0 correspondant en fait à l'aliment témoin, sans additif. L'aliment utilisé pour les tests était de l'aliment 2ème âge standard, différent de l'aliment « pois » (**Annexe 2**) et donc inconnu des animaux avant le début des tests (*cf.* **article n°6** pour la composition de l'aliment 2ème âge).

Tableau VI. Concentrations testées pour chacun des trois ingrédients (noyaux) fonctionnels (en ml/kg d'aliment). La concentration utilisée dans l'article n°6 est indiquée **en gras**. Les noyaux ont été fournis par les Laboratoires Phodé. N1 : Extraits de stevia (*Stevia rebaudiana*) et de plantes à fort taux en saponines ; N2 : Extraits d'orange (*Citrus sinensis*) ; N3 : Extraits d'épices à saveur brûlante.

	Ingrédient fonctionnel à tester		
Concentration (%)	N1	N2	N3
C0	0	0	0
C1	0,925	0,00375	0,01125
C2	**3,7**	0,0075	**0,045**
C3	14,8	**0,03**	0,18

Pendant une semaine, les animaux ont été acclimatés aux nouvelles conditions expérimentales (loges expérimentales, présence des expérimentateurs, aliment « pois »). Pendant cette semaine d'adaptation, les animaux recevaient 600 g d'aliment « pois » par jour, à raison d'un repas unique distribué dans la matinée. La semaine suivante, pendant quatre jours consécutifs, les animaux ont été habitués à manger pendant une durée limitée de 10 min. Durant cette phase d'habituation, les animaux recevaient quotidiennement une ration de 800 g d'aliment « pois » dans la matinée puis, la ration était retirée et pesée. Les refus étaient redistribués aux animaux 1h30 plus tard.

La dernière semaine, pendant quatre jours consécutifs, les animaux ont été soumis à la phase de tests. Pendant cette phase, les animaux recevaient leurs aliments expérimentaux à raison d'un repas de 10 min par jour distribué dans la matinée. Pour chaque groupe expérimental (N1, N2 et N3), l'ordre de présentation de chacune des concentrations (C0, C1, C2 et C3) a été alterné entre les jours et les animaux en suivant un paradigme de carré latin afin d'éviter tout biais expérimental. Après 10 min de repas, la ration était retirée et les refus pesés. Un complément d'aliment « pois » pour atteindre 1 kg d'aliment par jour était distribué 1 heure 30 plus tard. Le choix de mesurer les quantités ingérées pendant un repas très court de 10 min repose sur la volonté d'éliminer les différences d'ingestion qui pourraient apparaître sur une période de temps plus longue, et qui seraient liées à des variations inter-individuelles

dans les profils ingestifs (mangeurs rapides ou lents, repas longs ou repas très courts répartis dans le temps, *etc.*).

2.3. Analyses statistiques

Les données ont été analysées avec le logiciel R 2.14.1 (The R Foundation for Statistical Computing, 2011). Les quantités d'aliment ingéré ont été analysées avec des tests *t* de Student pour données appariées, ou des ANOVA à un facteur sur mesures répétées, suivies de tests *t* de Student corrigés pour les comparaisons multiples d'après la méthode de Benjamini et Yekutieli (Benjamini et Yekutieli, 2001). La normalité des données a été vérifiée par un test de Kolmogorov-Smirnov.

3. Résultats

Dans les trois lots (N1, N2 et N3), une forte variabilité interindividuelle a été observée en ce qui concerne la quantité totale d'aliment expérimental consommé lors des tests, toutes concentrations confondues. Pour les individus du lot N1, la consommation cumulée sur les 4 jours de tests était en moyenne de 1241 ± 267 g, avec une variation moyenne allant de 227 ± 11 g à 405 ± 25 g par jour. Pour les individus du lot N2, la consommation cumulée sur les 4 jours de tests était en moyenne de 1250 ± 188 g, avec une variation moyenne allant de 251 ± 64 g à 389 ± 41 g par jour. Pour les individus du lot N3, la consommation cumulée sur les 4 jours de tests était en moyenne de 1195 ± 308 g, avec une variation moyenne allant de 188 ± 11 g à 439 ± 33 g par jour.

Au sein de chaque lot expérimental, les animaux ont mangé l'aliment « pois » en phase d'habituation en plus grande quantité que l'aliment expérimental en phase de test, toutes concentrations confondues (**Tableau VII**). Cependant, aucune différence de consommation n'a été mise en évidence entre les groupes à la fois pendant la phase d'habituation ($F(2,29) = 0,08$; $P = 0,93$) et pendant la phase de test ($F(2,29) = 0,13$; $P = 0,88$).

Les résultats concernant l'effet des concentrations sur les niveaux d'ingestion spontanée sont présentés dans la **Figure 12**. Les animaux du lot N1 ($F(3,30) = 1,26$; $P = 0,31$) et du lot N3 ($F(3,24) = 2,24$; $P = 0,11$) n'ont présenté aucune préférence pour une concentration particulière. Pour le lot N2, l'ANOVA sur données appariées a révélé un effet dose ($F(3,33) = 5,07$; $P < 0,01$), caractérisé par un niveau d'ingestion supérieur de l'aliment témoin C0 par rapport à l'aliment C3 ($P < 0,05$).

Tableau VII. Quantité moyenne (S.E.M.) d'aliment « pois » (phase d'habituation) et d'aliment expérimental toutes concentrations confondues (phase de test) consommés en fonction des lots N1, N2 ou N3. * $P < 0,05$.

Lot	Consommation (g)		Test *t* pour données appariées		
	Habituation (Aliment « pois »)	Tests (Aliment expé.)	*t*	*P*-value	
Lot N1	352 ± 13	310 ± 13	2,74	0,02	*
Lot N2	342 ± 12	313 ± 11	2,28	0,04	*
Lot N3	341 ± 17	299 ± 14	2,58	0,03	*

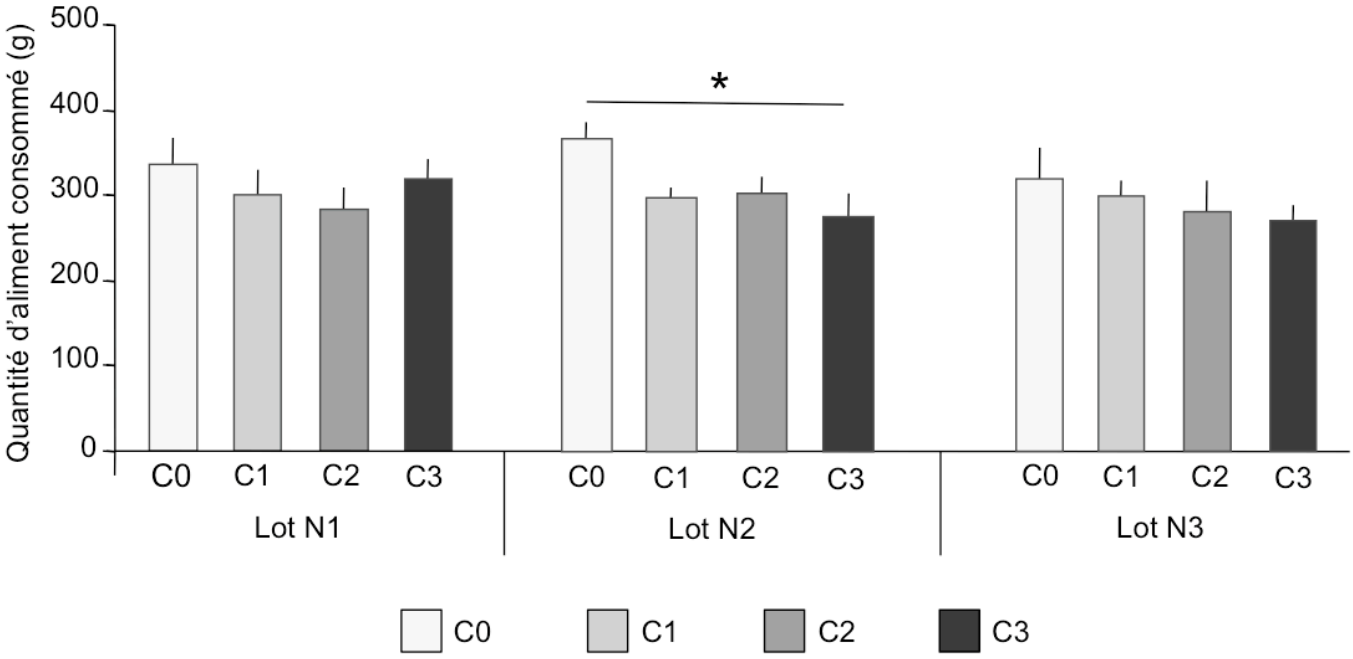

Figure 12. Quantité moyenne (S.E.M.) d'aliment consommé (en g) dans chaque lot expérimental (Lot N1, Lot N2 et Lot N3) en fonction de la concentration en additif (C0, C1, C2 et C3). * $P < 0,05$. Les concentration en additif de chaque aliment sont les suivantes : C0 = 0% (pour les 3 lots expérimentaux), Lot N1 : C1 = 0,925%, C2 = 3,7%, C2 = 14,8% ; Lot N2 : C1 = 0,00375%, C2 = 0,0075%, C3 = 0,03% ; Lot N3 : C1 = 0,01125%, C2 = 0,045%, C3 = 0,18%.

4. Conclusions

Les animaux n'ont montré de préférence marquée pour aucune concentration particulière, quelque soit l'additif testé. Cependant, pour l'aliment N2, il semblerait que les animaux aient préféré l'aliment sans additif (C0) à l'aliment le plus fortement concentré (C3), suggérant que l'ajout de cet additif dans un aliment standard aurait plutôt tendance à provoquer une baisse de la prise alimentaire, peut-être *via* une diminution de la palatabilité de l'aliment. On peut

néanmoins souligner la grande variabilité interindividuelle dans le niveau d'ingestion spontanée au sein des lots.

Par ailleurs, les analyses de comparaison entre les consommations moyennes enregistrées sur les quatre jours de la phase d'habituation (aliment « pois ») et de la phase de test (aliments expérimentaux, toutes concentrations confondues) ont montré une préférence marquée pour l'aliment « pois » comparé aux aliments expérimentaux. Pourtant, en conditions d'élevage classique, les animaux augmentent continuellement et spontanément leur prise alimentaire avec la croissance. La diminution de la consommation observée dans notre étude met en évidence d'une part une réponse néophobique pour l'aliment expérimental inconnu, et d'autre part souligne le fait que l'ajout des additifs dans l'aliment, quelque soit le dosage, diminue la palatabilité de l'aliment comparé à l'aliment « pois » dont la palatabilité est pourtant faible.

Dans le cadre de cette expérience, en situation optimale (non stressante), la néophobie pour l'aliment n'a donc pas permis de mettre en évidence l'effet de la concentration des additifs sur les préférences alimentaires des animaux. Il serait alors envisageable de s'affranchir de cette néophobie dans un premier temps, et de travailler sur une population fragilisée (stress social ou environnemental) afin d'évaluer l'efficacité des additifs selon la population considérée.

DISCUSSION GÉNÉRALE

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L'étude des mécanismes comportementaux et neurobiologiques qui sous-tendent la modulation hédonique du comportement alimentaire chez le porc, *via* notamment l'établissement des préférences et aversions, présente d'importants enjeux en termes d'applications aussi bien dans le domaine de la production porcine que de la recherche biomédicale. L'objectif principal de la thèse était donc de décrire ces mécanismes, notamment par le biais du développement d'un modèle porcin des préférences et des aversions alimentaires conditionnées, mais également par l'étude de l'établissement des préférences alimentaires spontanées. Plus particulièrement, au cours de nos travaux, nous avons cherché à déterminer lesquels, des signaux oraux (*e.g.* goût) ou viscéraux (*e.g.* apport calorique), étaient les plus déterminants pour la mise en place des choix alimentaires chez le porc juvénile. Les différentes questions de recherche ont été abordées grâce à une approche pluridisciplinaire mettant en œuvre des méthodes éthologiques, telles que le conditionnement classique et opérant et les tests de choix alimentaires, et des techniques d'imagerie cérébrale fonctionnelle, telles que la tomographie par émission de positrons (PET) et la tomographie par émission monophotonique (SPECT).

La discussion qui suit est structurée en quatre parties distinctes. Dans une première partie, nous reviendrons sur l'ensemble des résultats scientifiques issus de cette thèse, en détaillant notamment les composantes comportementales et cérébrales majeures qui semblent intervenir dans l'établissement et l'expression des préférences et aversions alimentaires chez le porc, au regard de la littérature existante chez d'autres espèces. Dans une seconde partie, nous aborderons les questions méthodologiques relatives à cette thèse en nous intéressant aux facteurs expérimentaux et individuels inhérents aux protocoles mis en œuvre dans cette thèse et qui pourraient avoir eu une influence sur la capacité de nos animaux à exprimer ou non des préférences et aversions alimentaires. Dans une troisième partie, nous évoquerons l'application de ces travaux dans les domaines de la nutrition et santé animale et humaine, en élevage et en recherche biomédicale. Enfin, nous terminerons cette discussion par des perspectives de recherche, en dégageant les différentes pistes à explorer à l'avenir pour compléter et enrichir ce travail.

Tableau VIII. Résumé des principaux résultats comportementaux et ingestifs obtenus et des principales caractéristiques expérimentales des expériences de conditionnement aversif ou préférentiel. US : stimulus inconditionné ; CS : stimulus conditionné ; i.d. : intra-duodénale ; N : nombre d'animaux utilisés.

Chapitre	Aversion Préférence	N	Repas/ Accès à l'eau	CS	US	Voie	Type de renforcement	Caractéristiques des sessions de conditionnement		Associations US/CS simultanées	Résultats principaux obtenus
								Nb	Durée		
I	Aversion	11	Repas : pendant session	Aliment aromatisé	LiCl 8%	i.d.	Nausées	4	30 min	3	Forte aversion à court terme, et maintien à moyen terme[2]
	Préférence		Eau : *ad lib*		Glucose 15% (90 kcal)		Calories				Pas de préférence, mais évitement à court terme (comparé à F_{NaCl}[3])
II-1A	Préférence	9	Repas : pendant session	Boisson aromatisée	Saccharose 1,125% (0,6 L = 27 kcal[1])	*Per os*	Calories + Goût sucré	3	7 h	2	Pas de préférence à court terme
II-1B	Préférence	8	Privation eau : 1 h avant début session	Boisson aromatisée	Saccharose 10% (1,11 L = 444 kcal[1])	*Per os*	Calories + Goût sucré	6	7 h	2	Préférence, mais à court terme seulement, disparaît dès le 2ème test
II-2	Préférence	9		Boisson aromatisée	Saccharine 0,37%	*Per os*	Goût sucré	4	7 h	3	Pas de préférence nette, mais augmentation (107%) de la consommation de la boisson associée à la maltodextrine comparé au témoin
					Maltodextrine 2,25% (2,53 L = 228 kcal[1])		Calories				
III	Préférence	9	Repas : après session	Boisson aromatisée	Saccharose 16% (CS++ : 0,6L = 384kcal[1])	*Per os*	Calories + Goût sucré	4	1 h	3	Pas de préférence nette, mais CS++ *vs* CS–[4] = 57% *vs* 43% Augmentation motivation pour CS++ (exploration abreuvoir) Modifications microstructure abreuvement (durée, nombre)
			Privation eau : hors session		Saccharose 16% (CS+ : 384 kcal)	i.d.	Calories + Goût sucré				

[1] Quantité moyenne d'eau de boisson consommée pendant les sessions de conditionnement et valeur énergétique équivalente lorsque le renforcement était ajouté directement dans l'eau boisson (*i.e. per os*).
[2] Les tests de choix réalisés la semaine suivant la fin du conditionnement reflètent l'apprentissage à court terme, et la 7ème semaine suivant le conditionnement, l'apprentissage à moyen terme.
[3] Traitement témoin associé à une infusion i.d. de NaCl dans les mêmes conditions de débit et de volume que les traitements LiCl et Glucose.
[4] Traitement témoin associé à une infusion i.d. d'eau distillée dans les mêmes conditions de débit de volume que le traitement CS+.

1. Mécanismes sous-tendant l'établissement et/ou le rappel des préférences et aversions alimentaires

1.1. Déterminants physiologiques et comportementaux

1.1.1. Préférences *vs* aversions alimentaires conditionnées

Le résumé des expériences de conditionnement préférentiel et aversif est présenté dans le **Tableau VIII**. Sur le plan comportemental, nous avons développé une aversion alimentaire conditionnée induite *via* des injections i.d. de LiCl, qui s'est caractérisée par une modification drastique des préférences alimentaires et du profil comportemental et ingestif (**article n°2**). Le développement d'aversions alimentaires conditionnées induites *via* le LiCl a été mis en évidence chez diverses espèces, notamment chez le modèle bovin (Olsen *et al.*, 1989 ; Ralphs et Stegelmeier, 1998), équin (Pfister *et al.*, 2007), ovin (mouton : Ginane et Dumont, 2006 ; Provenza *et al.*, 1994 ; agneau : Burritt et Provenza, 1996 ; Launchbaugh et Provenza, 1994), porcin (Gaultier *et al.*, 2011), caprin (Duncan et Young, 2002), rongeur (rat : Desgranges *et al.*, 2009 ; souris : Stafstrom-Davis *et al.*, 2001), ou même chez les reptiles (Paradis et Cabanac, 2004). D'autres substances ont été utilisées pour induire des aversions alimentaires, mais le LiCl s'est souvent révélé être la substance la plus efficace, puisqu'il permet l'acquisition d'une aversion marquée et très résistance à l'extinction (Ralphs et Stegelmeier, 1998). Meunier-Salaün *et al.* (2010) ont comparé l'efficacité de différents composés parmi lesquels le LiCl, l'apomorphine, la vératrine et l'érythrocine pour le développement d'une aversion alimentaire conditionnée chez le porc. Leurs résultats ont montré que seul le LiCl provoquait des réponses stéréotypées et des épisodes de vomissement. De plus, une injection i.d. s'est révélée plus efficace qu'une injection i.g. pour l'acquisition de l'aversion, avec des épisodes émétiques plus nombreux et plus de temps passé à réaliser des activités stéréotypées. L'ensemble de ces résultats suggère donc que les effets postingestifs négatifs engendrés par l'administration i.d. de LiCl représentent un stimulus particulièrement efficace pour l'établissement d'une aversion conditionnée robuste chez le porc juvénile.

Au contraire, le développement d'une préférence alimentaire conditionnée s'est révélé plus difficile à mettre en évidence, malgré le recours à des paradigmes variés (**Tableau I**). D'une part, le recours à des renforcements strictement caloriques *via* des infusions i.d. d'hydrates de carbone, comme le glucose à 15% (**article n°2**) et le saccharose à 16% (**article n°4**), ou encore par l'administration orale d'un composé calorique non sucré comme la mal-

todextrine à 2,25% (**article n°3**) n'ont pas permis d'aboutir au développement de préférences alimentaires. D'autre part, l'utilisation, en tant que renforcement gustatif, de la saccharine, un édulcorant artificiel non calorique connu pour avoir un goût sucré chez l'Homme, n'a pas non plus entraîné la mise en place de préférences (**article n°3**). Seule l'utilisation de renforcements combinant apport calorique et goût sucré *via* l'ajout dans l'eau de boisson de saccharose à 10% (**article n°3**) ou 16% (**article n°4**) a abouti à l'expression de préférences alimentaires. L'administration orale de saccharose 10% a permis d'aboutir au développement d'une préférence alimentaire significative mais seulement à très court terme (*i.e.* la semaine suivant le conditionnement). L'administration orale de saccharose 16%, bien que n'ayant pas permis la mise en évidence statistique d'une préférence, a tout de même entraîné une augmentation des réponses motivationnelles pour la solution pendant le conditionnement, et des modifications sensibles de la microstructure de l'abreuvement (augmentation du temps total passé à boire et de la durée des épisodes d'abreuvement) pendant les tests de choix ultérieurs.

Ainsi, nos résultats semblent indiquer que les aversions sont plus faciles à induire que les préférences alimentaires chez le porc juvénile. Comme évoqué dans la synthèse bibliographique (**article n°1**), les apprentissages alimentaires préférentiels ou aversifs ont de nombreuses caractéristiques communes, comme notamment la rapidité de leur acquisition et leur robustesse. Cependant, bien que certaines études soient parvenues à induire des préférences alimentaires robustes après un nombre très faible d'associations US/CS chez le rat (Ackroff *et al.*, 2009), plusieurs hypothèses peuvent expliquer qu'en pratique, les préférences soient plus difficiles à obtenir que les aversions. D'une part, comparés au malaise gastrique, un apport calorique ou la perception d'un goût agréable en bouche sont des signaux viscéraux et gustatifs de faible intensité, et seront donc moins facilement intégrés au mécanisme de conditionnement (Bellisle, 2006). Un malaise viscéral douloureux aura de plus grandes chances de provoquer chez l'animal une modification drastique et persistante des choix alimentaires, alors que l'apprentissage d'une préférence est un processus plus progressif nécessitant davantage d'associations (Bellisle, 2003). D'autre part, comme suggéré par Ginane et Dumont (2006), d'un point de vue adaptatif, les conséquences postingestives négatives provoquées par la consommation d'un aliment néfaste (*e.g.* plante toxique) représenteraient un coût important pour l'animal comparé aux bénéfices issus de la consommation d'un aliment calorique ou au goût agréable, d'où l'intérêt pour l'animal de retenir plus rapidement les aliments à ne pas consommer.

Pourtant, une multitude d'études utilisant des paradigmes expérimentaux similaires à ceux développés dans cette thèse ont rapporté l'établissement de préférences conditionnées induites *via* des renforcements gustatifs et/ou caloriques chez le modèle rongeur (*e.g.* Bonacchi *et al.*, 2008 ; Gilbert *et al.*, 2003 ; Reilly et Trifunovic, 2000 ; Sclafani *et al.*, 1997 ; Warwick et Weingarten, 1994), ou ruminant tels que les agneaux (Villalba et Provenza, 1997, 2000), les moutons (Ginane *et al.*, 2009) et les chèvres (Duncan et Young, 2002). Ces résultats nous amèneront donc, dans la suite de cette discussion, à nous interroger sur les raisons qui pourraient expliquer la difficulté à induire des préférences alimentaires marquées chez le porc (*cf. 2. Facteurs influençant l'acquisition et/ou l'expression des apprentissages*).

1.1.2. Renforcement gustatif *vs* renforcement calorique

L'objectif principal de cette thèse était de déterminer la voie d'action (sensorielle *vs* viscérale) la plus déterminante pour l'établissement des préférences et aversions alimentaires sur le plan physiologique et comportemental. Dans le cas des aversions alimentaires, le nombre important d'épisodes émétiques observés suite à l'administration de LiCl durant le conditionnement (**article n°2**) suggère que **l'intensité élevée du malaise intestinal induit par le LiCl** est un signal suffisamment intense pour induire une aversion chez le porc, même en l'absence de stimulus oral désagréable. À notre connaissance, aucune étude ne s'est intéressée à la mise en place d'aversion conditionnée par des renforcements gustatifs négatifs, comme par exemple l'ajout dans une boisson aromatisée d'un goût désagréable (*e.g.* amer). Pourtant, l'aversion spontanée pour l'amer a été largement rapportée chez les primates humains et non humains, les rongeurs et les porcs (*e.g.* Berridge, 2000 ; Michiels et al. 2012 ; Steiner *et al.*, 2001), et des études ont montré que le goût désagréable d'un aliment pouvait prévaloir sur un signal viscéral positif chez l'Homme (Zeinstra *et al.*, 2009). Des études ultérieures pourraient permettre (1) d'évaluer la possibilité de mettre en place une aversion *via* un renforcement négatif agissant sur la sphère sensorielle extéroceptive ; (2) comparer la robustesse et l'intensité des aversions obtenues *via* un renforcement négatif oral (goût désagréable) *vs* viscéral (malaise intestinal), et ainsi de caractériser les signaux les plus déterminants dans l'établissement des aversions alimentaires.

Les résultats des expériences de conditionnement préférentiel ont montré qu'un renforcement calorique seul, au même titre qu'un renforcement gustatif seul, ne suffisait pas à induire une préférence marquée chez le porc juvénile, du moins dans les conditions expérimentales de ce travail. Pourtant, chez les rats, l'utilisation indépendante d'un renforcement

oral ou viscéral est suffisante pour l'acquisition d'une préférence marquée (Drucker *et al.*, 1994 ; Lucas *et al.*, 1997 ; Myers et Hall, 2000 ; Warwick et Weingarten, 1994). Chez l'Homme, par contre, alors qu'un renforcement calorique seul serait suffisant pour induire des préférences robustes (Capaldi et Privitera, 2007), un renforcement oral seul semblerait inefficace (Yeomans *et al.*, 2008). Ces résultats mettent donc à jour des différences inter-espèces dans l'efficacité des renforcements gustatifs ou caloriques à induire des préférences conditionnées, et il semblerait que, chez le porc, **la combinaison entre goût plaisant et apport calorique** soit le **stimulus renforçateur le plus efficace** pour le conditionnement d'une préférence.

Cependant, nos expériences ne nous permettent pas de conclure sur l'absolue nécessité de cette combinaison, ni d'exclure totalement la possibilité d'induire une préférence en utilisant des renforcements sucrés ou caloriques indépendants. D'une part, il est possible que la quantité de calories apportées par les renforcements i.d. (glucose 15% : 90 kcal, maltodextrine 2,25% : 228 kcal, et saccharose 16% : 384 kcal) n'ait pas représenté un signal suffisamment intense pour modifier durablement les choix alimentaires. Dans l'**article n°3**, nous rapportons une consommation de la boisson précédemment associée à la maltodextrine 107% supérieure à celle de la boisson témoin pendant les tests de choix, suggérant un effet potentiellement renforçateur de la maltodextrine qui mériterait des expériences complémentaires. D'autre part, comme souligné dans la discussion de l'**article n°3**, des différences inter-espèces existent dans la perception du goût sucré de la saccharine, et il est possible que le goût de la saccharine ne soit pas perçu comme agréable par le porc. L'utilisation d'un autre édulcorant non calorique, comme l'aspartame, en tant que renforcement sucré permettrait de vérifier la possibilité d'induire des préférences *via* le goût sucré seul chez le porc. Une partie des résultats de cette thèse tend d'ailleurs à confirmer l'attrait des porcs pour le goût sucré. Ainsi, la perception de saccharose à 16% dans l'eau de boisson induit une augmentation des réponses motivationnelles (*e.g.* temps passé à explorer l'abreuvoir, **article n°5**). Dans le *chapitre IV,* l'ajout dans l'aliment d'un additif alimentaire fonctionnel à base de stevia, un édulcorant naturel au goût sucré, modifie la palatabilité de l'aliment, les porcs exprimant une préférence globale pour cet additif (**article n°6)**. De plus, bien que l'ajout des additifs dans un nouvel aliment après transition n'ait pas induit une augmentation des quantités ingérées, l'additif à base de stevia semblerait maintenir un niveau normal de consommation le jour de la transition. Nous pouvons donc supposer un effet positif du goût sucré sur la consommation

et les préférences alimentaires, et envisager la possibilité de réaliser un conditionnement préférentiel *via* le goût sucré seul chez le porc.

1.2. Déterminants neurologiques

Les travaux de la thèse visaient aussi à déterminer si la perception de stimuli alimentaires olfactifs, gustatifs et/ou viscéraux aux valeurs hédoniques marquées entraînerait l'activation différentielle de régions cérébrales connues pour être impliquées chez l'Homme ou d'autres modèles animaux dans la régulation hédonique du comportement alimentaire.

1.2.1. Des patterns d'activité cérébrale caractéristiques

Nos études d'imagerie ont permis de mettre en évidence des patterns d'activation spécifiques à la perception de stimuli alimentaires, qu'il s'agisse de la perception orale et nasale de flaveurs alimentaires aux valeurs hédoniques contrastées (**article n°2**) ou de la perception orale et viscérale du saccharose (**article n°5**). La perception de ces stimuli a engendré des différences d'activation dans les circuits neuronaux impliqués dans la reconnaissance primaire des stimuli sensoriels, et dans l'ensemble des volets motivationnel, affectif (plaisir) et cognitif (mémoire) de la régulation du comportement alimentaire.

Volets affectif et motivationnel. Comparées entre elles, les perceptions de flaveurs aversive, moins préférée ou préférée (**article n°2**) ont induit des différences d'activation dans l'OFC, l'ACC et l'IC, alors que nous ne sommes pas parvenus à mettre en lumière ces changements d'activité suite à la perception orale et/ou duodénale de saccharose (**article n°5**). Ces structures étant connues pour participer au traitement de la récompense (pour revue, Haber et Knutson, 2010) et à l'évaluation émotionnelle et hédonique des informations sensorielles (Kringelbach et Berridge, 2010), nous nous attendions pourtant à observer des changements d'activation de ces structures suite à la perception d'un stimulus hédonique tel que le saccharose. L'OFC (Rolls *et al.*, 2003 ; Royet *et al.*, 1999) et l'ACC (de Araujo *et al.*, 2005 ; Rolls *et al.*, 2003) sont également impliqués dans les processus d'évaluation hédonique des odeurs et dans la formation et la reconnaissance des flaveurs chez l'Homme (de Araujo *et al.*, 2003). De même, l'IC, qui émet des projections vers l'OFC et l'ACC, est impliqué dans le traitement primaire des odeurs et des goûts (de Araujo *et al.*, 2003 ; Small *et al.*, 2004). Ainsi, l'absence d'activation de l'OFC, de l'ACC ou de l'IC suite à la perception orale et/ou viscérale de saccharose suggère que leur activation suite à la perception de flaveurs serait davantage induite par le **traitement des informations olfactives et gustatives nécessaires à la**

reconnaissance des flaveurs (**article n°2**). Néanmoins, il est important de rappeler que les deux études reposent sur des techniques d'imageries fonctionnelles différentes. L'absence de différences significatives dans l'activation de ces structures suite à une acquisition par la technique du SPECT pourrait donc être dû à des limites méthodologiques, la résolution de la SPECT étant bien moins élevée que celle de la PET (**Tableau III**).

La perception combinée de saccharose aux niveaux oral et duodénal, comparée à une stimulation neutre, a aussi induit des modifications de l'activité cérébrale dans des structures impliquées dans l'évaluation de la valeur hédonique des stimuli d'ordre alimentaire, dans le traitement de la récompense et de la motivation. Ceci concerne notamment l'AMY, l'APFC, le PHC, le CAU et le PUT (pour revues, Fattore *et al.*, 2010 ; Schultz, 2000). Des différences d'activation ont également été mises en évidence au sein de ces structures suite à la perception de flaveurs aux valeurs hédoniques contrastées (**article n°2 ;** Gaultier *et al.*, 2011). Du point de vue du traitement central, ces résultats suggèrent donc que, chez le porc, les composantes affectives et motivationnelles du comportement alimentaire entrent en jeu à la fois lors du traitement de stimuli sensoriels **complexes** (flaveur) et de stimuli sensoriels **simples** (goût sucré du saccharose). Elles sont aussi impliquées lors de la perception d'un stimulus sensoriel (*e.g.* flaveur) qui a été **associé dans le passé** à des conséquences postingestives spécifiques (*e.g.* malaise intestinal) et d'un stimulus sensoriel (*e.g.* goût sucré) qui est **directement associé** à des conséquences postingestives spécifiques (*e.g.* apport calorique). Néanmoins, nos données ne nous permettent pas de déterminer avec certitude pour quelle raison seule la perception combinée orale et duodénale du saccharose est parvenue à induire des différences d'activation dans ces régions, alors que des stimuli oraux ou viscéraux indépendants n'y sont pas parvenus. Ces résultats pourraient s'expliquer par des limites méthodologiques, hypothèse renforcée par de récents travaux mettant en évidence chez le porc des activations différentielles dans ces régions (PUT, CAU, APFC) suite à la perception duodénale de glucose (Boubaker *et al.*, 2012).

Volet cognitif : Mémoire et expérience de l'individu. Nos résultats ont également révélé l'**impact de facteurs relatifs à l'expérience individuelle** sur les réponses cérébrales provoquées par la perception de stimuli alimentaires. Nous avons ainsi montré l'implication de régions cérébrales jouant un rôle dans les processus de mémoire (*e.g.* APFC, PHC, PeC, PUT, CAU) lors de la perception orale et viscérale de saccharose, stimulus auquel les animaux avaient été exposés auparavant lors de tests de ratio progressif (**article n°5**). Des résul-

tats similaires ont été mis en évidence lors de la perception de flaveurs connues (**article n°2**). Des études chez l'Homme ont souligné l'implication de l'APFC dans les processus mnésiques (Kouneiher *et al.*, 2009) et dans les processus d'associations entre un événement passé (*e.g.* prise alimentaire) et les informations relatives au contexte de cet événement (*e.g.* état interne, état subjectif ; Simons *et al.*, 2005). Le rôle du PHC et du PeC dans la mémoire a aussi été rapporté au vu de leurs étroites connexions avec l'hippocampe, une structure impliquée dans les processus cognitifs comme la mémoire et l'apprentissage chez l'Homme (pour revues, Fanselow et Dong, 2010 ; Furtak *et al.*, 2007 ; Kerr *et al.*, 2007). Enfin, les noyaux de la base, et notamment le PUT et le CAU, sont connus pour être impliqués dans la mémoire (Grahn *et al.*, 2008 ; White, 1997) et dans les processus de sélection de récompense en fonction de l'expérience individuelle (Muranishi *et al.*, 2011). Des expériences complémentaires comparant l'activation cérébrale induite par la perception de stimuli sensoriels chez des animaux ayant été soumis ou non à ces stimulations par le passé permettrait de vérifier l'impact de l'expérience sur les réponses cérébrales enregistrées au sein de ces régions.

1.2.2. Stimulation sensorielle *vs* stimulation viscérale

La tomographie par émission monophotonique (SPECT) nous a permis de démontrer que seule la perception combinée orale et viscérale du saccharose provoquait des réponses cérébrales marquées dans les structures impliquées dans le contrôle hédonique de la prise alimentaire, dans le traitement de la récompense et dans la motivation alimentaire et la mémoire (**article n°5**). Par contre, la perception indépendante de saccharose au niveau oral ou duodénal n'a pas suffi, dans ce contexte expérimental, à mettre de tels résultats en évidence, contrairement à ce qui a été rapporté lors de la perception i.d. de glucose chez le porc (Boubaker *et al.*, 2012). Ces résultats font écho à ceux obtenus par l'approche comportementale du conditionnement préférentiel : alors que l'association entre signaux viscéraux et oraux, notamment avec l'utilisation du saccharose comme renforcement (**article n°3**), entrainait la mise en place d'une réponse comportementale hédonique, des signaux renforçateurs oraux ou caloriques indépendants étaient relativement inefficaces. Ainsi, les résultats comportementaux couplés à l'approche d'imagerie cérébrale permettent de privilégier l'hypothèse selon laquelle la combinaison entre goût sucré et apport calorique serait nécessaire pour le développement de réponses alimentaires positives chez le porc. Ceci souligne bien la complémentarité et la synergie entre les deux approches expérimentales et la pertinence de leur combinaison. Une expérience complémentaire similaire à celle présentée dans le *chapitre I* et dans

laquelle les animaux seraient d'abord soumis à un conditionnement préférentiel *via* le saccharose, puis à l'imagerie suite à la perception des flaveurs ainsi conditionnées permettrait de mettre en parallèle les réponses comportementales et cérébrales engendrées par la perception d'une flaveur précédemment associée à un renforcement sucré et calorique.

2. Facteurs influençant l'acquisition et/ou l'expression des apprentissages

La première partie de la discussion a permis de souligner l'obtention rapide d'un modèle de l'aversion alimentaire conditionnée, mais aussi des difficultés à montrer l'expression d'une préférence alimentaire, malgré une littérature abondante chez le rongeur rapportant le développement de préférences alimentaires conditionnées *via* des renforcements gustatifs et/ou caloriques. Un certain nombre de facteurs inhérents aux paradigmes expérimentaux mis œuvre, ou aux sujets eux-mêmes, a pu influencer l'issue de nos expériences de conditionnements.

2.1. Facteurs liés au paradigme expérimental

2.1.1. Influence des variables temporelles

Au cours de ce travail de thèse, plusieurs paradigmes expérimentaux caractérisés par différents temps d'exposition au CS ont été utilisés. Le temps d'exposition au CS dépendait du nombre total de répétitions de chaque association US/CS (de 3 à 6 répétitions) et de la durée de chaque session de conditionnement et de tests de choix (entre 30 min et 7 h par session ; **Tableau VIII**). L'impact de ces contraintes temporelles sur la qualité de l'apprentissage et sur sa résistance à l'extinction a été évalué dans différentes études (Bouton et Sunsay, 2003). Ainsi, **le nombre d'associations US/CS pendant le conditionnement**, *i.e.* le nombre de répétitions de chaque session de conditionnement, semble avoir peu d'effet sur l'acquisition de l'apprentissage chez les rats et les souris (Gottlieb, 2008) ou les chèvres (Duncan *et al.*, 2007). Par ailleurs, la plupart des études menées chez les rongeurs ont mis en évidence des préférences marquées en utilisant un nombre d'associations équivalent à celui utilisé dans cette thèse (*e.g.* entre 3 et 4 répétitions ; Azzara et Sclafani, 1998 ; Bonacchi *et al.*, 2008 ; Gilbert *et al.*, 2003 ; Reilly et Trifunovic, 2000). Dans nos travaux, **la durée des sessions** était comprise entre 30 min (**article n°2**) et 7 h (**article n°3**) par jour, ce qui représente des durées relativement courtes si nous nous référons à la majorité des études réalisées chez le rat et qui utilisent des sessions de conditionnement de 23 h par jour (e.g. Azzara et Sclafani, 1998 ; Bonacchi *et al.*, 2008 ; Reilly et Trifunovic, 2000 ; Sclafani et Glendinning, 2005).

Ceci suggère donc qu'une augmentation de la durée d'exposition au CS *via* un allongement de la durée des sessions de conditionnement pourrait aboutir à des préférences plus marquées.

Il est possible que d'autres contraintes temporelles entrent en jeu dans la réussite de l'apprentissage, comme le temps séparant chaque session, ou encore la fréquence d'administration du renforcement (*i.e.* renforcement non systématique ; Bouton et Sunsay, 2003). Certains auteurs soulignent par exemple que **la durée des intervalles séparant les sessions** pourrait avoir un impact sur la réussite de l'apprentissage (Pavlov, 1960), des sessions de conditionnement plus espacées dans le temps produisant des réponses plus marquées que des sessions rapprochées chez les pigeons (Terrace *et al.*, 1975), les rats (Barnet *et al.*, 1995 ; Bouton et Sunsay, 2003) ou encore les abeilles (Deisig *et al.*, 2007). Dans la configuration où les sessions de conditionnement sont trop rapprochées dans le temps, l'effet du conditionnement précédent peut avoir une influence négative sur l'association lors du conditionnement suivant en agissant sur la mémoire à court terme (Sunsay *et al.*, 2004). Cette hypothèse pourrait en partie expliquer l'échec du conditionnement au glucose décrit dans le *chapitre I*, notamment lorsqu'il était précédé par une session de conditionnement aversif au LiCl générant des conséquences postingestives relativement intenses (**article n°2**). Il est possible que la réalisation de sessions de conditionnement séparées par des intervalles de temps plus importants (*e.g.* 1 association tous les 2 jours) aboutisse à l'établissement de préférences alimentaires plus marquées chez le porc.

2.1.2. Influence des facteurs liés au stimulus inconditionnel (US)

Villalba et Provenza (2000) ont montré que les agneaux développaient une préférence pour un aliment associé à un apport calorique obtenu *via* une infusion d'amidon dans le rumen si l'infusion était proportionnelle à la quantité d'aliment ingéré, mais pas si la quantité d'amidon infusée était fixe. De nombreuses études chez le rat (*e.g.* Ackroff *et al.*, 2001 ; Ackroff *et al.*, 2011 ; Azzara et Sclafani, 1998 ; Sclafani et Glendinning, 2005 ; Touzani *et al.*, 2009a, b, 2010) et le mouton (Ginane *et al.*, 2009) utilisent également des quantités proportionnelles entre CS et US, en suivant généralement un ratio 1:1. Dans notre cas, lors des conditionnements utilisant des renforcements i.d. (glucose, saccharose 16%), des quantités fixes de US ont été administrées aux animaux, quelle que soit la quantité d'aliment et/ou d'eau de boisson ingérée. L**'adéquation entre quantité de CS ingéré et d'US administré** pourrait ainsi être un facteur facilitant l'établissement d'apprentissages alimentaires, notamment dans le cas des apprentissages préférentiels. Des études ont néanmoins obtenues des

préférences robustes en administrant des quantités d'US fixes chez le mouton (Villalba et Provenza, 1997), la chèvre (Duncan et Young, 2002) et les rongeurs (Myers, 2007). Des essais complémentaires utilisant des quantités d'US proportionnelles à la quantité de CS ingéré permettraient d'évaluer cet effet d'adéquation chez le porc.

La **dose d'US administré** semble également être un critère important pour le conditionnement de préférences alimentaires, notamment celles induites par des renforcements caloriques. En effet, alors qu'un apport calorique peut représenter une conséquence postingestive positive, certaines études ont évoqué un effet inverse de ces calories sur la prise alimentaire. Ce phénomène, appelé l'**alliesthésie négative**, a été décrit pour la première fois par Cabanac (1971). L'alliesthésie négative est définie comme une diminution de la perception hédonique d'un stimulus gustatif suite à une augmentation de la charge énergétique viscérale, entraînant une moindre préférence pour ce stimulus. Cabanac *et al.* (1973) ont par exemple observé une baisse de la valeur hédonique d'un stimulus oral sucré après l'infusion i.g. de glucose, comparé à une situation sans infusion. Ce phénomène pourrait notamment expliquer pourquoi nous sommes parvenus à développer une préférence pour une eau de boisson additionnée de saccharose à 10% (apport calorique étalé sur la journée : 444 kcal/7 h, **article n°3**) alors que le conditionnement induit par l'ajout de saccharose à 16% dans l'eau de boisson (apport calorique rapide : 384 kcal/1 h, **article n°5**) n'a pas induit de préférences. Au contraire, l'absence de préférence pour la flaveur associée au glucose 15% (90 kcal, **article n°2**) pourrait être due à un niveau calorique trop faible. Des études complémentaires comparant différentes doses d'infusion i.g. de saccharose pourraient permettre de déterminer avec précision l'apport calorique idéal pour le développement de préférence alimentaire marquée.

2.1.3. Influence de la complexité de l'apprentissage

Les paradigmes de conditionnement mis en œuvre dans cette thèse impliquaient que les animaux réalisent soit deux (*i.e.* CS+ et CS–, **article n°3**), soit trois associations US/CS successives (*i.e.* CS++, CS+ et CS–, **articles n°3** et **n°4**). Chaque paradigme dans lequel l'animal réalisait trois associations successives s'est soldé par un échec, aucune préférence alimentaire n'ayant été exprimée lors des tests de choix. La seule préférence obtenue résultait d'un apprentissage moins complexe au cours duquel l'animal ne devait mémoriser que deux associations US/CS (*i.e.* solution F+ couplée au saccharose 10% et solution contrôle F–, **article n°3**). À partir de ces observations, nous avançons l'hypothèse selon laquelle la difficulté à induire une préférence alimentaire au cours de ces travaux pourrait être due à **des apprentis-**

sages trop complexes à réaliser pour le porc juvénile. Cette hypothèse est appuyée par des travaux menés chez les agneaux (Ginane *et al.*, 2009) qui ont montré le développement d'une préférence marquée lorsque les animaux devaient réaliser deux associations US/CS (deux fourrages associés à des infusions intra-ruménales d'amidon ou d'eau), mais pas lorsqu'ils devaient mémoriser trois associations (trois fourrages associés à des infusions intra-ruménales d'amidon, de caséine ou d'eau). Les performances d'apprentissage peuvent donc être réduites lorsque les animaux sont soumis à des associations US/CS multiples (Ginane *et al.*, 2009), ou lorsque les aliments à conditionner sont présentés simultanément pendant le conditionnement (Duncan et Young, 2002). Cependant, cet effet de la complexité de l'apprentissage doit être évalué en fonction du contexte et pourrait être lié à d'autres critères de variabilité. En effet, l'ensemble des expériences a été réalisé sur des animaux juvéniles et il est probable que des phénomènes de maturation et d'expérience rendent les adultes plus efficaces que des individus immatures (*cf. 2.2.2. Influence des caractéristiques individuelles*).

2.2. Facteurs liés aux individus

2.2.1. Influence de l'état interne de l'animal

Faim vs satiété. Chez de nombreuses espèces, l'état interne des individus est connu pour influencer les préférences alimentaires à la fois sur le long et le court terme (Kyriazakis *et al.*, 1999). Chez les rats, des animaux en état de privation alimentaire pendant le conditionnement consomment une plus grande quantité d'eau de boisson aromatisée associée à des infusions i.g. de glucose que des rats nourris *ad libitum* (Yiin *et al.*, 2005). Chez l'Homme, lors d'un conditionnement alimentaire induit *via* l'ajout de saccharose dans l'eau de boisson, la préférence pour la flaveur renforcée est plus importante lorsque les individus consomment la solution dans un état de faim que dans un état de satiété pendant le conditionnement (Mobini *et al.*, 2007). Il semblerait d'ailleurs que, chez l'Homme, la restriction alimentaire entraîne des réponses préférentielles plus marquées dans le cadre d'apprentissages flaveur/nutriments (Brunstrom et Mitchell, 2007) et flaveur/flaveur (Brunstrom et Fletcher, 2008).

Globalement, ces résultats suggèrent que **la privation alimentaire stimule la préférence** pour une flaveur associée à un renforcement gustatif ou calorique. Compte tenu du développement d'une préférence à court terme induite *via* le saccharose chez des animaux recevant un repas pendant le conditionnement (**article n°3**), nous nous attendions à obtenir des résultats plus marqués chez des animaux en état de privation alimentaire (**article n°4**),

mais le fait que les animaux étaient en état de privation alimentaire aussi pendant les tests de choix pourrait expliquer cet échec. Les animaux étant affamés, leurs choix auraient alors été guidés par des motivations d'ordre homéostatique plutôt qu'hédonique, la motivation de l'animal à consommer l'eau de boisson (précédemment associée à des calories) étant davantage guidée par la faim que par le plaisir. Il est possible que l'acquisition de la préférence ait eu lieu mais que l'animal, affamé, ait été incapable d'exprimer cet apprentissage par la suite. Cette explication est renforcée par l'observation, chez des animaux en état de privation alimentaire, de réponses motivationnelles identiques pour une récompense sucrée ou non, lors des tests de ratio progressif (**article n°5**). Ainsi, **alors que la privation alimentaire apparaît bénéfique à l'acquisition de l'apprentissage, elle pourrait être délétère à son expression lors des tests de choix**. La réalisation d'expériences utilisant des animaux en état de privation alimentaire seulement pendant le conditionnement pourrait permettre de vérifier cette hypothèse.

Les réponses cérébrales sont aussi fortement influencées par l'état de satiété des individus (Del Parigi *et al.*, 2002). Chez l'Homme, Smeets *et al.* (2006) ont montré que la consommation de chocolat jusqu'à l'état de satiété provoquait des changements de l'activité cérébrale dans certaines régions comme le cortex préfrontal, l'OFC, l'IC ou encore l'AMY, le PUT et le GP suite à la perception orale de chocolat. La perception de stimuli gustatifs simples (*e.g.* sucrose, acide citrique) dans un état de satiété, comparé à la condition à jeun, provoque une moindre activation dans les régions impliquées dans les processus de motivation et d'émotion, comme l'AMY, l'ACC et l'OFC (Haase *et al.*, 2009). L'activation de l'IC serait également sensible à l'état de satiété (Tataranni *et al.*, 1999). Enfin, Kringelbach *et al.* (2003) ont mis en évidence une corrélation entre l'activation de l'OFC et l'évaluation hédonique subjective de la flaveur d'un aliment liquide suite à la consommation jusqu'à satiété de cet aliment. De plus, il est important de noter les influences respectives des réponses comportementales, cérébrales et métaboliques sur la prise alimentaire. Domingos *et al.* (2010) ont par exemple suggéré l'influence du statut métabolique sur le jugement hédonique des stimuli et sur l'activité dopaminergique ; la baisse du taux de leptine suite à une privation alimentaire entraîne en effet une augmentation de la valeur hédonique du saccharose et de la libération de DA. Ces résultats soulignent là encore la pertinence d'une approche pluridisciplinaire pour l'étude des mécanismes de régulation de la prise alimentaire, et l'importance de **considérer le statut nutritionnel des individus** lors de l'interprétation de données issues de l'imagerie cérébrale fonctionnelle.

Influence de la privation en eau. Chez les rongeurs, de nombreuses études ont mis en évidence l'impact de la privation en eau sur l'ingestion et les préférences alimentaires. En situation de privation en eau sévère (36-48 h), des rats consomment des quantités équivalentes d'eau et de solutions de saccharose pendant de tests de double choix et lorsque les boissons sont présentées successivement, alors que ces mêmes rats normalement hydratés exprimaient au préalable une préférence marquée pour la solution de saccharose (Scalera, 2000 ; Scalera et Tarozzi, 2001). Selon Scalfera (2000), une déshydratation sévère affecterait les mécanismes gustatifs impliqués dans la reconnaissance des stimuli, entraînant une évaluation équivalente de la palatabilité de l'eau et de la solution de saccharose. Ces mécanismes pourraient agir *via* des modifications de la composition et de la production de salive générées par la privation en eau. En effet, des études ont mis en relation la composition (Neyraud *et al.*, 2012) et la production (Christensen *et al.*, 1984) de salive et l'évaluation hédonique de certains stimuli gustatifs (*e.g.* graisses). Une autre hypothèse proposée par Scalfera (2000) est que l'hyperosmolarité provoquée par la privation en eau pousserait les animaux à boire les solutions indépendamment de leur palatabilité afin de diminuer l'osmolarité plasmatique et ainsi rétablir un équilibre osmotique (Houpt *et al.*, 1999). On peut également supposer que l'osmolarité de la solution entre directement en compte dans le choix des animaux, une solution hyperosmolaire pouvant être perçue comme « moins hydratante », et donc moins palatable pour des animaux privés en eau, qu'une solution dont l'osmolarité est plus faible. Ainsi, des animaux privés en eau pourraient préférer une solution à l'osmolarité faible comparée à une solution hyperosmolaire, sans que, à notre connaissance, cette hypothèse ne soit appuyée par la littérature. Ces différentes hypothèses suggèrent que des **facteurs homéostatiques et osmotiques entrent en jeu dans les choix alimentaires d'animaux en situation de privation en eau**, atténuant ainsi l'impact des facteurs hédoniques sur les préférences.

Ces données pourraient expliquer l'absence de préférence marquée pour une eau de boisson précédemment associée à du saccharose 16% (384 kcal ingérées très rapidement, *i.e.* moins d'1 h) chez des animaux privés en eau depuis 23 h (**article n°4**), alors que nous avons obtenu une préférence pour une eau de boisson associée à du saccharose 10% chez des animaux sans privation en eau et dont l'ingestion de calories était répartie sur 7 h (**article n°3**). Bien que l'équipe de Scalfera n'ait pas démontré l'effet de cette privation en eau pour des délais de privation allant de 12 à 24 h, il est possible que l'état de soif de nos animaux ait suffit à modifier les mécanismes gustatifs, salivaires ou osmolaires, et ainsi empêcher ou perturber l'expression des préférences, en dépit des facteurs hédoniques.

2.2.2. Influence des caractéristiques individuelles

Influence du sexe des individus. L'influence du sexe sur les performances cognitives (apprentissage, mémoire) a été mise en évidence chez des animaux soumis à des conditionnements classiques et opérants (pour revue, Dalla et Shors, 2009). Chez les rongeurs, des études ont rapporté **une extinction plus rapide de l'apprentissage chez les femelles** que chez les mâles dans le cas d'une aversion gustative conditionnée par le LiCl (Choleris *et al.*, 2000 ; Randall-Thompson et Riley, 2003), alors que le sexe ne semblait avoir aucun effet sur l'acquisition d'un tel apprentissage. Cependant, cet effet sexe semble être exclusif des aversions conditionnées au LiCl puisque les auteurs n'ont pas réussi à mettre en évidence cette dichotomie sexuelle sur l'acquisition ou l'extinction d'une aversion gustative conditionnée *via* la morphine. Bien qu'aucune étude n'ait mis en évidence un effet sexe sur l'expression des apprentissages préférentiels, nos travaux ont été réalisés sur des animaux femelles et nous pouvons donc supposer que l'utilisation de mâles aurait pu induire des préférences plus robustes à l'extinction, notamment dans le cas de la préférence à court terme induite par le saccharose 10% (**article n°3**).

Des différences liées au sexe ont également été mises en évidence chez l'Homme quant à l'effet de la satiété sur l'activation de certaines régions cérébrales telles que le striatum ventral et le cortex préfrontal pendant une stimulation gustative (Smeets *et al.*, 2006). Haase *et al.* (2011) ont démontré que dans un état de faim, les femmes traitaient les stimuli gustatifs purs (*e.g.* acide citrique, NaCl) différemment des hommes, avec notamment une activation plus importante dans les régions du système limbique et les régions impliquées dans le traitement de la récompense et de la mémoire (*e.g.* IC, AMY, striatum dorsal, gyrus temporal). Des études ont aussi souligné l'influence du sexe sur l'activation de structures cérébrales impliquées dans la régulation de la prise alimentaire lors de la présentation de stimuli visuels alimentaires, avec une activation plus importante du DLPFC, de l'IC ou encore du PCC chez les femmes que chez les hommes (effet augmenté dans le cas d'aliments hypercaloriques comparés à des aliments peu caloriques ; Cornier *et al.*, 2010 ; Frank *et al.*, 2010 ; Killgore et Yurgelun-Todd, 2010). Ceci suggère un traitement cognitif plus marqué de ces informations chez ces premières. Enfin, Wang *et al.* (2009) ont montré que l'influence du contrôle cognitif (*i.e.* inhibition volontaire de la sensation de faim) sur les réponses cérébrales provoquées par une stimulation alimentaire était plus marquée chez l'homme que chez la femme.

La littérature existante permet de supposer que des activations cérébrales sensiblement différentes à celles trouvées dans nos travaux réalisés sur des animaux femelles, et l'établissement de préférences alimentaires plus robustes auraient pu être mises en évidence chez porcs mâles. Dans un souci de représentativité, il serait intéressant d'étudier ces phénomènes au sein d'un échantillon comportant à la fois des mâles et des femelles pour minimiser l'effet de ce facteur de variation potentielle. Des études complémentaires pourraient ainsi permettre de déterminer si ces différences sexuelles sont induites par une différence dans la perception des signaux alimentaires, et par voie de conséquence expliquer la différence entre les sexes dans la prévalence de désordres alimentaires et de l'obésité (Keel *et al.*, 2007).

Influence de l'âge des individus. Il est classiquement rapporté des différences dans l'expression d'une large variété de comportements entre des individus juvéniles et des adultes. Des travaux menés chez des porcs miniatures adultes ou sevrés ont décrit un effet du sexe sur la réactivité comportementale lors de tests d'isolement social et de confrontation à l'Homme chez des animaux adultes, mais pas chez les porcelets sevrés (Chataignier *et al.*, 2011 ; Val-Laillet *et al.*, 2011b). Ces résultats soulignent une évolution de la réactivité comportementale en fonction de l'âge des individus, qui peut être associée à des **phénomènes de maturation sexuelle**. L'implication des phénomènes de maturation et des changements développementaux a été ainsi mise en évidence lors d'expériences de conditionnements préférentiels *via* le saccharose menées chez des rats avant le sevrage (Myers et Hall, 1998) et après le sevrage chez des rats adultes (Myers et Hall, 2000). Chez les jeunes rats, un renforcement oral sucré a produit une modification des réponses appétitives pour la flaveur associée et un renforcement viscéral calorique a entraîné un changement des réponses consommatoires. Comparativement, chez les rats adultes, les deux types de renforcements ont induit des changements des réponses à la fois appétitives et consommatoires pour la flaveur associée.

L'expression du comportement alimentaire est également modulée par **l'expérience des individus** mettant en jeu des mécanismes d'apprentissage et de mémoire. En effet, un individu au stade adulte aura stocké un nombre considérable d'informations concernant les stimuli alimentaires qu'il aura rencontrés, ce qui lui permettra d'adapter de manière plus fine ses comportements ingestifs et ses choix alimentaires comparé à un individu juvénile (Myers et Hall, 2000). Ces mêmes auteurs soulignent néanmoins que les individus juvéniles seraient capables d'acquérir des apprentissages, au même titre que les adultes, mais avec une moindre efficacité pour la restitution de ces apprentissages lors des tâches post-apprentissages à cause

d'un défaut d'attention et de traitement des stimuli (Myers et Hall, 2000). Les animaux utilisés au cours de cette thèse étaient tous des animaux juvéniles. La réalisation d'études similaires sur des animaux adultes serait donc nécessaire pour vérifier l'existence de différences liées à l'âge dans les réponses comportementales observées lors de ce travail de thèse.

Influence des facteurs génétiques. Les conditions d'élevage actuelles ont nécessité un énorme effort de sélection génétique des animaux en vue de l'amélioration constante de critères de productivité, tels que les critères de prolificité chez les truies ou les critères de croissance chez les porcs. Cette sélection sur critères zootechniques s'accompagne inévitablement de modifications des caractéristiques comportementales comme cela a été montré chez les bovins (Grandin et Deesing, 1998) et chez les porcins (Breuer *et al.*, 2005 ; Holl *et al.*, 2010). Ces phénomènes de sélection peuvent ainsi entrainer des différences comportementales marquées entre les races concernant le comportement maternel (Meunier-Salaün *et al.*, 1991), les agressions (Breuer *et al.*, 2005), le comportement social (Le Neindre, 1989) ou la réactivité émotionnelle (Boissy *et al.*, 2005 ; Holl *et al.*, 2010 ; Le Neindre *et al.*, 1993).

De nombreuses études ont également mis en évidence un **effet de la race et des facteurs génétiques sur les stratégies de sélection alimentaire**, comme cela a été rapporté chez les volailles (Schütz et Jensen, 2001) et les souris (Ramirez et Fuller, 1976), et sur les **profils ingestifs** (Fernández *et al.*, 2011 ; Labroue *et al.*, 1994 ; Labroue *et al.*, 1997 ; Quiniou *et al.*, 1999 ; Renaudeau *et al.*, 2006). Ainsi, Labroue *et al.* (1994) et Fernández *et al.* (2011) ont décrit deux types d'animaux selon les races, les « porcs grignoteurs », réalisant de nombreux repas de courte durée s'étalant tout au long de la journée, et les « porcs mangeurs de gros repas », qui réalisent quelques repas de longue durée chaque jour. Fernández *et al.* (2011) ont aussi rapporté des « mangeurs rapides » et des « mangeurs lents ». Ainsi, les porcs de race Large White seraient des grignoteurs et des mangeurs rapides alors que les porcs de race Landrace seraient des mangeurs de gros repas et des mangeurs rapides (Fernández *et al.*, 2011 ; Quiniou *et al.*, 1999). Dans nos travaux, plusieurs races croisées issues des races Large White et Landrace ont été utilisées. Il est donc probable qu'une étude préalable des profils ingestifs ait révélé une grande variabilité au sein de nos échantillons d'individus (*e.g.* quantités totales ingérées, vitesse d'ingestion, distribution des repas au cours de la journée, *etc.*). Il aurait donc été intéressant **de caractériser les individus en fonction de leurs profils ingestifs** (*e.g.* mangeurs rapides *vs* mangeurs lents, ou grignoteurs *vs* mangeurs de gros repas) afin de minimiser l'impact de ces différences inter-individuelles lors des tests de choix. Enfin, il

faut noter que les facteurs individuels intra-lignée doivent également être pris en compte puisqu'il est admis que les performances d'apprentissage et de mémoire d'individus, au sein d'une même lignée, peuvent différer, comme cela a été mis en évidence chez la grande limnée (*Lymnaea stagnalis* ; Sugai *et al.*, 2007) ou chez le rat âgé (Hok *et al*., 2012).

Ainsi, une multitude de facteurs peuvent avoir un impact important sur les choix et les préférences alimentaires, les capacités d'apprentissage et de mémorisation ainsi que sur les réponses cérébrales induites par la perception de stimuli alimentaires. Bien que l'ensemble de ces facteurs mériterait des études complémentaires, cette tâche est rendue particulièrement difficile par les **nombreuses interactions et influences respectives** qu'exercent ces facteurs les uns sur les autres (*e.g.* effet lignée limité par l'expérience : Sclafani, 2007 ; interaction entre dimorphisme sexuel et type de renforcement : Randall-Thompson et Riley, 2003 ; interaction effet sexe et faim/satiété : Smeets *et al.*, 2006).

3. Validité du modèle porcin pour des applications en élevage et/ou nutrition humaine

3.1. Applications en élevage porcin

En élevage porcin, les animaux sont soumis à de nombreux changements de leur environnement, concernant les volets du logement (passage d'un logement en groupe à un logement individuel), social (séparation de la mère au sevrage, réallotements) et alimentaire (transition alimentaire ; Meunier-Salaün et Picard, 1996). Des changements alimentaires interviennent par exemple au cours de la croissance (*e.g.* d'un aliment 1er âge à un aliment 2ème âge), mais également au sevrage (du lait maternel à un aliment solide), et chez les truies reproductrices (de l'aliment gestation à l'aliment lactation après la mise-bas). Ces changements nécessitent des capacités d'adaptation et, dans certains cas, entraînent des réponses comportementales qui peuvent être des signes de stress (Dong et Pluske, 2007). Parmi ces réponses associées au stress, une diminution de la prise alimentaire peut être observée lors de ces périodes de changements. Un des objectifs de la thèse était donc de déterminer de quelle manière la prise alimentaire pouvait être **maintenue ou stimulée** lors d'une période de changement alimentaire, notamment *via* la compréhension des mécanismes de traitement des signaux sensoriels et viscéraux intervenant dans l'établissement des préférences alimentaires chez le porc.

Les travaux présentés dans le *chapitre IV* n'ont pas abouti au développement de préférences alimentaires marquées vis-à-vis d'additifs alimentaires fonctionnels, ou additfs sensoriels. Néanmoins, l'ajout de l'additif à base de stevia dans le nouvel aliment tendait à mainte-

nir les taux de consommation le jour de la transition (**article n°6**). Les résultats obtenus dans les expériences de conditionnement préférentiel soulignent la difficulté à développer des préférences alimentaires robustes chez le porc juvénile, mais montrent que le recours à un renforcement sucré et calorique pourrait être le meilleur moyen de stimuler/maintenir la prise alimentaire. Cependant, plusieurs limites à l'extrapolation de nos résultats pour des applications pratiques en élevage sont à prendre en compte, compte tenu des différences notoires entre les conditions (*i.e.* environnement physique et social, pratiques d'alimentation, *etc.*) de nos expérimentations et les pratiques classiques en élevage de production.

Les réponses comportementales des animaux vis-à-vis des aliments sont influencées par des facteurs sociaux et d'expérience comme cela a été démontré chez les agneaux (Burritt et Provenza, 1996), les porcelets (Meunier-Salaün *et al.*, 1997 ; Oostindjer *et al.*, 2009 ; Oostindjer *et al.*, 2010), les ratons (Galef, 1985) ou les enfants (Birch, 1999 ; Hursti et Sjödén, 1997 ; Wardle et Cooke, 2008). Galef (1985) a mis en évidence l'importance du **contexte social** sur les choix alimentaires en démontrant qu'un rat conditionné négativement vis-à-vis d'un aliment suite à une infusion de LiCl montrait une reprise de la consommation de l'aliment après une exposition à des individus naïfs ayant mangé le même aliment au préalable. Meunier-Salaün *et al.* (1997) ont montré des résultats similaires sur des porcelets conditionnés de manière aversive vis-à-vis d'un aliment additionné de concanavaline A (*Concavanavalia uniformis*). Ainsi, l'influence des congénères suffirait à renverser les effets d'un apprentissage que nous avons pourtant défini comme robuste et résistant à l'extinction, suggérant **un rôle prépondérant du facteur social dans la mise en place de choix alimentaires**. Ce rôle passe en particulier par un phénomène de **facilitation sociale** qui se traduit par l'influence des congénères sur la tendance à inhiber, engager ou renforcer la performance d'un comportement (Harlow, 1932). Dans nos travaux, les animaux étaient systématiquement logés dans des cages individuelles, limitant ainsi l'impact de l'influence sociale sur leurs choix alimentaires. Aussi, nous pouvons faire l'hypothèse que, dans le cas d'un logement en groupe, la facilitation sociale aurait pu entraîner l'établissement de préférences généralisées pour un aliment ou, au contraire, l'évitement systématique d'un autre aliment. En termes pratique, le recours à des individus « modèles », conditionnés à préférer un aliment nouveau, pourrait par exemple maintenir des niveaux stables de consommation à l'échelle du groupe dans les élevages, en diminuant l'impact de la néophobie chez les congénères naïfs pendant les phases de transitions.

Il apparaît aussi délicat d'extrapoler nos résultats, obtenus sur des animaux juvéniles sevrés, à l'ensemble des stades de vie des porcs d'élevage, compte tenu **des effets de maturation et d'expérience** sur le développement et l'expression des comportements. Des études supplémentaires à différents stades de développement (*e.g.* truies reproductrices, porcelets au sevrage) permettraient d'évaluer les interactions et effets respectifs de la maturité sexuelle et de l'expérience sur la mise en place des choix alimentaires. Par exemple, la possibilité de transposer un apprentissage préférentiel acquis sur de l'eau de boisson vers un aliment solide évoquée dans l'**article n°3** mériterait d'être testée sur des animaux au moment du sevrage, c'est-à-dire pendant le passage d'une alimentation lactée à un aliment solide concentré.

La dernière limite à l'extrapolation de nos résultats pour des applications en élevage concerne la difficulté rencontrée dans le transfert d'une préférence acquise pour une eau de boisson vers un aliment solide (**article n°3**). De nombreuses études ont montré le **caractère généralisable des préférences alimentaires**, notamment chez les ruminants, ces généralisations concernant le passage d'un aliment solide à un autre aliment solide. Par exemple, les agneaux sont capables de généraliser une aversion ou une préférence conditionnée *via* des infusions ruminales de LiCl ou d'amidon, respectivement, d'un aliment connu vers un aliment nouveau, à condition que les deux aliments contiennent la flaveur conditionnée (*e.g.* origan, noix de coco ; Launchbaugh et Provenza, 1994 ; Villalba et Provenza, 2000). Cette différence peut venir du fait que les ruminants sont, au cours de leur vie, exposés à de nombreux types d'aliments simultanément (différents fourrages pour les animaux élevés en pâturage, comportement de tri, *etc*.). Au contraire, les porcs sont le plus fréquemment élevés en intérieur où ils ne sont pas confrontés à une telle diversité alimentaire et sont souvent soumis à une succession d'aliments uniques. Ces **différences de contexte environnemental** pourraient donc expliquer la difficulté des porcs à réaliser une généralisation de ces apprentissages à des aliments ayant des caractéristiques organoleptiques sensiblement identiques.

3.2. Applications en recherche biomédicale

La compréhension des facteurs qui déterminent les choix alimentaires et la quantité d'aliment consommé chez les animaux constitue une étape importante pour **la mise en oeuvre de stratégies alimentaires** chez l'Homme, pour éviter le développement de comportements alimentaires néfastes et encourager l'établissement d'un comportement alimentaire sain (Bellisle, 2003 ; Wardle, 2007). Ceci est un enjeu majeur en santé humaine face à un environnement alimentaire caractérisé par une disponibilité accrue d'aliments très palatables et une explosion

de problèmes de santé liés à l'alimentation (suralimentation, surpoids, obésité ; Popkin et Doak, 1998 ; Stein et Colditz, 2004). Comme cela a été évoqué dans l'**article n°1**, le porc est un modèle idéal pour l'étude du comportement alimentaire chez l'Homme. Le fait de travailler sur des animaux juvéniles représente un intérêt supplémentaire en termes d'applications biomédicales. En effet, bien que les choix alimentaires évoluent tout au long de la vie, l'enfance et l'adolescence représentent des périodes charnières pour la mise en place du répertoire alimentaire. C'est en effet durant ces périodes que l'individu affine ses choix (préférences, aversions) en fonction de son expérience (nouveauté, apprentissage, mémoire), sous l'influence de facteurs environnementaux, et notamment du contexte social et familial (variété des aliments proposés, expositions répétées, *etc.* ; Birch, 1999 ; Hursti et Sjödén, 1997 ; Wardle et Cooke, 2008). Dans une optique d'action préventive, il paraît donc important de déterminer comment se mettent en place les choix alimentaires pendant l'enfance et l'adolescence.

Les travaux présentés dans le *chapitre III* montrent l'importance de la concordance entre signaux oraux et postoraux lors de la perception de stimuli sucrés et caloriques (*i.e.* saccharose) pour l'activation des structures cérébrales impliquées dans la régulation hédonique de la prise alimentaire. En effet, la seule perception du goût sucré du saccharose en l'absence d'apport calorique n'a pas suffi à induire de modification de l'activité cérébrale. Ces résultats offrent des perspectives intéressantes en termes de recommandations alimentaires pour la limitation de la prise calorique et la diminution de la prévalence de l'obésité au niveau mondial. L'explosion de la disponibilité sur le marché d'édulcorants artificiels non caloriques comme l'aspartame amène en effet la communauté scientifique à poser de nouvelles questions de recherche concernant l'effet de ces composés sur le comportement alimentaire. Comme proposé par Haase *et al.* (2009), du fait de leur absence de valeur nutritive, les édulcorants artificiels entraînent des réponses cérébrales plus faibles car leur goût sucré est assimilé à une stimulation qui n'apporte pas de calories « récompensantes ». Cette moindre activation pourrait à terme provoquer une augmentation de l'attractivité envers des aliments caloriques pour compenser ce manque d'activation cérébrale dans les zones cérébrales du plaisir. Ces résultats sont d'ailleurs en accord avec les hypothèses proposées par Val-Laillet *et al.* (2011a) dans leur étude sur des miniporcs obèses. Ces auteurs proposent que, chez des porcs dont l'obésité a été induite par une alimentation riche en graisses et en sucres, l'hyperconsommation de ces aliments très palatables aurait pu induire une sorte d'habituation. Ainsi, les individus obèses augmentent continuellement la quantité d'aliment consommé pour main-

tenir une stimulation suffisante des zones cérébrales du plaisir, dont le métabolisme basal est réduit chez les sujets obèses. Nos résultats pourraient donc indiquer que **l'utilisation d'édulcorants artificiels à valeur nutritive réduite ne soit pas le meilleur moyen de limiter la prise calorique** chez l'Homme et que des stratégies préventives en terme d'éducation alimentaire devraient être envisagées.

L'étude des aversions alimentaires pourrait également permettre de développer de nouvelles stratégies pour améliorer l'état nutritif des patients en milieu clinique, notamment des cancéreux sous chimiothérapie ou des patients en gériatrie. Cette amélioration du statut nutritionnel est d'une importance capitale puisqu'une sous-nutrition ou malnutrition peut entraver la réussite des traitements et induire une diminution considérable de la qualité et de l'espérance de vie des patients (Berteretche *et al.*, 2004 ; Crogan et Pasvogel, 2003 ; **article n°1**). Nos travaux montrent que des épisodes émétiques induisent des aversions robustes chez le porc. Des études ont pourtant montré que l'utilisation de traitements antiémétiques chez les patients n'était pas suffisante pour prévenir la mise en place d'aversions et rétablir une prise alimentaire normale (Schwartz *et al.*, 1996). Ainsi, il serait intéressant de tester, sur notre modèle porcin de l'aversion alimentaire conditionnée, les **différentes stratégies alternatives qui pourraient permettre de rétablir une prise alimentaire normale suite à l'établissement de ces aversions**. Ces stratégies pourraient par exemple reposer sur l'utilisation d'additifs alimentaires agissant sur les propriétés hédoniques des aliments, comme ceux que nous avons testés dans le *chapitre IV*.

Pour conclure, l'étude de la mise en place du comportement et des choix alimentaires chez le porc représente des opportunités intéressantes pour le développement d'outils aidant au maintien d'un comportement alimentaire adapté et de stratégies pour la prévention des habitudes alimentaires délétères. Néanmoins, il est important de noter que, chez l'Homme, l'établissement des choix et du répertoire alimentaire ne repose pas entièrement sur les facteurs d'expérience et d'apprentissage, ni sur la mise en place d'aversions et préférences conditionnées. De nombreux autres facteurs interviennent parmi lesquels les facteurs sociaux (catégorie socio-culturelle, revenus), familiaux (pratiques alimentaires éducatives différentes selon les familles), culturels (repas à heures fixes, adaptation de la taille des repas), environnementaux (publicité, médias), génétiques, et psychologiques (évaluation émotionnelle du contexte entourant le repas, régimes et restrictions alimentaires volontaires ; Bellisle, 2003 ;

Birch, 1999). Il est donc important de prendre ces nombreux facteurs en considération avant d'extrapoler à l'Homme les résultats obtenus dans des études effectuées chez l'animal.

4. Perspectives

4.1. Quid de la régulation du comportement alimentaire chez les adultes ?

L'ensemble de nos travaux a été réalisé sur des animaux juvéniles. Ce choix était motivé par des contraintes d'objectifs (*e.g.* amélioration des transitions alimentaires chez les animaux juvéniles en élevage) et par des contraintes méthodologiques (*e.g.* taille et poids des animaux adultes non adaptés aux dispositifs d'imagerie cérébrale). Le choix de travailler sur des animaux juvéniles entraîne inévitablement des limites d'extrapolation des résultats sur des animaux adultes, arrivés au terme de leur maturation physiologique, comportementale et neurologique. Une des perspectives intéressantes de ces travaux serait donc d'étudier ces mécanismes chez des animaux adultes, ou de caractériser l'évolution de ces mécanismes entre les stades précoce et adulte par la réalisation d'un suivi longitudinal d'une cohorte d'animaux. Les races de miniporcs s'avèreraient une solution optimale pour pouvoir réaliser ce type d'études longitudinales ou tout simplement pour étudier ces processus chez des animaux adultes. En effet, de nombreuses espèces de miniporcs destinés à la recherche biomédicale ont été décrites (Köhn, 2011), comme la race Pittman-More ou Yucatan. Plusieurs études ont d'ores-et-déjà utilisé le miniporc comme modèle pour la recherche biomédicale, notamment sur les problématiques liées à l'obésité (Val-Laillet *et al.*, 2010a ; Val-Laillet *et al.*, 2010b ; Val-Laillet *et al.*, 2010c ; Val-Laillet *et al.*, 2011a). Le type miniporc présente les avantages d'un poids limité et d'une petite taille à l'âge adulte, ce qui le rend plus facile à gérer expérimentalement et facilite la réalisation d'imagerie fonctionnelle sur des animaux adultes. Des études complémentaires sur ce modèle animal sont donc à envisager.

4.2. Quid des préférences alimentaires en situation d'aliment unique en élevage ?

Alors que les ruminants ont souvent le choix entre différents fourrages et plantes herbacées (Duncan et Young, 2002 ; Duncan *et al.*, 2007), les porcs en élevage sont rarement exposés à des situations de diversité alimentaire. Dans la plupart de nos expériences, nous avons eu recours à des tests de double choix alimentaires pour évaluer les préférences spontanées ou conditionnées des animaux. Or, les réponses comportementales observées dans une situation de diversité alimentaire peuvent être bien différentes de celles observées dans une situation où un seul aliment est présenté (Meunier-Salaün et Picard, 1996). Ainsi, alors que

l'utilisation de tests de double choix semble tout à fait pertinente pour les ruminants ou encore pour étudier les problématiques liées à l'alimentation humaine (ruminants et humains étant en contact avec une large variété d'aliments au cours de leur vie), l'utilisation de **tests d'aliment unique** serait une méthode plus fiable pour préciser les préférences des porcs en élevage.

Le recours à des **tests de ratio progressif (PR)** est un autre moyen de tester la motivation et les préférences et alimentaires lors d'une situation où un aliment unique est présenté. Dans les travaux de l'**article n°4**, les animaux devaient appuyer sur un bouton en suivant un ratio d'appuis croissant afin d'obtenir une récompense sucrée (saccharose 5%) ou non au saccharose 5%, et associée ou non à un apport énergétique *via* une infusion i.d. de saccharose 16% ou d'eau. La diversité des informations à traiter simultanément (goût sucré ou non de la récompense, apport énergétique ou non, difficulté croissance du travail à effectuer, *etc*.) a pu rendre la tâche trop complexe, ce qui expliquerait la difficulté à mettre en évidence des différences de motivation pour les différentes récompenses. Une procédure alternative serait de soumettre les animaux à un conditionnement préférentiel *via* le saccharose pour un aliment aromatisé, et d'évaluer ensuite la motivation pour l'aliment aromatisé comparé à un aliment aromatisé neutre pendant des tests de PR. En complément, les animaux pourraient être soumis à des séances d'imagerie PET suite à la perception des flaveurs ainsi conditionnées afin de visualiser les différences d'activation obtenues suite à la perception de flaveurs préférées, et les comparer à celles obtenues suite à des stimulations aversives (**article n°2**).

4.3. Quid des mécanismes moléculaires cérébraux sous-jacents ?

Dans cette thèse, l'implication des structures cérébrales impliquées dans la reconnaissance et l'évaluation hédonique des stimuli sensoriels, dans la motivation alimentaire et les processus cognitifs ont été mises en évidence chez le porc, étayant les similitudes Homme/porc rapportées dans de précédentes études (Boubaker *et al.*, 2012 ; Gaultier *et al.*, 2011 ; Val-Laillet *et al.*, 2011a). Selon toute vraisemblance, les différences d'activation au sein de ces structures reflètent certainement des modifications de l'activité de systèmes de neurotransmetteurs impliqués dans la régulation du plaisir et de la motivation (*cf. synthèse bibliographique*). Parmi ces neurotransmetteurs, nous pouvons citer la dopamine (DA) qui est impliquée dans les processus de motivation alimentaire et du traitement de la récompense (Barbano et Cador, 2007), ou encore les opioïdes impliqués à la fois la motivation (« wanting ») et le plaisir (« liking ») pour des récompenses alimentaires (pour revues, Peciña *et al.*, 2006 ; Smith et Berridge,

2005, 2007). Enfin, nous pouvons évoquer la sérotonine (5-HT) qui semble également avoir un rôle important dans le contrôle de la prise alimentaire, mais avec une action plus marquée sur la voie de régulation homéostatique (pour revue, Lam *et al.*, 2010). Des approches moléculaires plus fines pourraient donc compléter notre travail en explorant les mécanismes moléculaires impliqués dans ces processus.

La première technique qui pourrait apporter des éléments de réponse intéressants est l'**immunohistochimie** qui consiste à localiser les protéines (antigène, Ag) contenues dans les cellules d'une coupe de tissu *via* l'utilisation d'anticorps spécifique (Ac). Des essais préliminaires ont été réalisés au cours de cette thèse pour mettre au point une technique de marquage de la DA et de la 5-HT chez le porc (**Figure 13**) *via* l'**immunohistochimie par fluorescence**. Cette technique de double marquage consiste à marquer l'Ag d'intérêt (DA ou 5-HT) avec un Ac primaire anti-dopamine ou anti-sérotonine, respectivement. Puis, des Ac secondaires marqués de fluorochromes différents et ayant une forte affinité pour l'un des Ac primaires sont ajoutés au milieu. C'est la fluorescence émise par les fluorochromes qui est alors perçue lors des observations au microscope à fluorescence (*essais préliminaires,* **Figure 14**). Un autre principe d'immunohistochimie qui pourrait être utilisé pour compléter nos travaux est le **marquage de la protéine c-Fos**, marqueur de l'activité neuronale dans les noyaux des neurones. Grâce à cette technique, quelques études ont relié l'activité du système dopaminergique avec l'évaluation de la palatabilité et des caractéristiques sensorielles des aliments (Domingos *et al.*, 2011). Lee *et al.* (2008) ont aussi montré un effet inhibiteur des saponines sur la libération de DA dans le noyau accumbens induite par la prise de cocaïne chez le rat. Les résultats présentés dans l'**article n°6** montrent qu'un des additifs fonctionnels testés, contenant des saponines, modifiait la palatabilité de l'aliment et les préférences alimentaires. Le marquage de la protéine c-Fos pourrait permettre de caractériser les interactions entre comportement alimentaire, DA et composés alimentaires, comme les saponines.

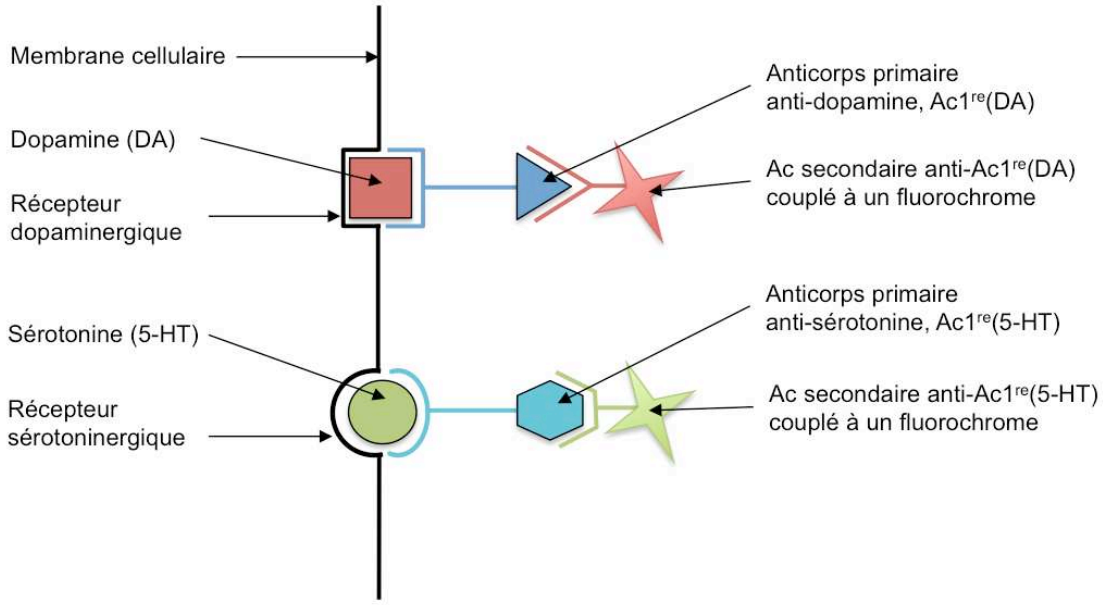

Figure 13. Principe de l'immunofluorescence à double marquage dans le cas de la dopamine (DA) et de la sérotonine (5-HT). Un anticorps primaire (Ac 1re) anti-DA ou anti-5-HT va venir se fixer sur l'antigène d'intérêt qui lui est spécifique, *i.e.* la DA ou la 5-HT, respectivement. Puis, des anticorps secondaires (Ac 2re) vont venir se fixer sur l'Ac 1re qui lui est spécifique. Chaque Ac 2re est muni d'un fluorochrome qui va se révéler lors de l'observation sous un microscope à fluorescence. La DA sera donc marquée en rouge, et la 5-HT en vert.

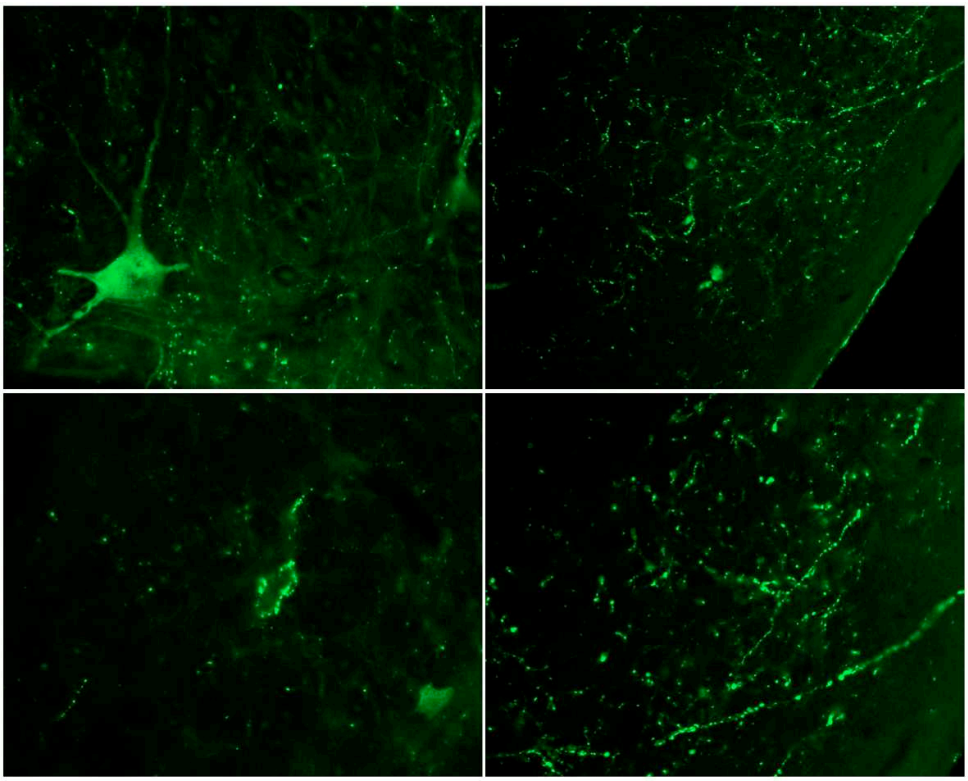

Figure 14. Marquage par immunofluorescence de la sérotonine (5-HT) dans des coupes histologiques du bulbe rachidien de porcs juvéniles. À gauche, le marquage semble révéler des corps neuronaux dans lesquels la sérotonine est produite et libérée. À droite, le marquage semble révéler les fibres axonales qui transportent ensuite la sérotonine jusqu'aux structures concernées. Ces images ont été obtenues grâce à un microscope à fluorescence et sont issues d'essais préliminaires réalisés au cours de la thèse.

Un des principaux inconvénients de l'immunohistochimie est que cette technique nécessite de sacrifier les animaux, ce qui représente de nombreux désavantages, comme l'impossibilité de réaliser des études longitudinales ou de tester plusieurs traitements sur les mêmes animaux. Le recours à l'**imagerie moléculaire** offre la possibilité de contourner cet obstacle et a déjà permis de souligner des similitudes entre la distribution, le développement et les fonctions de certains neurotransmetteurs chez l'Homme et chez le porc, comme notamment la 5-HT (Cumming *et al.*, 2007 ; Ettrup *et al.*, 2011) ou la DA (Lind *et al.*, 2007 ; Minuzzi *et al.*, 2006). La **technique de PET couplée au ^{11}C-raclopride,** un radioligand qui se fixe aux récepteurs dopaminergiques D_2, est une méthode largement employée pour évaluer la libération de DA à la suite d'une stimulation sensorielle dans des régions cérébrales d'intérêt comme le striatum (Lind *et al.*, 2005). Néanmoins, la courte demie-vie du ^{11}C-raclopride (20 min) implique d'avoir un cyclotron (accélérateur à particules permettant la formation d'isotopes radioactifs) à proximité du site d'étude, limitant ainsi l'utilisation de cet isotope. Des techniques reposant sur l'utilisation d'isotopes radioactifs dont le temps de demie-vie est plus long peuvent cependant être évoquées pour l'étude de l'activité dopaminergique. Parmi ces isotopes, nous pouvons cité le **^{123}I-ioflupane**, qui permet de mesurer l'expression du transporteur dopaminergique (DAT) en utilisant la SPECT (Del Sole *et al.*, 2010), ou encore le **^{18}F-fallypride**, qui permet de mesurer la disponibilité des récepteurs dopaminergiques $D_{2/3}$ *via* la PET (Rominger *et al.*, 2011). Le recours à de telles techniques d'imagerie cérébrale fonctionnelle moléculaire permettrait donc de compléter nos travaux en caractérisant, de manière moins invasive que l'immunohistochimie, les mécanismes moléculaires sous-tendant les processus de régulation du comportement alimentaire au sein des régions cérébrales déjà caractérisées. Certaines études chez l'Homme ont montré que l'obésité et/ou les addictions alimentaires étaient associées à des modifications de la disponibilité en récepteurs dopaminergiques D_2 (Volkow *et al.*, 2008 ; Wang *et al.*, 2001), suggérant l'intérêt de telles études en terme de recherche biomédicale.

CONCLUSION GÉNÉRALE

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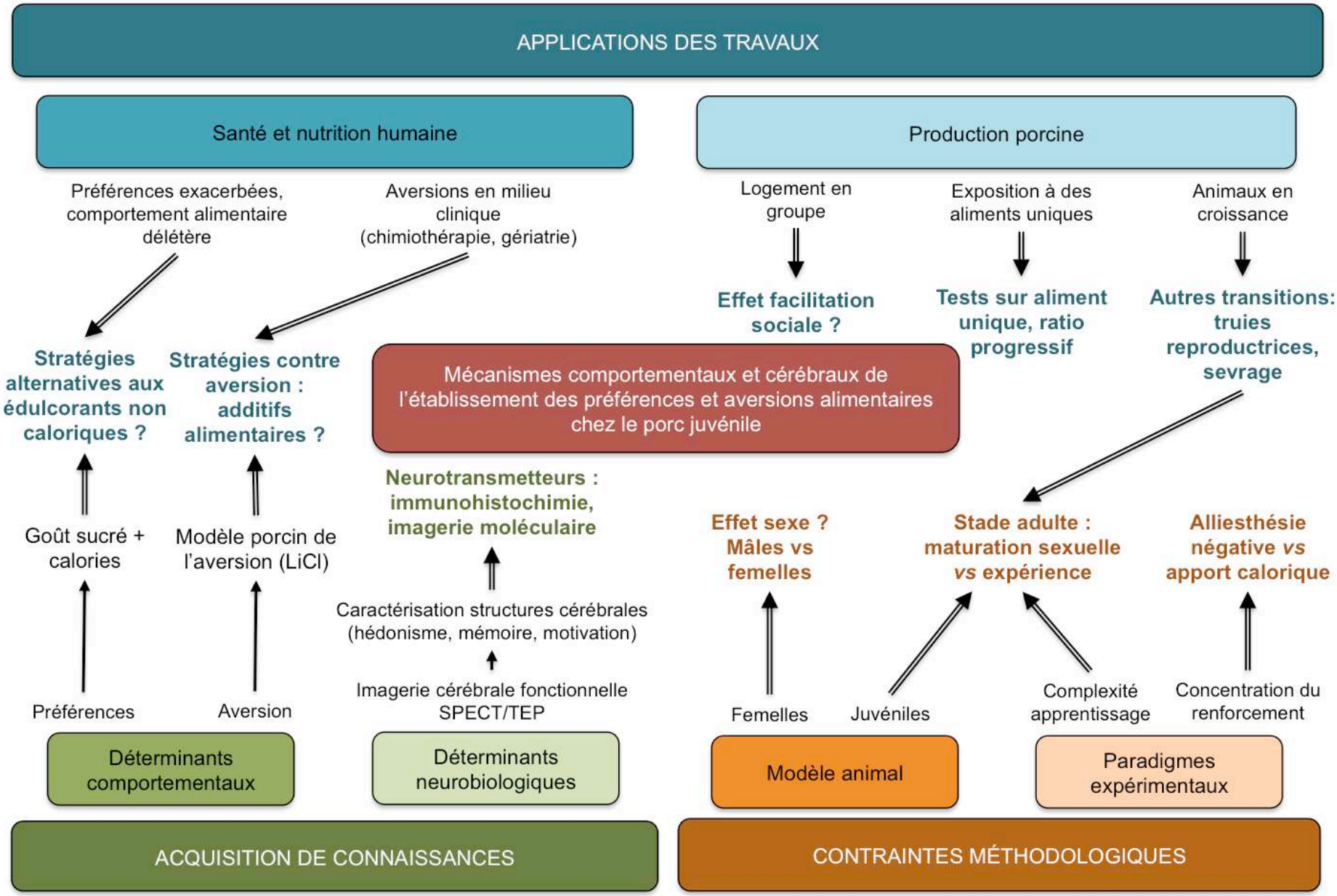
Les résultats de cette thèse ouvrent de nombreuses perspectives de recherche en termes d'acquisition de connaissances sur la régulation de l'ingestion et sur les mécanismes qui sous-tendent la mise en place des choix alimentaires (**Figure 15**). En effet, de nombreuses questions de recherche émergent de nos travaux, par exemple : quels sont les mécanismes moléculaires qui sous-tendent les modulations de l'activité cérébrale mises en évidence au cours de nos travaux pendant la perception de stimuli alimentaires ? Comment se mettent en place les choix alimentaires chez des animaux adultes, et comment les phénomènes de maturation et d'expérience influent-ils sur ces processus de régulation du comportement alimentaires ? Nos travaux offrent également des opportunités intéressantes d'application, avec pour finalité le maintien et la stimulation de la prise alimentaire en production animale, ou l'établissement d'un comportement alimentaire sain dans le domaine de la nutrition et de la santé humaine. Ainsi, le recours à une approche pluridisciplinaire mêlant techniques d'imagerie cérébrale et méthodes de mesure du comportement présente de nombreux atouts, tant dans la diversité des domaines scientifiques couverts que des concepts à décliner. Néanmoins, cette approche est aussi une stratégie complexe dont les limites, notamment méthodologiques, doivent être sciemment considérées avant toute tentative d'extrapolation et de généralisation des résultats.

Figure 15. Diagramme récapitulatif de la thèse. Objectifs, enjeux, résultats et perspectives de recherche en fonction de quatre grands axes : applications en élevage et en recherche biomédicale, acquisition de connaissances théoriques et contraintes méthodologiques.



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ANNEXES

ANNEXE 1

Evaluation de la douleur post-chirurgicale

SENAH

Unité Mixte de Recherches
Systèmes d'Elevage, Nutrition Animale et Humaine

N° Animal:
Date et heure chirurgie:
Type chirurgie:

		Date et heure				
		:	:	:	:	:
Appréciation globale subjective	Pas de Douleur ⇩ Douleur intense	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Attitude générale	Parmi les symptômes suivants: •présente des modifications respiratoires •gémit •vousse le dos •reste figé en posture antalgique •s'agite ou est abattu •perd l'appétit •regarde, mordille ou lèche la zone opératoire •boite,se déplace difficilement ou est réticent à se déplacer - Aucun signe présent - 1 seul présent - 2 à 4 présents - 5 à 8 présents	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ 0 1 2 3
Comportement interactif	•Est attentif et répond positivement aux caresses •Répond timidement •Ne répond pas immédiatement •Ne répond pas ou répond de façon agressive	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Réaction à la manipulation de la zone opératoire	•Pas de réaction visible ou audible - après 4 manipulations •Réaction(s) visible(s) ou audible(s) - à la 4e manipulation - à la 2e et 3e manipulation - à la 1ére manipulation ou non évaluable	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Intensité de la réaction	•Aucune réponse •Répond faiblement, essaye de se soustraire •Tourne la tête ou vocalise •Tente de fuir ou d'agresser ou non évaluable	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3	0 1 2 3
Score total	1 à 4 : Douleur légère 5 à 9: Douleur modérée 10 à 15: Douleur sévère					

Traitements et actions:

Sauf préconisations contraires dans le protocole expérimental. Pour un porc de 30 kg

- •Douleur légère - Aspegic (1 flacon toutes les 12 heures, IV)
- •Douleur Modérée - Spasfon (1 ampoule toutes les 6 heures, IV) + Aspegic (1 flacon/12 heures, IV)
- •Douleur Sévère - Chlorydrate de Morphine (0,1 à 0,5 ml SC à répéter toutes les 2 heures)

En cas de douleur sévère ne diminuant pas avec les opiacées penser aux conditions de terminaison de l'expérience

ANNEXE 2

Composition des aliments expérimentaux utilisés au cours de la thèse

L'**aliment 1er âge** a été utilisé exclusivement dans le *chapitre IV*, dans l'expérience concernant l'effet d'additifs alimentaires sur la prise alimentaire lors d'une transition alimentaire aliment 1er âge-aliment 2ème âge. L'**aliment 2ème âge standard** a été utilisé dans l'ensemble des expériences en tant qu'aliment expérimental (*e.g.* témoin) ou en tant que régime de base de nos animaux. L'**aliment « pois »** a été utilisé dans les *chapitres II* (**article n°5**) et *IV* en tant qu'aliment « hors expérience » car ses caractéristiques organoleptiques diffèrent de celles de l'aliment standard. L'**aliment Sucrose 5%** est un des aliments expérimentaux utilisés lors des tests de ratio progressif dans le *chapitre III* (**article n°5**).

	Aliments expérimentaux			
Composition centésimale (%)	Aliment 1er âge	Aliment 2ème âge standard	Aliment 2ème âge Sucrose 5%	Aliment 2ème âge Pois
Blé		23,20	22,04	13,92
Mais		25,00	23,75	15,00
Orge	45,30	24,10	22,90	14,46
Sucrose			5,00	
Pois				40,00
Tourteau de soja	17,50	22,60	21,47	13,56
Protéines de soja	2,50			
Huile végétale	2,30	0,50	0,48	0,30
Lactosérum douux	20,00			
Lait réengraissé à 40%	8,00			
Carbonate Calcium	1,41	1,13	1,07	0,68
Phosphate monocalcique	0,80	0,97	0,92	0,58
Sel		0,40	0,38	0,24
Mélange oligo-vitamines	0,50	0,50	0,48	0,30

Mécanismes comportementaux et neurobiologiques de l'établissement des préférences et aversions alimentaires chez le porc : applications en nutrition et santé animale et humaine

Résumé

L'étude des mécanismes comportementaux et cérébraux sous-tendant la modulation hédonique de la prise alimentaire chez le porc juvénile présente d'importants enjeux en termes d'applications en production porcine et en recherche biomédicale. L'objectif de cette thèse était de décrire ces déterminants par le biais du développement d'un modèle porcin des préférences et aversions alimentaires conditionnées, en utilisant des méthodes comportementales et des techniques d'imagerie cérébrale fonctionnelle. Sur le plan comportemental, nous avons validé un modèle porcin de l'aversion alimentaire conditionnée, obtenue *via* des injections duodénales de chlorure de lithium et caractérisée par une modification drastique des choix alimentaires. Nos résultats suggèrent également que les préférences alimentaires sont plus difficiles à induire que les aversions, et qu'un renforcement calorique et sucré, comme le saccharose, représente un stimulus efficace pour l'induction d'une préférence chez le porc. Sur le plan neurobiologique, l'exposition à des flaveurs alimentaires aux valeurs hédoniques contrastées et la perception combinée de saccharose aux niveaux oral et viscéral ont engendré des différences d'activation dans les circuits neuronaux impliqués chez l'Homme et les rongeurs, dans l'évaluation hédonique des stimuli sensoriels, la motivation, le traitement de la récompense (amygdale, cortex préfrontal, noyaux de la base) et la mémoire (hippocampe, cortex parahippocampique). Des études complémentaires sont nécessaires pour déterminer dans quelle mesure des facteurs de variation liés à l'animal ou aux paradigmes expérimentaux ont pu influencer l'expression des conditionnements.

Mots-clés : porc, conditionnement pavlovien, calories, saccharose, circuit de la récompense.

Behavioural and neurobiological mechanisms underlying the establishment of food preferences and aversions in pigs: Applications in animal and human health and nutrition

Abstract

The study of the behavioural and neurobiological mechanisms underlying the hedonic modulation of food intake in juvenile pigs has interesting opportunities of applications in the fields of pig husbandry and biomedical research. Our aim was to describe these determinants through the development of a pig model of conditioned food preferences and aversions, using behavioural conditioning methods and functional brain imaging techniques. On the behavioural side, we managed to develop a pig model of conditioned food aversion induced by duodenal injections of lithium chloride, and characterised by strong and robust changes of food choices. Our findings also suggest that food preferences are more difficult to condition than aversions, and that a sweet and caloric reinforcement, such as sucrose, is an efficient stimulus for food preference conditioning in pigs. Using brain imaging techniques, we managed to highlight specific patterns of brain activation following exposure to food-related stimuli. Both exposure to food flavours with contrasted hedonic values and combined oral and duodenal sucrose sensing triggered differential activation in brain networks known in humans and rodents, to be involved in the recognition and hedonic evaluation of sensorial stimuli, motivation, reward processes (amygdala, prefrontal cortex, basal nuclei), and memory (hippocampus, parahippocampal cortex). Further studies, however, are needed to investigate to what extent some factors of variation relating to the animals or to the experimental paradigms may have influenced expression of the conditioning.

Key-words: pigs, pavlovian conditioning, calories, sucrose, reward circuit.