Deep brain stimulation for obesity in the normal non human primate: A preclinical approach.

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THESE

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Deep brain stimulation for obesity in the normal non human primate: A preclinical approach.

Stimulation cérébrale profonde hypothalamique pour l'obésité chez le primate non humain : Une approche préclinique.

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Abstract

Object: Deep brain stimulation (DBS) has become an effective therapy in a variety of brain disorders. Recently, Hypothalamic DBS in cases of chronic intractable cluster headache has revived the interest in this region, which is also well-known to be involved in food intake and energy balance regulation. In the other hand, risks and problems related with implantation in this area has raised several questions regarding the safety of this approach. In this study, the authors proposed an Intraventricular "floating" electrode inserted in the third ventricle adjacent to the ventromedial hypothalamus (VMH) in freely moving *Macaca fascicularis* to modulate food intake and weight and as a potential treatment of morbid obesity.

Methods: Five adults *Macaca fascicularis* (4 subjects and 1 sham) monkeys were implanted stereotactically in the third ventricle contiguous to the VMH with chronic indwelling 3389 and 3388 Medtronic electrodes used for Deep Brain Stimulation (DBS). The study was divided in two phases: acute tests and chronic 8-weeks trials. In the acute tests, the meal size, eating time and locomotor activity were recorded after short periods of electrical stimulation (ES) in 24 hrs fasting animals at different frequencies and intensities of stimulation, in order to obtain the most effective sets of ES parameters able to reduced food intake (FI) and consequently weight and fat during chronic stimulation. In the chronic trials, three cycles of continuous ES of 8 weeks each were performed at the most effective frequency reducing FI in the acute test (or 80Hz), at 130Hz (considered High Frequency ES and used in Parkinson Disease DBS) , and 30Hz (considered Low frequency ES and used in Pain DBS). Body Mass Index, weight, fat content, subcutaneous skinfolds and hormones were measured during baseline and at the end of each 8 week stimulation trials.

Results: ***Results:*** During ***Acute 24 hrs-fasting trials*****,** there was a decrease in FI in all subjects at 80 Hz, (mean 15 ±4.4%). During ***Chronic 8 weeks stimulation trials*****,** a decrease in weight and BMI was observed in three out four monkeys at 80 HZ (mean 8% ± 4.4%), and slight increase at 130HZ (mean 2% ± 2.5) and at 30HZ (mean 5%±2,93). Fat mass decreased at the end of 80 Hz trials to ratio 0.82±0.08. (18% reduction).Subcutaneous skinfolds were reduced in all four subjects at 80 Hz and slightly increased at 130 Hz. Sham monkey remained stable. FI increased during off stimulation period (washout) following effective weight loss. Glucose also increased during hyperphagic period. Hormones and Leptin did not show significative variations in relation to different frequencies stimulation. No major adverse effects were recorded.

Conclusion: We conclude that stimulating the VMH region throughout an Intraventricular approach might modulate acutely food ingestion and induce a sustained decrease in weight and in fat content in normal non obese human primates.

Key words*: deep brain stimulation, obesity, ventromedian hypothalamus, Macaca fascicularis, Intraventricular approach*

Résume

Objet: La stimulation cérébrale profonde (SCP) est devenue une thérapie efficace dans une série de maladies cérébrales. Récemment, dans les cas des algies vasculaires de la face résistantes au traitement (intraitables), chroniques, la SCP hypothalamique a suscité un nouvel intérêt pour cette région, également bien connue pour son implication dans la régulation de la prise alimentaire et de la balance énergétique. Cependant, les risques et les problèmes connexes liés à l'implantation dans cette aire cérébrale ont soulevé plusieurs questions concernant la sûreté de cette technique chirurgicale. Dans cette étude, les auteurs ont proposé l'implantation d'une électrode intraventriculaire insérée dans le troisième ventricule au niveau de l'hypothalamus ventromedial (VMH) chez des singes *macaca fascicularis* non obèses dans le but de moduler la prise alimentaire et le masse corporelle des sujets. Cette méthode de SCP pourrait s'avérer être un traitement potentiel de l'obésité morbide.

Méthodes: Cinq singes de *macaca fascicularis* adultes (4 sujets et 1 contrôle ou sham) ont été implantés de façon stéréotaxique dans le troisième ventricule. Une électrode chronique Medtronic®, habituellement utilisée dans le cadre de la SCP chez les patients atteints de la maladie de Parkinson, a été positionnées dans l'espace intraventriculaire adossée à la paroi de ce dernier au niveau du VMH. Dans la première phase de l'étude, le comportement alimentaire de chaque animal (durée du repas, quantité de nourriture avalée) et son activité motrice ont été enregistrés et analysés en fonction différents paramètres de stimulation (fréquence et intensité) après une période de jeun de 24 heures. Dans la seconde phase du protocole, trois cycles de stimulation intraventriculaire de 8 semaines chacun ont été réalisés à 130Hz, à 80Hz et à 30Hz, suivi des périodes de « washout » de 4 semaines entre les périodes« on - stimulation ». L'index de masse corporelle, le poids (masse corporelle), la « teneur « en graisse, l'épaisseur cutanée et les concentrations hormonales ont été mesurés au début de l'étude pour établir une ligne de base et après chaque session de stimulation.

Résultats: Lors de la première phase du protocole réalisée sur des animaux a jeun depuis 24 heures**,** nous avons remarqué une diminution de la prise alimentaire comprise entre 11 et 19% chez tous les sujets stimulés à une fréquence 80 hertz. A partie de ces résultats, **,** une diminution de la masse corporelle et du BMI (body mass index indice de masse corporelle) ont été observés chez trois de quatre singes lors des phases de stimulation chronique à une fréquence de 80 hertz : la moyenne de perte pondérale était de 8± 4.4%. Une augmentation de 2-6 ± 2.5% et de 5 ±2,93 %de la masse corporelle a été observée respectivement chez les animaux stimulés à une fréquence de 130Hzet de 30Hz. Une diminution importante des épaisseurs sous-cutanées ( )a été observée pour chacun des quatre sujets à une fréquence de 80 hertz et dans une moindre mesure, une augmentation de cette variable ( ) a été remarquée une fréquence de 130 Hz. Tout au long de l'étude, les variables relevées sur le singe Sham sont restées stables. Sur la durée de l'étude, aucun effet potentielle ment délétère n'a été remarqués sur les animaux.

Conclusion: La stimulation de la région de VMH par voie intraventriculaire pourrait s'avérer efficace pour moduler le comportement alimentaire et induire une diminution soutenue de la masse corporelle caractérisée par réduction de la masse graisseuse chez les primates non humains non obèses.

Mots clés*: stimulation cérébrale profond, obésité, noyau ventromédian, Macaca fascicularis, Implantation Intraventriculaire*

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Abbreviations

µm	micrometers
3T	3 Tesla
3V	Third ventricle
6-OHDA	6-hyrdroxydopamine
ac	anterior commisure
AC	Abdominal circumference
AF	Abdominal fat
AGRP	Aglouti related peptide
ANOVA	analysis of variance
AP	anteroposterior
ARC	arcuate nucleus
ATP	adenosine triphosphate
BDNF	brain derived neurothophic factor
BFS	Best and more effective frequency stimulation
BG	basal ganglia
BIA	bioimpedance analysis
BMI	Body Mass Index
CART	Cocaine- and amphetamine-regulated transcript
CCK	cholecystokinin
Cl	Chloride
cm	centimeter
CM	centromedian nucleus of the thalamus
cm/s	centimeter/second
CNS	central nervous system
CRH	corticotrophin release hormone
CSF	Cerebrospinal fluid
DA	dopamine
DAT	dopamine transporter
DBS	deep brain stimulation
DM	diabetes mellitus
DMH	nucleus dorsomedial nucleus
DNA	deoxyribonucleic acid
DXA	Dual energy X-ray absorptiometry
ECW	Extracellular water
ES	electrical stimulation
FFA	free fatty acid
FI	Food Intake
FL	fasciculus lenticularis
FSH	Follicle stimulating hormones
FT	fasciculus thalamicus
fx	fornix

g	grams
G/l	grams/liter
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
GDH	glutamate dehydrogenase
GH	growth hormone
GHS-R	growth hormone secretagoge receptor
Glu	glutamate
GP	globus pallidus
GPe	globus pallidus externus
GPi	globus pallidus internus
GPil	lateral segment of the GPi
GPim	medial segment of the GPi
H1	field H1 of Forel
H2	field H2 of Forel
HDL	High density lipoproteins
HFS	high frequency stimulation
hGH	l'hormone de croissance de recombinaison humaine
hr	hour
Hz	hertz
i.m.	intramuscular
i.v.	intravenous
ic	internal capsule
IC	internal capsule
ICV	Intracerebroventricular
ICW	Intracellular water
If	nucleus arcuate or infundibular nucleus
iGluR	ionotropic glutamate receptor
INSERM	Institut National de la Santé et de la Recherche Médicale
IPG	implantable pulse generator
IS	Iliac skinfolds
K	potassium
KA	kainic acid or kainate
Kg	kilogram
LB	Lewy body
L-dopa	levodopamine
LFS	Low frequency stimulation
LH	Luteinizing hormones
LHA	Lateral Hypothalamus area
lt	lamina terminalis
Lv	lateral ventricle
m	meters
mA	milliamperes
MB	midbrain
MC-1	Melanocortin receptors 1
MC-3	Melanocortins receptors 3
MC-4	Melanocortins receptors 4

MCH	Melanin Concentrating Hormone
MDmc	magnocellular division of the mediodorsal nucleus
MFB	median forebrain bundle
mg	milligram
mGluR	metabotropic glutamate receptor
mL	milliliter
MM	medial mammillary nucleus
mm	millimeter
MP	la maladie de Parkinson
MPEP	2-methyl-6-(phenylethynyl)-pyridine
MPP^+	1-methyl-4-phenylpyridinium
MPPP	1-methyl-4-phenyl-4-propionoxypiperidine
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
mRNA	messenger ribonucleic acid
msec	milliseconds
MSH	Melanocyte-stimulating hormone
n	number or sample size
NA	number of averages
NA	Noradrénaline
Na	sodium
NADH	(reduced form of) nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
NPY	neuropeptide Y
NPY/Y1	neuropeptide Y1 receptor
NST	le noyau subthalamique
NTS	Nucleus of solitary tract
OMS	Organisation mondiale de la santé
opt	optic tract
PARS	poly-ADP-ribose synthetase
PBS	phosphate buffered saline
PD	Parkinson's disease
PD	proton density magnetic resonance image
PET	positron emission topography
Pf	parafascicular nucleus
PFA	perifornical area
PN	pons
Po	posterior hypothalamic nucleus
POMC	pro opiomelanocortin
Pop	the preoptic nucleus
PPN	pedunculopontine nucleus ou le noyau pédonculopontin
pps	pulse par seconds
PTP	permeability transition pore
PVG/PAG	periventricular grey matter/peri aqueductal gray matter
Pvn	paraventricular

PVN	Paraventricular nucleus
R:L	right to left ratio
RARE	Rapid Acquisition with Relaxation Enhancement
RF	radio frequency
RMP	resting membrane potential
RNA	Ribonucleic Acid
ROS	reactive oxygen species
rpm	revolution per minute
sb	The subthalamus
SCP	stimulation cérébrale profonde
SHF	la stimulation à haute fréquence
SN	substantia nigra
SN	substantia nigra ou la substance noire
SNc	substantia nigra pars compacta
SNC	système nerveux central
SNr	substantia nigra pars reticulata
SOC-3	suppressor of cytokine signalling - 3'
SOD1	superoxide dismutase-1
SPE	stimulation plus efficace
SPECT	single positron emission computerized tomography
Sr	supraoptic recess
SS	subscapular skinfolds
ST	the corpus striatum
STN	subthalamic nucleus
T2	transversal relaxation time
T3L	Triiodothyronine Libre
T4L	thyroxine libre
TBW	Total body water
TCA	tricarboxylic acid
TE	echo time
Th	thalamus
TH	tyrosine hydroxylase
TH^+	tyrosine hydroxylase positive
TND	transneuronal degeneration
TRH	Thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UPDRS	unified Parkinson's Disease rating scale
V	Volts
V3	Third ventricle
VA	ventral anterior (nucleus of the thalamus)
VAmc	magnocellular division of the ventral anterior nucleus
Vim	ventralis intermedius (nucleus of the thalamus)
VL	ventrolateral (nucleus of the thalamus)
VLDL	very low density protein
VLo	ventrolateral oralis
VMH	ventromedial
VTA	ventral tegmental area

w	weeks
WHO	World Health Organization
WO	washout
zi	zona incerta
ZI	zona incerta
ZTV	la zone tegumentaire ventrale
α-MSH	α-melanocyte stimulating hormone
µg	microgram
µs	microsecond

Work related to the thesis

Torres N, Chabardès S, Piallat B , Devergnas A, and Benabid A ***Hypothalamic deep brain stimulation for obesity in the non obese non human primate*** Acta Neurochir (Wien). 2008 Sep;150(9):933-1012 : Abstracts of the Proceedings of the XVIIIth Congress of the European Society for Stereotactic and Functional Neurosurgery (ESSFN) : 5-8 October 2008, Rimini, Italy.

Torres N, Chabardes S, Piallat B, Devergnas A, Benabid A ***Deep brain stimulation for obesity in the normal non human primate: A preclinical approach.*** In preparation

Chabardes S, **Torres N**, Piallat B, Benazzouz A, Wallace B, Nicolaidis S, Benabid A ***Acute and Chronic stimulation at high and low frequencies of the ventromedian and lateral nuclei of the hypothalamus in the rat: effects on weight and food intake.*** Submitted to European journal of Neuroscience

Benabid A, Chabardes S .Seigneuret E, **Torres N.** ***Intraventricular stimulation for targets close to the midline: Periaqueductal Gray, posterior hypothalamus, anterior hypothalamus, subcommissural structures.*** Acta Neurochirur(Wien)2006 Oct;148(10):I-LXIV

Work related to Deep Brain Stimulation

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Piallat B, Chabardes S, Devergnas A, **Torres N,** Allain M, Barrat E, Benabid A ***Monophasic but not biphasic pulses induce Brain Tissue Damage during Monopolar High Frequency Deep Brain Stimuation*** Neurosurg 64(1) January 2009(in press)

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Introduction et Résumé (En Français)

INTRODUCTION

L'obésité est une pathologie complexe caractérisée par l'accumulation excessive du tissu adipeuse excessive. Elle est définie en termes de index (indice) de masse corporelle (BMI), calculé en kilogramme/ (m) ². Bien que le BMI soit une variable continue, les études épidémiologiques basées sur le risque de comorbidities ont permis des classifier des groupes de populations. Un BMI entre 18 et25 est considéré comme normal ; entre 25-29.9 on parle de surpoids et a plus de ou égal à 30, de l'obésité (Korner et caractérisée, 2003).Il n'existe pas de consensus concernant la définition de l'obésité morbide mais, en général,dans la littérature de la chirurgie bariatrique (Brolin, 1992)on considère que plus de 45 kilogrammes au-dessus de poids idéal représente une obésité morbide. L'obésité atteint actuellement, au niveau mondial, des proportions épidémiques, avec plus de 1 milliard d'adultes en surpoids dont 300 millions d'obèses .L'obésité (représente) 2 à6% (voir même 7 %) des (dépenses) (coûts totaux) des soins de santé dans plusieurs pays développés .Les coûts réels sont beaucoup en fait plus importants car toutes les comorbidities associées a l'obésité ne sont pas prises en compte dans les calculs (OMS 2003).

Avoir un poids excessif augmente sensiblement le risque de comorbidités associée comme :le diabète type – II (DM), l'hypertension artérielle, la dyslipidemia, la cardiopathie ischémique, l'insuffisance cardiaque,l'accident vasculaire cérébrale, la lithiase biliaire, la stéatose hépatique, l'arthrose, le syndrome d'apnée de sommeil, ainsi que le cancer endométrial, du sein, de la prostate, ou du colon. L'obésité est associée également à une diminution de la qualité de vie (Roe et Eickwort, 1976), à un haut

risque des comorbidities (Must et al., 1999), et a une espérance de vie réduite de cinq à 20 ans (Fontaine et al, 2003).

Le traitement de l'obésité inclut des mesures non pharmacologiques, des agents pharmacologiques et la thérapie chirurgicale. Les traitements non pharmacologiques sont la thérapie comportementale, l'exercice physique et les régimes hypocaloriques. Le problème principal du traitement non pharmacologique demeure dans la difficulté à suivre des régimes restreints en calories et d'augmenter l'activité physique.

Les médicaments utilisés à long terme comme le sibutramine et l'orlistat entraînent une perte de poids discrète de 4 - 6 % sur une période de 6 mois dans des études contrôlés et en relation directe avec la observance au régime, à l'exercice physique, et a la thérapie comportementale. D'autres médicaments comme les agents de libération de la NA (Noradrenaline) entraînent une perte de poids significative par rapport au placebo dans des études à court terme, mais ce type de médicaments est approuvé seulement pour des courtes périodes d'administration.

La chirurgie bariatrique reste une alternative efficace dans l'obésité morbide. Néanmoins, certains patients présentant obésité morbide restent toujours de mauvaises candidates pour ce genre d'intervention. Un exemple de intervention chirurgicale est la chirurgie de « bypass » gastrique deRoux en Y, ou une petite poche gastrique empêche les patients de manger de grandes quantités à chaque repas. Dans l'étude complémentaire ayant le plus long suivi après chirurgie par« bypass gastrique », Pories et autres ont rapporté un maintien de la perte d'excès du poids de 58%, de 55% et de 49% (le poids excessive a été définie comme le poids en excès par rapport au poids idéale prévu pour les patients) à 5, 10, et 14 ans post-opératoirement respectivement. Cependant, les complications apparaissent entre 15-55 %de patients après la chirurgie

bariatrique, et le taux de mortalité peri-chirurgical est environ 1.5 % même dans les centres qui ont une expérience. (Pories et MacDonald, 1993). Les procédures les plus sûres et les plus simples (comme les gastroplasties en bande réglable) ne sont en général pas si efficaces.

En conclusion, il existe une nécessite réelle pour des procédés plus efficaces et plus sûrs pour traiter l'obésité morbide résistante (au traitement usuel). (Korner et Aron, 2003)

Tandis que l'obésité a longtemps été considérée comme un trouble comportemental, la découverte de l' hormone leptine en 1994 a catalysé (stimule) le champ de la recherche d'obésité en démontrant l'existence d'un signal humoral afférent du tissu adipeux au système nerveux central. L'évidence suggère qu'une fois que le tissu adipeux s'accumule, un système hormonal neuroendocrine d'autoréglage empêche sa diminution , rendant la perte de poids volontaire difficile(Zhang et autres, 1994). En conséquence, la modulation des circuits de cerveau apparaît comme une stratégie de recherche clinique valable dans l'obésité. Les avances dans notre connaissance (La connaissance de plus en plus approfondie) de l'anatomie et de l'électrophysiologie fonctionnelle des circuits neuronaux appropriés à cette condition) peuvent (peut) dévoiler des nouvelles cibles et donc des applications potentielles de la neuromodulation dans l'obésité. Dans notre étude, ces cibles potentielles, impliquées dans la physiopathologie de cette maladie, sont explorées et des nouvelles directions du traitement sont discutées.

Récemment, suite au développement du nouveau matériel et des techniques d'électrophysiologie, la stimulation électrique cérébrale profonde(SCP) a été employée dans le traitement des troubles obsessionnels compulsifs, de la douleur, des troubles

de mouvement(Benabid et autres, 1998), (Benabid et autres, 2001 ; Cosyns et autres, 2003 ; Gabriels et autres, 2003) et de l'épilepsie réfractaire au traitement médical (Benabid et autres, 2002) (Benabid, Koudsie et coll 2001). Cet outil permet maintenant d'explorer des nouvelles cibles et d'élargir les options thérapeutiques disponibles.

Certaines caractéristiques de cette méthode expliquent sa popularité. Les paramètres de SCP peuvent être ajustés a un seuil approprié évitant ainsi les effets indésirables sur les structures de voisinage(Blomstedt et Hariz, 2006). Contrairement à la lésion stereotaxique , la SCP fournit l'avantage de la réversibilité et la possibilité de doser l'intensité électrique (Deuschl et autres, 2006) diminuant le risque de déficit neurologique permanent important. La SCP est adaptable : malgré le fait que les mécanismes fondamentaux de la SCP restent largement inconnus (Benabid et autres, 2005a ; Fraix et autres, 2004), si les paramètres électriques appropriés sont bien choisis, la SCP peut exciter ou inhiber les structures neuronales , ouvrant ainsi un éventail d'applications cliniques potentielles. L'identification de la fréquence comme un facteur clé pour la modulation des structures de SNC a permis l'apparition d'un grand nombre d'applications qui va encore augmenter à l'avenir (Benabid et autres, 2005b).

Le déséquilibre entre la prise alimentaire et la dépense énergétique produit une augmentation du poids chez les individus pour lesquels l'identification des signaux périphériques hypothalamique est faible (Leptine, ghrelin, glucose ou insuline)(Heini et autres, 1998). L'activation des centres hypothalamiques sensibles a ces signaux périphériques en utilisant la SCP a basse fréquence ou l'inhibition des centres nerveux en hyperactivité utilisant la stimulation a haute fréquence,peut changer le « set point »

du poids corporelle dans les patients avec une obésité morbide (Benabid et autres, 2005a ; Benabid et autres, 2005b)

OBJECTIFS

Le but de cette étude est d'évaluer les effets de la stimulation hypothalamique ventromediane chronique sur le comportement alimentaire, le métabolisme, et le poids corporel global chez le primate. L'étude essaie de:

- Reproduire d'autres travaux qui ont observe l'effet aigu de la stimulation de VMH sur la prise alimentaire(Takaki et autres, 1992) ;
- Déterminer si le positionnement intraventriculaire d'électrode peut stimuler le VMH à partir du troisième ventricule ;
- Observer les effets de la stimulation de VMH à des différentes fréquences sur le comportement du primate non humain employant la technologie des électrodes de stimulation profonde du cerveau déjà disponible pour l'application humaine.

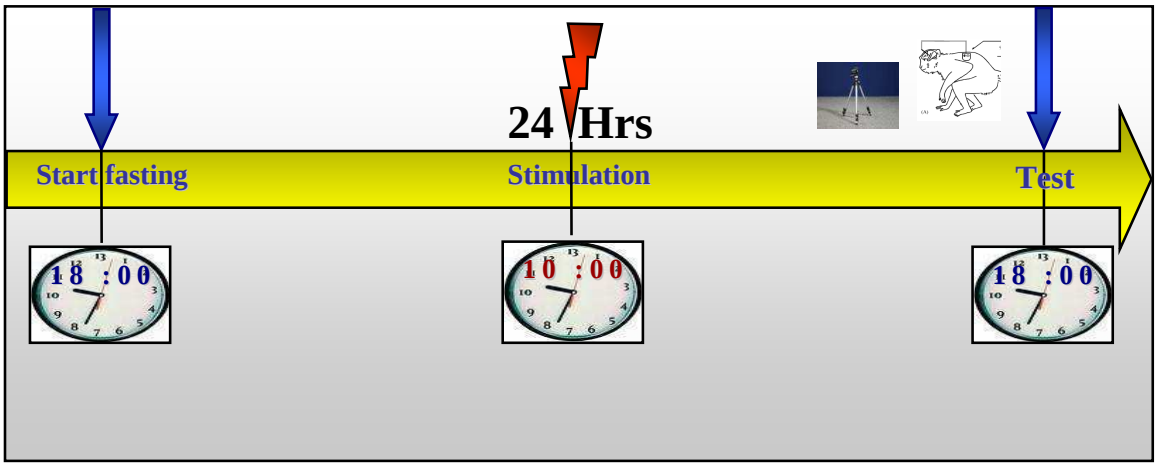
Jusqu'ici la recherche des effets de la stimulation profonde chronique de cerveau au niveau de l'hypothalamus médial chez des animaux supérieurs n'a été jamais effectuée. Dans notre étude, nous avons passé en revue l'efficacité et la sûreté de la stimulation chronique dans ce modèle de régulation alimentaire et du poids. Les résultats de ces expériences vont nous permettre d'évaluer la possibilité de réaliser des études cliniques qui permettront valider notre approche dans le traitement de l'obésité morbide.

DESIGN DE L'ETUDE

Cinq singes *Macaca fascicularis* de poids normaux (H4, H5, H7, H8 et H10) ont été acclimatés à deux repas quotidiens. Quatre d'entre eux on été implantés avec une électrode (3388 et 3389 DBS électrodes Medtronic®, Richmond MN USA)au niveau du

troisième ventricule et du noyau de VMH/DMH et reliées a un dispositif implantable type pacemaker (Soletra® Medtronic, Richmond MN USA). Un cinquième singea ete implanté au niveau hypothalamique,avec le même type d'électrode, mais sans connexion au stimulateur implantable type « pacemaker »

Phase 1 : Consiste dans l'étude des contacts d'électrode pour identifier les plus efficaces en la réduction de la prise alimentaire ainsi que ceux qui produisent le moins des effets secondaires après l'usage de différents paramètres de stimulation. Après une période de jeun de 24 heures, l'animal a reçu la stimulation d'onde bi phasique pendant 8 heures, à des fréquences et des intensités différentes. Un repas standard comprenant la prise alimentaire journalière habituelle a été donne a17h00 heures. La latence du déclenchement de l'alimentation, le temps dépense pendant l'alimentation et la quantité totale de nourriture consommé ont été enregistrés. L'activité motrice globale pendant la stimulation a été également enregistrée et analyse *a posteriori* à l'aide d'un logiciel de reconnaissance des images et du comportement. Chaque animal a subi entre 4 et 5 sessions de stimulations dans lesquelles des repas ont été présentés dans les mêmes conditions (température, type de nourriture etc.),en changeant uniquement les valeurs de la stimulation. La combinaison des paramètres qui réduisaient effectivement la taille de repas sans effets secondaires inacceptables ontété choisis pour la stimulation chronique. Le but principal de la phase 1 a été d'obtenir une fréquence qui puisse réduire effectivement la prise alimentaire. Cette fréquence (SPE : stimulation plus efficace) a été utilise dans le étude chronique (phase 2) Le schéma 1.



Le schéma 1: Diagramme montrant le protocole d'essai de la stimulation aiguë: l'animal a jeun pour 24 heures, après on réalise la stimulation électrique pendant 6-8 heures et la présentation du repas standard la taille du repas, le temps employée pour s'alimenter et la locomotion étant mesurés.

Phase 2 : Evaluation des effets de la stimulation continue chronique intraventriculaire sur le comportement de l'alimentation et sur le poids corporel global. **Paradigme 1 :** Les singes H5 et H7 ont reçu une stimulation électrique, pendant 8 semaines, à une fréquence (SPE) qui a produit la diminution de la prise alimentaire. Les singes H8 et H10 ont reçu une stimulation à haute fréquence simultanément (HFS), utilisant des paramètres proches à ceux de la stimulation subthalamique pour la maladie de Parkinson (130 hertz). Le singe H4 a servi de control. **Paradigme 2 :** Les singes H8 etH10ont reçus à leur tour la stimulation électrique, pendant une période de 8 semaines, à la fréquence SPE .Les singes H5 et H7 ont été stimule également a HFS pour la même période et le singe H4 n'a reçu aucune stimulation. Les animaux ont été périodiquement surveillés : poids, nourriture et niveaux hormonaux et électrolytes. **Paradigme 3 :** Les singes H5, H7, H8, 10 ont tous reçu la stimulation considérée à basse fréquence (LFS) (30 hertz) employé dans diverses pathologies (douleur, freezing etc.). Entre les paradigmes, des périodes de 4 semaines en « off-stimulation »

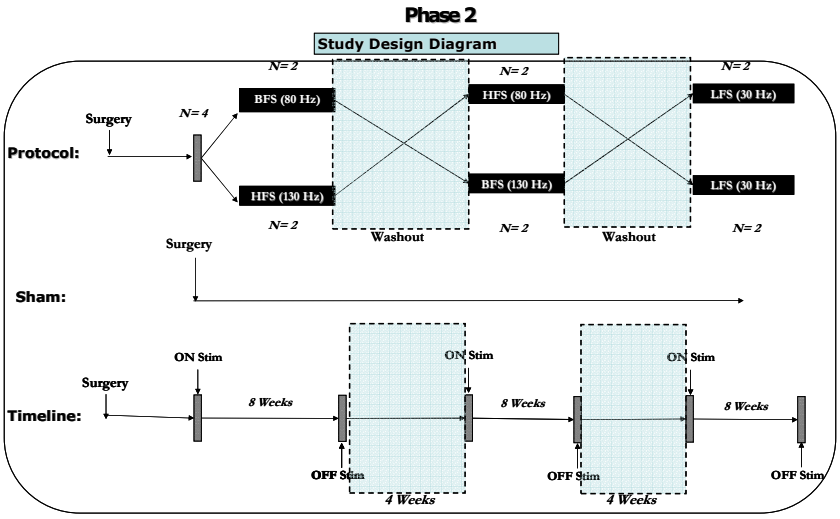
(« washout periods ») ont été programmes pour éviter le chevauchement des effets biologiques entre les divers paramètres donnant des résultats antagoniques (« *carry-on* effet »).

ANALYSE STATISTIQUE

L'analyse statistique des données présentées a été faite en utilisant les tests suivants :

-Le test de Kruskal-wallis a été employé pour réaliser des multiples comparaisons non apparies non paramétriques entre les différentes fréquences de stimulations pendant les tests de stimulation aigue de la phase 1

-Pour des comparaisons multiples entre les groupes, on a utilise le test non paramétrique pairé ANOVA à une voie, le test de Friedman suivi du test post hoc de Dunn. Les variables ont été transformées dans des rapports, représentant les pourcentages de variation en relation à la valeur du premier jour de la période de stimulation.

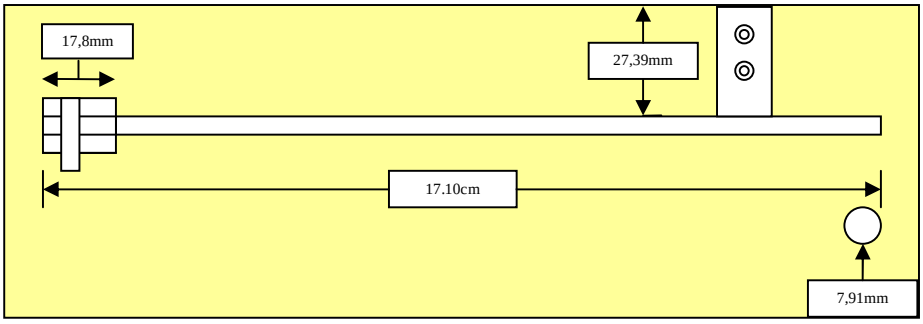


Le schéma2: Protocole pour SCP chronique à différentes fréquences. Entre chaque paradigme, une période de « *washout* »de 4 semaines a été réalisée afin d'éviter le effet « carry on »

Chirurgie Pour Les Electrodes Intraventriculaires Hypothalamiques

Anesthesie Et Soin Peroperatoire

Chaque animal a été anesthésié en utilisant la ketamine (Imalgene®, MERIAL Lyon France) (dose de charge de 20 mg/kg, après dose de maintenance a 5 mg/kg) suivie de l'administration du diazépam (0.25 mg/kg IV, IM). L'anesthésie consistait dans une combinaison de ketamine et de diazépam en plus de la lidocaine à 1% pour l'anesthésie locale du cuir chevelu et des muscles. Le NaCl 0.9% a été infusé en intraveineux sans interruption pendant l'opération, en maintenant une voie de accès vasculaire pour l'administration de médicaments et pour la prise de sang. L'intubation et la ventilation assiste n'ont pas été nécessaires.



Le schéma3 : Le porte électrode de Kopf employé pour exécuter la ventriculographie et pour diriger un tube guide a travers le Foramen du Monro permettant à l'électrode SCP de glisser dans le troisième ventricule

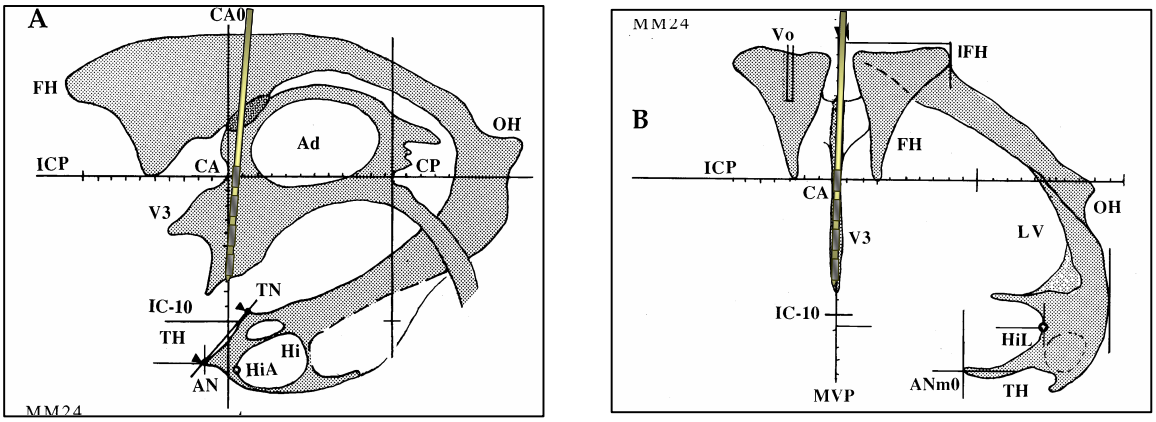
Procédure Stereotaxique

La chirurgie stereotaxique a été réalisée en utilisant un cadre stereotaxique de Kopf® pour les grands animaux avec un kit adaptateur pour le primate (David Kopf Instruments, USA). Le cadre a été modifié en enlevant un morceau du bloc central pour visualiser mieux le troisième ventricule sur la ventriculographie (AP) antero postérieure. La procédure usuelle

utilise un cadre attaché à une base fixe placée dans une salle spécialement conçue pour employer le tele-radiographie trans-opératoire. Les cliches de face et la fluoroscopie de profil peuvent être obtenus avec cette installation spéciale qui place la tête du singe loin des sources de rayons de X, en diminuant la déformation. Nos méthodes chirurgicales sont basées sur la ventriculographie et la visualisation chirurgicale directe des repères internes (commisure antérieure, commisure postérieure, hauteur du thalamus, troisième ventricule, infundibulum). Ces procédures sont conçues principalement pour viser les structures centrales dans le cerveau (comme les ganglions de la base). L'approche, employée couramment chez l'homme ((Talairach et Szikla, 1980))et adapté pour les singes (Percheron et autres, 1986) est utile pour viser les structures peri ventriculaires (comme dans la matière grise peri aqueductale de l'humain). Après la fixation de la tête des singes dans le cadre des clichés de face et du profil ont été obtenus pour éviter la rotation dans le plan sagittal ou coronal. La ventriculographie a été exécutée utilisant la solution de contraste Iopamiron *(Iopamiron® 200, iode 200mg/mL, Bracco, Italy)* et en introduisant un trocar rigide ventriculaire (diamètre de 0.8 millimètre) à 2mm de la suture sagittale et a 70° du plan horizontale, attache a un porte électrode de Kopf .

Tous les films ont été traités dans une salle noire à cote de la salle d'opération animale. Apres avoir traverse 16 -20 millimètres de cortex nous trouvons les ventricules latéraux. Chez les animaux en position assise, la pression du LCR est neutre. Comme il n'y a aucune résistance à la introduction du cathéter, la meilleure manière de trouver les ventricules est de retirer le guide des que la profondeur appropriée est atteinte. Le niveau de solution saline à l'intérieur de trocar baisse lorsque les ventricules latéraux sont traversés. Environ 2 ml de Iopamiron sont injectés rapidement après 0.5 ml d'air introduit afin de enfin de repérer la

position du trocar. La radiographie est obtenue immédiatement, car le produit est rapidement évacué. Le foramen de Monro est alors visualisé dans les deux projections et un deuxième cathéter est dirigé sous la fluoroscopie directement dans le foramen. Une électrode de SCP est alors avancée a l'intérieur du deuxième cathéter et le guide est retiré a l'entrée du troisième ventricule. Sans guide, l'électrode de SCP glisse facilement a la partie antérieure du troisième ventricule près des corps de mamillaires. Les vues sagittales et coronales sont obtenues et compares a la ventriculographie initiale.



Le schéma4 : Projections de face et de profil de la ventriculographie du singe montrant la position réelle d'électrode. Les parois ventriculaires sont proches des contacts actifs des électrodes. La coordonnée postéro antérieure(y) était postérieur de 2.3 millimètres de CA. La coordonnée dorsoventral (z) a été déterminée par le plancher du troisième ventricule. La coordonnée latérale (x) était mesure par rapport a la ligne médiane du troisième ventricule [modifié de (Percheron, 1997)].

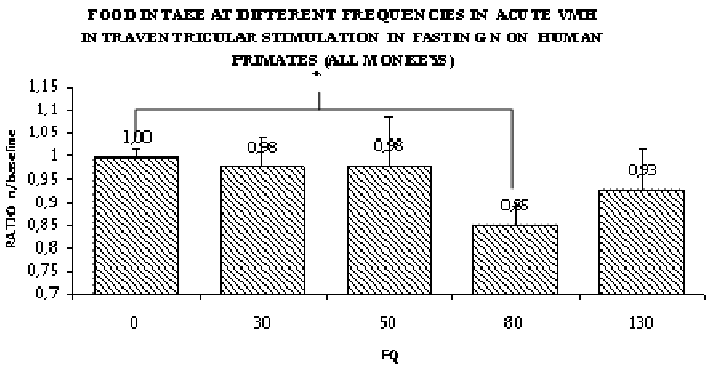
RESULTATS

Réponses Aiguës A La Stimulation De VMH Par l'Intermediaire Des Electrodes Intraventriculaires

Après la stimulation aiguë de chaque animal, nous avons trouvé que la stimulation monopolaire à 80 hertz, 2 volts, reduit la quantité du repas à 0.85 ± 0.04 de la valeur de

base (soit 15% ± 4% de réduction). Le temps de repas reste inchangé et la vitesse de déplacement a une tendance a l'augmentation pendant la stimulation. Ces paramètres ont été utilises ensuite pour le protocole chronique de stimulation.

Le schéma5 : Changement de la quantité de repas exprimée par des rapports (*n/ligne de base)* pour les singes stimulé au niveau VMH par voie intraventriculaire à des différentes fréquences, après 24 heures de jeûne. (TEST de Friedman *de* mesures répétées non paramétriques ANOVA avec des comparaisons de post-test de Dunn). La valeur *p< *0.05* a été considéré significative). Ces résultats obtenus pendant la stimulation aigue, ont été utilises pour la stimulation chronique.



Protocole De Stimulation Chronique

Poids, Composition Graisse Corporelle Et Mesures Indirectes De Graisse

Les animaux ont été pesés à des intervalles réguliers. À chaque fois, des anesthésiques intraveineux ont été utilises pour la sédation (Imalgene®, laboratoire Merial Lyon France) à la dose de charge de 10-30 mg/kg suivis par des doses de maintenance ajustes a la réponse clinique. Une bonne sédation est exigée pour un usage adéquat du la Bioimpedance (BIA).

L'analyse d'impédance Bioélectrique (BIA) est une méthode utilisée généralement pour estimer la composition du corps. La BIA détermine réellement l'impédance électrique, ou l'opposition au passage d'un courant électrique a travers les tissus de corps. Les valeurs obtenues peuvent alors être utilisés pour calculer la teneur en eau du corps entier (TBWr). Cette quantité total d'eau peut être utilisée pour estimer la masse graisseuse de corps par différence avec le poids corporel.

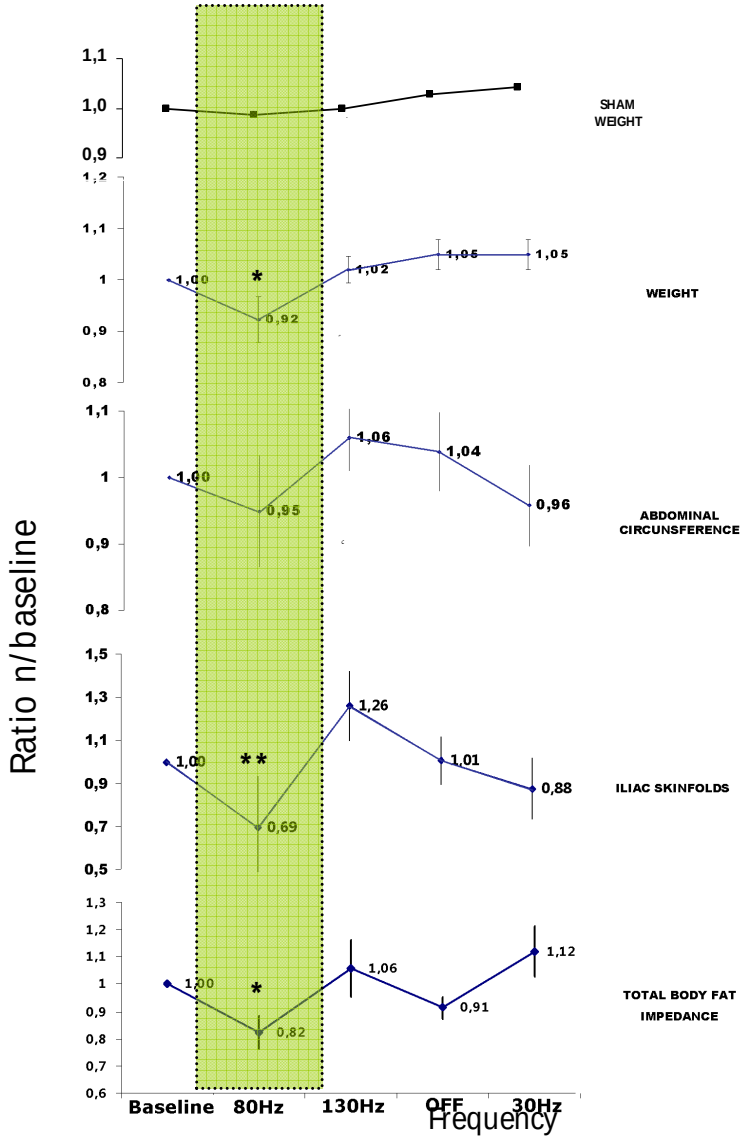
Pour mesurer la BIA l'individu doit rester immobile au moins 15 minute pendant l'essai pour assurer la reproductibilité de chaque test. Les poignets et les chevilles ainsi que les aires occipitale et sacrée ont été rasées et ont servi aux placement d'électrodes de Xitron®. En utilisant le logiciel Hydra pour le post traitement, l'analyse de la composition corporelle de l'animal a été effectuée par multiples mesures de l'impédance. Les résultats ont été stockés pour une l'analyse a posteriori. En même temps, les épaisseurs cutanées iliaque et sous-scapulaire, ainsi que la circonférence abdominale ont été mesurées. Les valeurs de base des individus au début de l'étude sont décrites dans le tableau 1 :

Tableau1 : ***CARACTÉRISTIQUES DES ANIMAUX***

Singe	Sexe	taille (cm)	Poids (kg)	BMI	Circonférence abdominale	pli sous-cutanée souscapulaire	pli sous-cutanée iliaque	Fi
H4	M	37.00	7.20	52.59	45.00	6.92	16.55	340.82
H5	M	41.00	7.60	45.21	31.50	4.00	9.47	407.23
H7	M	39.00	7.00	46.02	36.50	5.36	9.87	410.95
H8	M	40.50	7.28	44.38	35.00	5.52	6.44	466.22
H10	M	41.00	6.10	36.29	32.00	4.60	3.55	376.36

Tableau 1 : Valeurs de base caractéristiques pour chacun des cinq singes. La taille des animaux a été mesurée entre la couronne (tête) et la base de la queue et utilisée pour calculer l'index du poids corporel. La prise alimentaire a été obtenue comme la moyenne de l' ingestion des sujets pendant la période « off-stimulation » avant le début des tests.

Graphique 1 : Poids, circonférence abdominale et épaisseur cutanée iliaque et subscapulaire pendant le protocole chronique de stimulation aux différentes fréquences pendant la stimulation chronique a des différentes fréquences chez le *Macaca fascicularis*

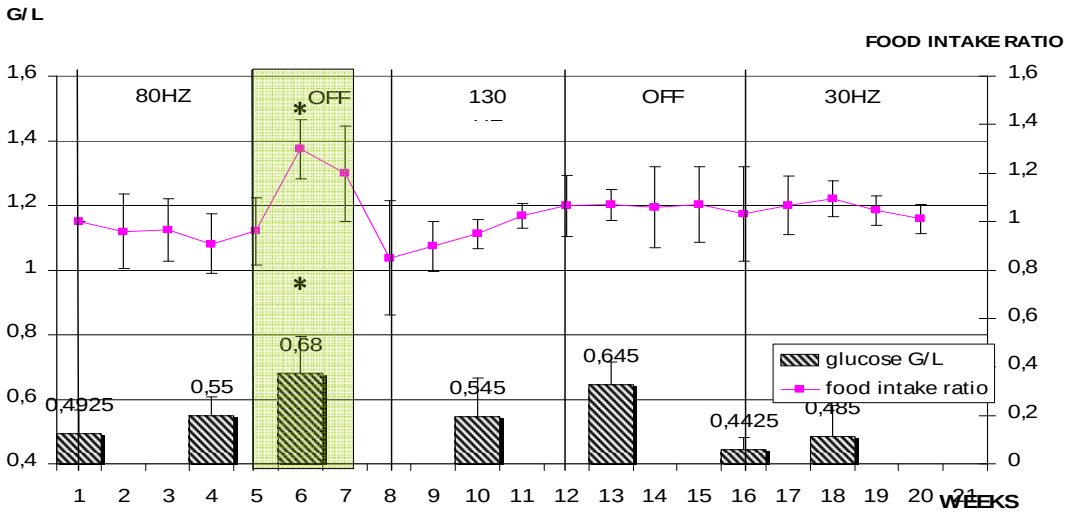


Graphique 1 : Poids, circonférence abdominale et épaisseur cutanée iliaque et subscapulaire pendant le protocole chronique de stimulation aux différentes fréquences (* *p< 0.05* ** essai paired non paramétrique de *p<0.01* Friedman avec des comparaisons de post-test de Dunn). La *ligne de base* signifie les valeurs « off stimulation » avant le début de l'étude, et WO signifie « washout » la période off-stimulation après le paradigme II. Chaque période de stimulation a duré 8 semaines. (stimulation monopolaire continue à 2 volts et à 0.6 ms durée d'impulsion) ; la première courbe represente l'évolution du poids du singe ***sham*** au cours des mêmes périodes de 8 semaines.

La réduction moyenne globale du poids était de 8% à une fréquence de 80Hz (* *p<0.05).* Le poids a légèrement augmenté à une fréquence de 130 hertz (2% en moyenne, écart-type 2.5) et à celle de 30 hertz (5% ±3). Les plis sous-cutanés autour de la taille sont le reflet de la réduction de poids; les épaisseurs cutanées iliaques ont diminué de 0.69 ±0.08 par rapport a la ligne de base et la circonférence abdominale a montré une tendance a la diminution. Le contenu de graisse corporelle, mesuré avec l'aide de le bioimpedancemeter, a montré une diminution significative de la teneur en graisse à 0.817 ± 0.1 pendant stimulation a (80Hz) (graphique 1)

Changements De La Prise Alimentaire Et Du Glucose Pendant La Stimulation Chronique

Graphique 1 Changements de la prise alimentaire et du glucose pendant la stimulation chronique du *Macaca fascicularis*



On a observé une augmentation significative de la prise alimentaire des sujets pendant la période *off* après la stimulation efficace à une fréquence de 80 Hz (± 1.29 0.12, $p<0.05$). Cet hyperphagie a duré pendant la période off, diminuant… brusquement au début de la stimulation a HFS 130 hertz (0.90 ± 0.10 à semaine 14) et restant autour de la ligne de base pendant le reste des épreuves. Le singe « Sham » a eu une tendance d'augmentation de la prise alimentaire vers la fin de la durée de l'étude.

Le glucose est représenté en g/l.Pendant la période de « washout », on a observe une augmentation significative de la glucose plasmatique à 0.68 g/l (essai pairé… non paramétrique de $p<0.05$ Friedman avec des comparaisons de post-test de Dunn). Cette élévation en glucose plasmatique correspond à une période de hyperphagie après des

stimulations efficaces à 80 hertz (graphique 9). Le glucose s'est maintenu dans des valeurs normales vers basses pendant le reste de l'étude (valeurs de référence pour macaca 0.66± 0.15 G/L)

Changements hormonaux et des valeurs biologiques (laboratoire) pendant la stimulation

Les changements des taux des hormones hypothalamiques ont été étudiés pendant les tests. Des normes précises concernant des facteurs de libération hypothalamiques spécifiques pour le genre de Macaca ne sont pas disponibles. Nous utilisons le dosage des hormones pour évaluer si les changements, vu la teneur en graisse et les variations du poids, pourraient refléter un déséquilibre hormonal. Les résultats ont pu être regroupés en : axe corticotrope (GH , cortisol) , axe thyréotrope (TSH, T3L et T3L) et axe gonadotrope (prolactine, FSH, LH et testostérone). Les résultats sont présents dans le tableau ci-dessous :

Hormones pendant la stimulation chronique à différentes fréquences dans V3

HORMONES	LIGNE DE BASE		80 HERTZ		130 HERTZ		30HZ		VALEURS DE RÉFÉRENCE		
CORTISOL	600.75	±148,9	700.13	±146	510.00	±104,3	840.2	±326,	275	689	nmol/l
GH	6.52	±1,79	6.59	±3,74	6.72	±6,97	7.40	±7,9	3	60	uUI/ml
T3L	4.88	±0,74	5.71	±0,72	5.54	±0,61	6,29*	±0,43	3	6	pmol/l
T4L	10.23	±1,27	9.66	±0,97	10.40	±1,03	12.84	±3,10	12	22	pmol/l
FSH	2.43	±1,13	2.25	±1,14	2.54	±1,24	2.59	±1,96	1.3	11.5	mUI/ml
PROLACTINE	97.75	±70,8	223.88	±112	123.13	±62,71	263.0	±341,	30.3	212.1	uUI/ml

Tableau 2 : Valeurs hormonales pendant la stimulation V3 intra ventriculaire chez le singe *Macaca fascicularis*

En jaune sont marques sont les hormones qui ont des valeurs au dessus des valeurs de référence publiées dans la littérature. *p=0.056* (l'essai pairé non paramétrique de Friedman

avec des comparaisons de post-test de Dunn) toutes les valeurs ont été obtenues en employant les valeurs normales pour les humains.

Axe Corticotrope : ***Le cortisol*** a présenté une grande variation entre les essais sans pour autant avoir une différence significative entre les valeurs de base et les valeurs a la fin de chaque période de stimulation. Les niveaux moyens du cortisol étaient plus élevés …que les valeurs de référence à une fréquence de 30 Hz et de 80 Hz. ***L'hormone de croissance (GH)*** a été dose à l'aide de l'hormone de recombinaison humaine (hGH-RIACT de CISBIO) (tableau 2). Les différences trouvées n'étaient pas statistiquement significatives.

Axe Thyreotrope : Thriiodothyroxine (***T3 libre***) a eu une tendance d'augmentation à une fréquence de 30 Hz de stimulation (6.29 *p*= 0.056), valeur légèrement supérieure aux valeurs de référence. La ***T4 libre*** n'a présenté presque aucune variation entre les différentes fréquences de stimulation. L'hormone stimulant de la thyroïde (TSH) n'a pas pu être analysée car la réaction croisée entre le TSH du test humain et celui de genre Macaca est faible, ce qui n'est pas le cas avec les primates non humains de ordre « supérieur » comme les chimpanzés ou les gorilles (genu *Pan, Pongo* et *Gorilla* de la famille *Hominidae*).

Axe Gonadotrope : Les variations de l'hormone ***FSH (hormone follicule stimulant)*** n'étaient pas statistiquement significatives. La prolactine a eu des valeurs très variables, avec des valeurs moyennes légèrement supérieures à la référence à une stimulation a 80 et 30 Hz , mais sans valeurs statistiquement significatives. Les niveaux de la ***LH*** (hormone lutéinisante) étaient non discernables chez deux singes. Il y avait une

tendance a l'augmentation des niveaux de la ***testostérone*** pendant la stimulation. Les deux hormones (LH et testostérone) semblent être corrélées a l'augmentation de la fréquence (avec des valeurs en croissance a mesure que la fréquence de stimulation augmente). Cependant, les différences observées ne sont pas statistiquement significatives.

Changement des valeurs de la Leptine pendant la stimulation

On a observé une grande variabilité dans les niveaux de la leptine pendant l'expérience, même chez le sujet « sham » non stimulé. Les différences observées ne sont pas statistiquement significatives (ANOVA pairé non paramétrique : test de Friedman). En général le singe « sham » a des niveaux plus élevés de leptine, etant donné son BMI supérieur, par rapport aux autres sujets et ces niveaux semblent augmenter pendant l'expérience. A la fin de l'expérience et avec le stimulateur en « off » toute la population étudie a présenté une augmentation des niveaux absolus de la leptine.

Résumé des analyses de laboratoire

Plusieurs conclusions intéressantes peuvent être tirées de la stimulation V3 chronique hypothalamique dans les primates non humains. En premier lieu, les électrolytes Na et K, une indication indirecte d'adipsie…. et de la déshydratation, sont restées dans les valeurs normales de référence tandis que les valeurs pour le chlorure ont été augmentés pendant la stimulation a une fréquence de 80 hertz ($p<0.05$) mais les valeurs absolues sont demeurées dans les normales pour les *espèces de Macaca.*

L'autre conclusion positive concernait la glycémie. Pendant la période de « off-stimulation », après la stimulation efficace qui produit la réduction de poids (80Hz), le

glucose sérique a augmenté de façon significative ($p<0.05$) coïncidant avec une augmentation de la prise alimentaire (hyperphagie de rebond).

Le GH, le cortisol, la prolactine, et le FSH ont présenté une variabilité élevée sans démontrer une différence statistiquement significative entre les fréquences. Les valeurs de cortisol et de prolactine à 80Hz et à 30Hz ont été légèrement augmentées.

Il y a une tendance d'augmentation des valeurs de T3 libre à LFS (low frequency stimulation - stimulation a une basse fréquence) (30Hz) ($p=0.056$). Aucune différence significative n'a été vue dans T4L.

La LH était indétectable chez deux singes .La testostérone était dans des valeurs normales mais avec une tendance a l'augmentation avec la fréquence. La Leptine a présenté une variabilité élevée sans pour autant suivre une tendance qui la rapproche au comportement de la graisse ou du poids corporelle pendant l'étude. Cependant, après la fin de l'expérience (en « off stimulation ») la leptine a eu une tendance a augmenter ses niveaux dans tous les sujets

DISCUSSION

A notre connaissance, le présent étude est le seul a démontrer la diminution efficace du poids total et de la masse de graisse dans les primates non humains et non obèses après la SCP au niveau hypothalamique. C'est également la première fois que une approche intraventriculaire a été utilisée pour moduler les structures hypothalamiques chez les singes. Dans notre étude l'utilisation de cette nouvelle et moins traumatique (invasive ?) approche au niveau des structures médianes centrales de cerveau est essentielle. Elle est facile a exécuter et raisonnablement sure pour les sujets. (Suite au caractère préclinique de ce travail), Les valeurs des différent hormones et électrolytes

ont été mesurées dans le sérum sans montrer des modifications qui pourraient entraîner des troubles métaboliques ou endocrinologique. L'application dans des protocoles cliniques humains bien cibles parait être donc possible.

Des détails des différents résultats sont discutés ensuite.

Modulation du poids et de la teneur en graisse

La conclusion la plus importante de cette étude est que le poids et la masse graisseuse peuvent être modulés chez le primate non humain en utilisant des paramètres électriques proportionnés… de stimulation de la région médiale hypothalamique. Les animaux ont présente une réduction de 8 a 10 % du poids corporel et de 18 % de la teneur en graisse à la fin de la semaine 8, après la stimulation à une fréquence de 80 Hz. Les autres fréquences utilisées n'ont produit aucun changement de poids significatif. La réduction du poids a été accompagnée par une réduction de la masse graisseuse et d'une réduction des épaisseurs cutanées iliaques et une tendance dans la réduction de la circonférence abdominale. La réduction du poids a été faite principalement sue la teneur en graisse produite localement, c'est-à-dire a l'endroit ou la graisse s'accumule,par ex l'abdomen ou la région iliaque. La graisse localisée dans la région souscapulaire n'a pas changé pendant l'expérience.

La réduction de la graisse abdominale a été rapportée chez l'homme associée a une réduction du risque des maladies associées a l'obésité comme l'hypertension ou le diabète (Larsson et autres, 1989 ; Pender et Pories, 2005). Plusieurs auteurs ont montré la modulation dans le poids chez les rats après la lésion des structures latérales dans l'hypothalamus ou la stimulation électrique dans l'hypothalamus ventromedian (Anand et Brobeck, 1951 ; Bielajew et autres, 1994 ; Sani et autres, 2007 ; Stenger et autres,

1991) mais les résultats chez les singes sont limités et loin de être définitifs (Lacan et autres, 2008). Ainsi , plusieurs rapports contradictoires ont été publiés (Robinson et Mishkin, 1968)

La contradiction des résultats dans différentes publications a une possible raison méthodologique. Pour la plupart, ils emploient des paramètres de stimulation (fréquence, tension etc.) venant de la stimulation de ganglions de la base chez les patients présentant une maladie de Parkinson (Takaki et autres, 1992). Dans cet contexte et compte tenu de la complexité de la région, il est peut-être important d'étudier les paramètres de la stimulation électrique de façon plus exhaustive. Dans cette étude, le protocole aigu de stimulation a choisi les paramètres (fréquence, voltage etc.) selon la réduction aiguë de la prise alimentaire. Cette longue période de essai peut se révéler a être essentielle et comme une période clé pour obtenir la réduction de poids souhaitée.

Un autre élément diffèrent dans les études publiées par rapport au travail actuel est la période d'observation. Les protocoles très courts (Lacan et autres, 2008) ont produit probablement la modulation dans la prise alimentaire , mais ils ont échouées a montrer une perte effective du poids. L'évaluation à long terme est peut-être nécessaire. Le travail actuel a présenté une longue période de stimulation aiguë et un protocole « cross-over » avec des périodes « on-stimulation » de 8 semaines et période « off-stimulation » de 4 semaines. De plus longues périodes de stimulation sont nécessaires dans plusieurs pathologies pour réaliser d'avantage de bénéfice comme par exemple en la dystonie et les troubles obsessionnels (Krauss et autres, 2003 ; Vercueil et autres, 2001)

Un élément final qui distingue cette étude des autres et pourrait expliquer les divergences, est l'optimisation de la position finale du contact active. L'optimisation du

ciblage du VMH dans la littérature est faite en mettant le contact directement dans le centre du noyau. Il est possible que l'intensité des champs électriques appliques prés des aires hypothalamiques, antagonistes a l'action du VMH, puisse rendre difficile l'évaluation des effets de cette stimulation électrique du VMH sur le poids. Le problème de l'approche intra parenchymateuse consiste dans le fait que les aires de fonction opposée au VMH, notamment le hypothalamus latéral, peuvent être a leur tour modulées par la stimulation électrique a l'intérieur du noyau. La stimulation intraventriculaire, qui au premier regard est moins précise spatialement, présente l'avantage de couvrir des aires hypothalamiques médiales de façon complète (aires qui sont plus susceptibles d'entraîner une de perte poids), évitant ainsi l'hypothalamus latéral ,un secteur de fibres et de population neuronale éparse/clairseme (Beltt et Keesey, 1975).

Modification de la prise alimentaire

La prise alimentaire était stable pendant les périodes de stimulation. Mais pendant le période « off stimulation », il y a eu une augmentation de 25% de la prise alimentaire ($p<0.05$), juste après la réduction efficace du poids et de la masse graisseuse. L'hyperphagie a été accompagnée d'une augmentation du glucose et du poids. Dans la littérature sur l'obésité on trouve habituellement le même phénomène : des périodes de gain de poids et une hyperphagie après la perte efficace de poids (Hensrud et autres, 1994 ; Masuo et autres, 2005). Dans la première partie du travail actuel, les tests aigus ont démontré la diminution de la prise alimentaire, aux fréquences particulières, chez les singes soumis au jeun. Ces paramètres qui ont induit la réduction en prise alimentaire pendant des épreuves aiguës, ont aussi produit la réduction du poids et de la graisse

corporelle pendant le protocole chronique de stimulation de 8 semaines. Ces résultats suggèrent qu'un effet comportemental sur la prise alimentaire a été obtenu pendent la stimulation aigue. Mais, cet effet aigu diminue dans le temps et un effet métabolique de nature catabolique s'installe en produisant finalement la dépense d'énergie nécessaire pour provoquer la perte de poids et la réduction de la masse graisseuse

En conclusion, l'augmentation de la prise alimentaire et la reprise du poids pendant le période « off stimulation » après la perte efficace de poids pose la question de la période du temps pendant laquelle la stimulation devrait continuer pour produire un BMI stable et probablement établir un nouveau «set point» pour la masse graisseuse chez les sujets.

Changements Hormonaux Et Du Ionogramme Sanguin

La stimulation électrique hypothalamique pourrait en théorie induire plusieurs troubles endocrinologique secondaires à la sécrétion des facteurs de libération dans le système porte hypothalamo-hypophysaire (Fink, 1976 ; Martin et Reichlin, 1970 ; Martin et Reichlin, 1972). Certains de ces troubles pourraient expliquer la perte de poids et la réduction de la masse de graisse pendant la stimulation efficace de l'hypothalamus médial (par ex l'hyperthyroïdisme) (Martin et Reichlin, 1970).

Plus important encore, certaines de ces conditions endocrinologiques pourraient empêcher ou retarder l'application clinique de notre étude. En plus des hormones, les électrolytes et le glucose pourraient produire des effets secondaires indésirables, comme la déshydratation (Szczepanska-Sadowska et autres, 1979) ou le diabète et pourraient mettre en risque l'état de santé de l'animal et potentiellement des patients humains. Ainsi, pour évaluer la sûreté de la procédure, il est nécessaire de tenir compte.

non seulement de la tolérance chirurgicale ou des symptômes comportementaux aigus, mais également des résultats biologiques et hormonaux.

Par contre, la stimulation électrique efficace de V3 semble produire un état catabolique associé a l' hyper métabolisme, qui, a son tour, produit une augmentation des dépenses énergétiques corporelles (Bielajew et autres, 1994).Les tests de laboratoire aident donc à mettre en évidence certaines causes de la perte du poids.

Les ***électrolytes*** sont restés dans les valeurs normales de référence, et seulement la valeur du chlore a augmentée de manière significative toutefois restant dans les normes pendant la stimulation a une fréquence de 80Hz. Dans la littérature, la lésion hypothalamique latérale et la stimulation électrique du VMH (Anand et Brobeck, 1951 ; Bernardis et Bellinger, 1996) (Stenger et autres, 1991 ; Teitelbaum et Epstein, 1962) chez les rongeurs ont été souvent accompagnés de l'adipsie et l' hypernatrémie pendant la perte de poids. Dans notre cas, il était difficile de mesurer la prise d'eau pour plusieurs raisons techniques donc les électrolytes nous ont fourni au moins une idée du degré de la déshydratation produite. Le syndrome adipsie - hypernatremie n'était pas présent dans notre étude, signifiant probablement que le centre de la soif n'a pas été inclus dans la stimulation ou au moins les mécanismes compensatoires se sont maintenus.

Le glucose est resté dans les valeurs normales de référence pendant toute l'expérience. Néanmoins, une augmentation de la glycémie a été remarque juste après la réduction du poids corporel et de la teneur en graisse après la stimulation efficace de V3. Pendant la période de « off stimulation » , une augmentation de la prise alimentaire et du poids ont été également notés. Mais cette augmentation des niveaux de glycemia, s 'est maintenue dans la gamme de référence normale publiée pour le macaca. Ce

résultat indique que des mécanismes d' homéostasie pancréatique compensent en juste proportion cette période anabolique.

Le GH, le cortisol, la prolactine, le FSH, ont présenté une grande variabilité. Les valeurs de la ***LH et la testostérone*** semblent avoir une tendance à l'augmentation pendant la stimulation (à toutes les fréquences) mais aucune différence significative n'a été trouvée. Egalement, aucun changement n'a été trouvé dans l'axe Thyrotropine, excepté le ***T3*** libre, qui a une tendance d'augmentation quand la stimulation était à une fréquence de 30 Hz (p=0.056). La stimulation électrique du VMH et probablement de DMH ne produit pas la sécrétion directe des facteurs de libération hypothalamiques. Cependant, il est bien connu que la stimulation du VMH est associée à un tonus sympathique élève, avec une augmentation du taux de change de la noradrénaline dans la graisse brune du rongeur (Minokoshi et autres, 1986).Le tonus sympathique associé à la dépense énergétique basique métabolique augmente peut conduire aux changements des hormones de stress (Vissing et autres, 1989). Il se peut qu' un mécanisme compensateur ait été actif dans notre étude et cela aide à expliquer pourquoi les hormones sont restées dans des niveaux de référence. Par ailleurs des mesures additionnelles du taux métabolique devraient être faites dans la chambre métabolique pour mesurer la consommation d'O2, la chaleur, la fréquence cardiaque,le taux respiratoire et d'autres variables reflétant l'état catabolique, ce qui nous permettra de comprendre le mécanisme de la modulation du poids pendant la stimulation hypothalamique.

L'approche V3 intraventriculaire par rapport a celle intra parenchymateuse, pourrait s'avérer importante pour éviter des aires voisines antagonistes. Quelques secteurs

proche de l'hypothalamus ventromedial peuvent effectivement sécréter des facteurs de libération hypothalamiques mettant en risque l'état de santé général du sujet implanté.

Enfin les niveaux sériques de ***leptine*** étaient très variables et n'ont pas suivi un modèle discernable, aucune différence statistique n'étant pas retrouvée. La leptine est un signal d'adiposité proportionnel à la quantité de la teneur en graisse. Ainsi, les niveaux de leptine devraient diminuer après un traitement de perte de poids efficace. Cependant, les auteurs ont constaté que pendant la perte de poids, la leptine ne suivait pas exactement la teneur en graisse chez les sujets stimules. Les échantillons de leptine ont été traités après une longue période de stockage et ont été envoyés congelés en carbo-glace. En conséquence, le stockage et le transport sont peut-être en partie responsables de la variabilité élevée de la leptine. Autre explication serait que la durée de l'étude n'a pas été suffisamment longue pour entraîner des modifications significatives dans les valeurs de la leptine. Aussi la diminution de la teneur de la graisse n'était peut être pas si importante pour produire une variation des niveaux de leptine.

Evaluation des risques

La démonstration récente de l'efficacité de la SCP au niveau de l'hypothalamus postérieur a ouvert plusieurs voies de recherches liées aux divers cibles hypothalamiques (leone et autres, 2005). Malheureusement, quelques soucis de sûreté sont a envisager (Pinsker et autres, 2008). Ces soucis nous ont incités à explorer des

nouvelles approches pour atteindre d'une manière moins invasive ces secteurs médiaux du cerveau. Les électrodes intraventriculaires de la SCP ont, en théorie, un risque comparable à l'insertion d'un cathéter ventriculaire dans le cas de l'hydrocéphalie.

Le première problème de sûreté est le procédé chirurgical. Dans notre étude la chirurgie a été bien tolérée chez tous les animaux. L'un d'entre eux a une infection au niveau du stimulateur implanté, ce qui nous a forcé à retirer la pile. Après le rétablissement complet, cet animal a été utilise comme « sham ». Pendant les épreuves aiguës, quand les singes ont été enregistrés et analysés, aucun changement comportemental n'a pas été remarque. Dans le protocole chronique, aucun effet secondaire évident n'a été vu. De façon générale, les singes étaient sains, sans signes de douleur ou réduction du mouvement. Quelques mouvements stéréotypés ont été observés chez deux singes, mais ils ont été liés aux longues périodes de captivité dans de petites cages. Quand les animaux ont été mis dans de plus grandes cages et en paires, les mouvements stéréotypés se sont arrêtés.

Le facteur de limitation le plus important pour l'usage de cette stimulation dans un environnement clinique pourrait être, en théorie, la rupture de l'équilibre hormonal provoquée directement par la stimulation et la sécrétion des hormones de libération hypothalamiques produisant des états potentiellement dangereux (comme l'acromégalie ou l'hyperthyroïdisme). L'analyse soigneuse du sérum prélève nous a permis de conclure que la stimulation n'induisaient pas le changement des éléments essentiels de plasma qui pourraient devenir une menace pour les animaux. On a observé les sujets au cours d'une longue période, presque trois ans du moment de la chirurgie à l'euthanasie. Pendant toutes ces années, les singes ont bien toléré le matériel (IPG et électrodes). La position de l'électrode n'a pas change pendant l'étude, maintenant

l'hypothalamus stimulé, sans produire des lésions aux parois du V3 ou a d'autres secteurs.

En conclusion, ce procédé pourrait être adapté aux sujets humains dans des essais cliniques contrôlés sans risques majeurs évidents aux patients.

CONCLUSION

Les nouvelles indications pour la **SCP** commencent à se développer au fur et a mesure que nos connaissances de l'anatomophysiologie de diverses régions du système nerveux central augmentent . Ainsi, ses caractéristiques de réversibilité et de minime agressivité tissulaire permettent aux chercheurs d'explorer de nouveaux secteurs dans le **SNC**. Les résultats encourageants de la modulation de l'hypothalamus postérieur dans les algies vasculaires de la face et les expériences récentes menées au niveau de l'hypothalamus ventromedian dans les modèles d'obésité chez les rongeurs ont incité a une recherche plus approfondie dans ce secteur. Notre étude est le premier, à notre connaissance, à mettre en évidence une réduction claire du poids et de la teneur en graisse corporelle chez les primates non humains après la **SCP.** La réduction du poids et de la graisse n'ont pas été suivies d'un déséquilibre hormonal, des électrolytes, ou d'autres effets indésirables qui pourraient empêcher l'application chez le sujet atteint d'obésité morbide. Finalement, un élément central dans notre étude, cette nouvelle approche, ou plutôt la renaissance et l'adaptation de l' ancienne stimulation intra ventriculaire pour atteindre les structures médiales, s'est avéré a être une technique sans complications importantes, stable dans le temps et probablement plus efficace dans la modulation des structures médiales hypothalamiques

Introduction (In English)

INTRODUCTION

CHAPTER I OBESITY

Definition and Classification

Obesity is a complex disorder characterized by the accumulation of excess adipose tissue. It is defined in terms of Body mass Index (BMI), calculated as weight (kg)/ height (m) ^{2}. Although BMI is a continuous variable, epidemiological studies based on risk of comorbidities have permitted to classify groups of populations. A BMI less than 25 is considered to be normal; 25-29.9 is overweight and greater or equal to 30. obese (Korner and Aronne, 2003)The definition of morbid obese is less consensual, but in general a 45 Kgs over ideal weight is used in the bariatric surgery literature(Brolin, 1992). The risk of diabetes, hypertension, and dyslipidaemia increases from a BMI of about 27 Kg (Ezzati et al., 2005)

Obesity has reached epidemic proportions globally, with more than 1 billion adults overweight - at least 300 million of them clinically obese. Obesity accounts for 2-6% of total health care costs in several developed countries; some estimates put the figure as high as 7%. The true costs are undoubtedly much greater as not all obesity-related conditions are included in the calculations (Who, 2004)

Being overweight substantially increases the risk of morbidity from a number of conditions, including type 2 diabetes mellitus (DM), hypertension, dyslipidemia, coronary heart disease, congestive heart failure, stroke, gallbladder disease, hepatic steatosis, osteoarthritis, sleep apnea, and endometrial, breast, prostate, and colon cancers. Obesity is associated with diminished quality of life(Roe and Eickwort, 1976) high risk of comorbidities (Must et al., 1999)and reduced life expectancy by five to 20 years

(Fontaine et al., 2003). Obesity has been shown to decrease life expectancy by 7 years at the age of 40 years(Peeters et al., 2003). The increase in risk of death with each unit increase in BMI declines progressively with age but remains substantial until the age group of 75 years and older(Stevens et al., 1998). What is not yet fully confirmed is whether intentional weight loss in obese individuals prolongs life as well as reducing risk. Preliminary evidence suggests a 30-40% reduction in diabetes related mortality with moderate (less than 10 % of bodyweight loss)(Williamson et al., 1995). People with newly diagnosed diabetes who lost 10 Kg in their first year of management were found to have gained a further 4 years of life(Lean et al., 1990).

Abdominal fat (AF)distribution is also associated to comorbidities, reflected in the so called metabolic syndrome (large waist circumference, abnormal concentrations of triglycerides, HDL cholesterol, and fasting glucose and hypertension)(Alberti and Zimmet, 1998). Obese men and women with a large visceral adipose tissue depot are at a particular high risk; even a preponderance of visceral AF might be related to even a higher risk in lean subjects. The mechanism behind this association has been elucidated to some extent. An increase visceral fat accumulation results in an increase in portal free fatty acid(FFA) concentration (Larsson et al., 1992)and causes elevated hepatic gluconeogenesis(Peiris et al., 1988) and very low density (VLDL) protein secretion(Bostrom et al., 1988) as well as a decrease hepatic insulin clearance; the resulting hyperinsulinemia and insulin resistance together with increased gluconeogenesis as well as FFA-induced reduction of peripheral reuptake of glucose will lead to a reduced glucose tolerance and in term to a non insulin dependent diabetes(Randle et al., 1963). A reduced fibrinolitic activity caused by the hyperinsulinemia(Vague et al., 1986) associated with high levels VLDL increases the

chances of having myocardial infarction(Hamsten et al., 1985). Hyperinsulinemia have a permissive role in the development of hypertension. It seems at least hypothetically possible to link diabetes, hypertrigliceridemia, hypercholesterolemia, low HDL levels reduced fribrinolisis and hypertension to elevated portal FFA concentration due to an increased visceral adipose tissue depot.(Sjostrom, 1992)

For that motive is of the utmost importance to define not only BMI (equal to weight divided by height) and indirect measures like subcutaneous fat skinfolds but to determine body composition by compartment, in the study of the obesity patients and for evaluating risks.

Obesity Physiology and Treatment

Treatment of obesity includes non pharmacological measures, pharmacological agents and surgical therapy. Non pharmacological treatments include behavior therapy, exercise, and calorie-restricted diets. The main issue remains to overcome barriers to compliance with diet and physical activity. Lately, very strict diets such as the low carbohydrate Atkins diet have become popular. They have been shown to have good effects on blood pressure and glucose control. These effects are, however short lived and not superior to standard approaches over the longer term. Dietary trials for weight loss and maintenance have yet to show benefit in life expectancy.

The criteria of the US National Institute of Health or the European Union for the use of pharmacotherapy include a BMI of at least 27 kg/m² with a persistent comorbidities or a BMI of at least 30 kg/m².Long term use drugs like sibutramine and orlistat have demonstrated a discreet 4 - 6 % weight loss over a 6 month period in controlled trials, depending upon the intensity of the diet, exercise, and behavioral program administered.

Other agents (Noradrenergic-releasing agents) induce more weight loss than placebo in short term studies, but this kind of agents are only approved for short period using. Objections to pharmacotherapy linger, however. These concerns were fuelled by the withdrawal of flenfluramine and mixture of ephedrine and caffeine, which has led to a rigorous demand for evidence when obesity drugs are evaluated.

For the surgery, some patients with morbid obesity still remain poor candidates for standard Roux en -Y-gastric bypass, in which a small gastric pouch prevents those patients from eating large quantities at a single meal. In the longest follow up study of patients after bypass, Pories et al reported 58%,55% and 49% loss of excess weight (defined as the patient's weight minus the patients estimated "ideal" body weight) at 5, 10, and 14 years postoperatively. However, complications occur in 15-55 percent of bariatric patients, and the peri-surgical mortality rate is about 1.5 percents even in experience centers.(Pories and MacDonald, 1993) Safer procedures, as laparoscopic adjustable banded gastroplasty are simpler but are not as effective. Large room for more effective and safer procedure still exists (Korner and Aronne, 2003)

While obesity has long been consider a behavioral disorder, discovery of the hormone leptin 1994 catalyzed the field of obesity research by demonstrating the existence of an afferent humoral signal from adipose tissue to the central nervous system. Current evidence suggests that once adipose tissue accumulates a system of overlapping neuroendocrine hormones prevent it from diminishing, making volitional weight loss difficult (Zhang et al., 1994).In consequence, modulation of brain circuits emerges as a valuable clinical and research strategy in obesity. Advances in our knowledge of the functional anatomy and electrophysiology of the relevant neural circuitry underlying this condition may unveil novel targets and applications of neuromodulation in obesity.

CHAPTER II HYPOTHALAMUS PHYSIOLOGY

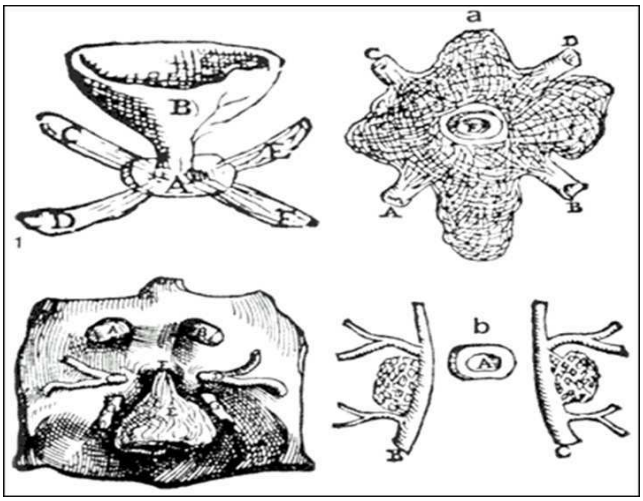


Figure 6 Plates from the seventh book of the first edition (1543) of the Fabrica by Andreas Vesalius, showing what is believed to be the oldest anatomical images in Western literature of the hypothalamic-pituitary unit. (Courtesy of the Library of the Department of Human Anatomy of the University of Bologna, Italy, with permission (From Toni R., Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective, Pituitary 3: 83-95, 2000).

Figure 7 Description of the functional role exerted by the cerebral third ventricle, as reported by Mondino de' Liuzzi in Anothomia. (A) Original front page of Anothomia in a XIV century edition; (B) Original text (in brackets) in medieval Latin (from the 1316 A.D. manuscript kept at the Società Medica Chirurgica in Bologna, Italy); (C) a portion of the Latin fragment shown in (B) containing the most important concepts; (D) English translation shown in (B). (From Toni R., Ancient views on the hypothalamic-pituitary-thyroid axis: an historical and epistemological perspective, Pituitary 3: 83-95, 2000).

Central Nervous System Control of Food Intake and Weight

For most of us the content and amount of food that we eat varies considerably from one meal to the next and from one day to the next. Our common experience, therefore, seems at odds with the hypothesis that food intake is highly regulated. Emotions, social factors, time of day, convenience and cost are but a few of the variables that are not biologically regulated, but nonetheless affect meal-to-meal energy intake. As a consequence, daily energy intake is variable both within and among individuals, and is not well correlated with daily energy expenditure. However, despite short-term mismatches in energy balance, most of us match cumulative *energy intake* to *energy expenditure* with great precision when measured over a period that spans many meals. This phenomenon reflects an active regulatory process, termed *energy homeostasis* that promotes stability in the amount of body energy stored in the form of fat (Schwartz et al., 2000) .For more than a century, increasingly sophisticated methods have been applied to the problem of how the brain contributes to the physiology of energy homeostasis and the pathogenesis of obesity. Although it is overly simplistic to reduce a behavior as complex as feeding to a series of molecular interactions, discoveries over the past few years have identified signalling molecules that affect food intake and that are critical for normal energy homeostasis. The application of molecular genetics to mice has been especially important in this effort. For example, several monogenic forms of human obesity were identified by searching for mutations homologous to those causing obesity in mice(Clement et al., 1998; Montague et al., 1997; Vaisse et al., 1998). Although such monogenic obesity syndromes are rare (Barsh et al., 2000)the successful use of murine models to study human obesity indicates that substantial homology exists across

mammalian species in the functional organization of the weight- regulatory system. More importantly, the identification of molecules that control food intake has generated new targets to develop in the treatment of obesity and related disorders. Optimism that we may soon enter an era of improved obesity treatment, therefore, seems justified. In the last decade, the combination of genetic and physiological techniques has made possible great progress in the identification of metabolic hormones and their relationship to key neuronal system in the hypothalamus. The adipose hormone, leptin, is a crucial signal that conveys metabolic information from the periphery to the hypothalamus, where melanocortin system seems to have a fundamental role in the brain response to peripheral metabolic status.

Because of the enormous toll on human health taken by obesity and related disorders, an improved understanding of the control of food intake is an important priority. However, the growing number of molecules implicated in energy homeostasis raises nearly limitless possibilities for how body-weight regulation might occur(Schwartz et al., 2000).

Energy Homeostasis Model

The increase of food intake following a fasting period is a good and simple example of food intake regulation. The consequent recovery of lost body weight and the gradual return to normal levels of energy intake is a evidence that a regulatory process that is precise and robust are in action. To explain this phenomenon, Kennedy proposed in 1957 a model in which inhibitory signals coming from body fat stores act in the brain to reduce food intake. When reductions in these fat stores are detected and the level of the inhibitory signals decrease, food intake increase until energy deficit is corrected.

This model, however, does not explain how energy intake is controlled during individual meals(Kennedy, 1950). Twenty years later, Gibbs and Smith proposed that signals generated during a meal (termed satiety factors), including peptides secreted from the gastrointestinal tract, provide information to the brain that inhibits feeding and leads to meal termination (Gibbs and Smith, 1986)

Adiposity and Peripheral Signals

Insulin was the first hormonal signal to be implicated in the control of body weight by the central nervous system (CNS)(Woods et al., 1979). The subsequent demonstration that profound hyperphagia and obesity of *ob/ob* mice results from autosomal recessive mutation of the gene encoding leptin, a hormone secreted by adipocytes, provided compelling evidence of a second adiposity signal(Zhang et al., 1994). Studies demonstrated that both insulin and leptin fulfill criteria that should be met by any candidate adiposity signal. Both hormones circulate at levels proportionate to body fat content(Bagdade et al., 1967; Considine et al., 1996) and enter the CNS in proportion to their plasma level(Baura et al., 1993; Schwartz et al., 1996a). Leptin receptor and Insulin receptors are expressed by brain neurons involved in energy intake(Baskin et al., 1999a; Baskin et al., 1988; Cheung et al., 1997) and the administration directly in the brain of either peptide reduces food intake(Campfield et al., 1995; Weigle et al., 1995; Woods et al., 1979), whereas deficit the one hormone does the opposite(Sipols et al., 1995; Zhang et al., 1994). To date this are the two molecules that fulfill these criteria.

Different mechanism underlies the association of insulin and leptin with body fat content. The effect of weight gain to reduce insulin sensitivity seems to explain how insulin but not leptin, varies according to fat stores(Schwartz et al., 1997). As weight increase,

insulin secretion must increase in both the basal state and in response to meals to compensate for the increase in insulin resistance if normal glucose homeostasis is to be maintained(Kahn et al., 1993; Polonsky et al., 1988). Failure to achieve this by pancreatic B cells can cause hyperglycemia and probably contributes to types 2 diabetes with obesity. Increase insulin secretion as obesity progresses is thus hypothesized to increase insulin delivery to the brain, where it helps to limit further weight gain.

Mechanisms involved in leptin secretion are quite different. The rate of insulin-stimulated glucose utilization in adipocytes is a key factor linking body fat mass to leptin secretion(Mueller et al., 1998). It may involve glucose flux through the hexosamine pathway(Wang et al., 1998). Because acute change in energy balance acute change adipocytes glucose metabolism, ***leptin secretion can transiently become dissociated from levels of total body fat.*** For example, food deprivation acutely lowers plasma leptin concentration much more rapidly and to a greater extent than would be expected from the decrease of body fat content. This exaggerated early decline in leptin levels helps to explain why compensatory responses are activated before energy stores are substantially depleted

Several observations help to realize the role of leptin in central energy homeostasis. Important evidence of this role is the relation between ***leptin resistance and obesity.*** The hypothesis that leptin resistance can occur in association with obesity was first suggested by the finding of elevated plasma level of leptin in obese humans(Considine et al., 1996). This hypothesis suggests that obesity occur as a reduced action of leptin in the brain and that affected individuals are unlikely to respond to pharmacological agents. Leptin resistance is well documented in mice(Campfield et al., 1995) and

rats(Chua et al., 1996) bearing leptin mutant receptors, but also mice that develops obesity for other causes like genetic ablation of thermogenic brown adipose tissue(Mantzoros et al., 1998) or with lack of melanocortin 4 receptors(Marsh et al., 1999) or even mice feed with highly palatable high fat diet(Campfield et al., 1995). Mechanisms implicated in leptin resistance are varied. The decrease ability of circulating leptin to enter brain interstitial fluid, where it can bind to neuronal leptin receptor is one of mechanism studied. Impaired transport through the blood-brain barrier might be involved. Like insulin, leptin uptake into the brain is facilitated by leptin receptors expressed by endothelial cells(Bjorbaek et al., 1998b) in the blood brain barrier. Whether dysfunction in this barrier can cause obesity remains to be demonstrated, but the finding that leptin in the cerebrospinal fluid in obese patients is low in comparison with plasma levels is consistent with this theory(Caro et al., 1996). Reduced leptin-receptor signal transduction is another potential cause of leptin resistance. Leptin receptor activation can induce expression of a protein that inhibits further leptin signal transduction, called 'suppressor of cytokine signalling - 3' (SOC-3)(Bjorbaek et al., 1998a). This is an area of active study and SOC-3 role remain to be determined. Finally, a failure of one or more neuronal system in the circuits activated by the leptin signal will manifest as a leptin resistance. The neuronal effectors pathways key role in energy balance is crucial to understand central mechanism of food intake and weight control (Figure 8)

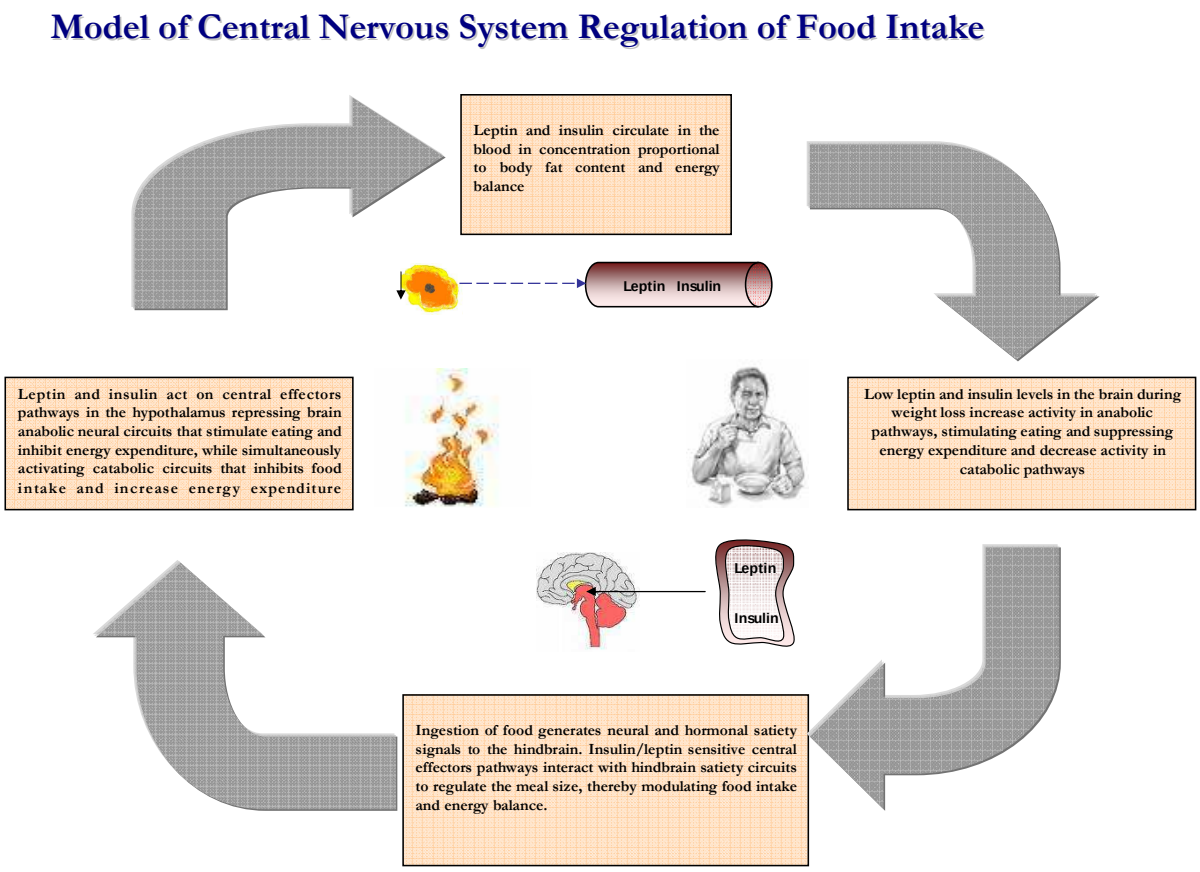


Figure 8: Model of Central Nervous System regulation of Food intake:

Neuropeptide Effectors of Adiposity Signals:

Several neuropeptides hypothalamic pathways has been signaled as candidate mediators between adiposity signals leptin and insulin in the CNS.

Prominent among anabolic effectors pathway is neuropeptide Y. NPY research was initiated in late 1984 by Clark et al in which the first evidence of NPY induced feeding was presented (Clark et al., 1985; Levine et al., 2004). When applied intracerebroventricularly, NPY induce a robust feeding response(Clark et al., 1985), which are not been paralleled by the administration of any other substance, like melanin-concentrated hormone(Qu et al., 1996), orexin/hypocretin(Sakurai et al., 1998b), lateral

hypothalamic orexigenic peptides. Consequently, continuous or repeated central administration of NPY leads readily to obesity. NPY meets the criteria of an anabolic signalling molecule: gene expression and secretion of NPY are increased when body fat store are depleted (Schwartz et al., 2000)Leptin and NPY stories seem to be linked. NPY producing neurons in the arcuate nucleus respond to the levels of leptin(Mercer et al., 1996). In conjunction with this, it was also found that obesity in leptin deficient mice is reduced by elimination of NPY(Billington et al., 1991; Zarjevski et al., 1993). Research in NPY continues and is now focus in a particular subset of neurons in the arcuate nucleus. These neurons also produce Aglouti related peptide (AGRP)(Hahn et al., 1998), which has been shown to be essential in the function of the melanocortin system, the key player in the hypothalamic feeding regulation (Horvath and Diano, 2004)

Melanocortins Suppress Food Intake

Candidate catabolic effectors signalling molecules have an opposite set of characteristics. Neuronal synthesis of these molecules augmented in response to increased adiposity signal in the brain. Among the peptides involved, melanocortin system(Cone, 1999) stands out as being remarkable both for its complexity and for its importance to energy homeostasis.

Melanocortins are peptides that are cleaved from the pro opiomelanocortin (POMC) molecules and that exert its effects by binding to the members of a family of the Melanocortins receptors(Cone, 1999). A role for melanocortin signalling in the control of energy homeostasis first emerged after the cloning of the MC3- and MC4-receptor genes and the demonstration that they are expressed primarily in the brain(Mountjoy et al., 1994). This discovery was followed by evidence that a synthetic agonist of these

receptors suppresses food intake, whereas a synthetic antagonist has the opposite effect(Fan et al., 1997)55. The report that mice lacking the MC4 receptor (owing to gene targeting) are hyperphagic and very obese(Huszar et al., 1997)56 indicates that tonic signalling by MC4 receptors limits food intake and body fat mass. Mice heterozygous for the deleted MC4 allele also become obese, although less so than homozygous knockouts(Huszar et al., 1997)56. Lack of a full complement of central MC4 receptors, therefore, predisposes to hyperphagia and pathological weight gain. This finding has since been extended to humans with MC4-receptor mutations.

Further evidence for the importance of melanocortin signalling came from studies of agouti mice and the cloning agouti gene identified protein (agouti) that acts as an antagonist of Melanocortin 1(MC-1) receptor normally expressed in hair follicles. By reducing MC 1 signalling, increased cutaneous agouti lightens the color of the coat of the animal(Miller et al., 1993). Agouti mice express agouti protein in tissues throughout the body and consequently develop obesity and a yellow coat color. The obesity is due to ectopic agouti production within the brain, where it antagonizes MC 4 receptors(Cone, 1999). Further studies have indicated the important place of antagonist of CNS melanocortin receptors (MC 3, MC 4) as a body weight regulation. The cloning of AGRP gene(Shutter et al., 1997) identified a peptide AGRP, with homology to agouti that is an antagonist of MC 3 and MC 4. Consistent with the role of anabolic signal, AGRP intraventricular injection causes hyperphagia and the increase in food intake is sustained after a week from a single injection(Hagan et al., 2001). Their action as an antagonist of the melanocortin system and the duration of action are a fascinating new area for further investigation.

The Hypothalamus Affects Feeding

The role of hypothalamic regions were first revealed in lesion studies in which destruction of the hypothalamic ventromedial, paraventricular and dorsomedial nuclei induced hyperphagia(an abnormal increase in appetite and food intake). By contrast, Lateral hypothalamic lesion induced hypophagia (reduced food intake) which could lead to animals dead. These lesion studies were strikingly precise in signalling which hypothalamic areas were implicated in promotion or suppression of feeding. In several studies, physiological observation of obese animal strains also supported the idea that humoral signals, arising from the periphery might inform the brain sites about overall energy needs. These ideas echoed some Sherrington's suggestions about feeding made some years before that feeding might be regulated the same way respiration, peripheral signal affect the blood changing their composition and thereby influencing the brain.

The observation that some mouse or rat mutants, including *db/db* (*lepr/lepr*) and *ob/ob* (*lep/lep*) mice and *fa/fa* (*lepr/lepr*) rats, become strikingly obese led to the crucial discovery of the adipose hormone, leptin, as a humoral signal that can centrally regulate metabolism(Elmquist et al., 1999; Zhang et al., 1994).The primary genetic defect of this animal is abolished leptin production or impaired leptin receptors. In humans, obesity cases are reported when either lack of circulating leptin or mutations in leptin receptors are found .Leptin is liberated by adipose tissue and acts as humoral sign that carries information about fat stores. Leptin receptors could be found in the arcuate nucleus and also in the ventromedial hypothalamus, which are implicated as a primary target as a feedback signalling leptin. These original findings and the intriguing nature of leptin

signalling raised expectation of a practical medical approach, but time as proved otherwise.

There are others peripheral and metabolic signals that seem to be important for central metabolic weight and feeding regulation like glucose, insulin, and cholecystokinin, glucagons like peptide, ghrelin, and pancreatic polypeptide. Ghrelin is produce by the stomach and his discovery (Kojima et al., 1999)revived the century old theory that stomach-drive mechanism can regulated food intake. It is a peptide hormone which was discovered in 1999 and is an endogenous ligand for the growth hormone (GH) secretagoge receptor (GHS-R) (Kamegai et al., 1999) Ghrelin stimulates GH secretion in healthy humans in a dose dependent manner and is a strong orexigenic and adipogenic molecule in mammals. Inhibiting ghrelin-receptor expression in the hypothalamus of transgenic rats decrease GH secretion, food intake, and body fat mass (Ueno et al., 2005)suggesting that in the hypothalamus, the ghrelin receptor is important in regulating GH secretion and energy homeostasis. Ghrelin induces weight gain and adiposity. Intracerebroventricular (ICV) Injection of ghrelin to free feeding rats during both light and dark phase increases food intake in a dose dependent manner. Its orexigenic activity is independent of the GH signalling pathway. IV Administration in human increase energy intake from a buffet lunch by 28 3.9% and also increase the visual analogue score for appetite. These data indicate that ghrelin is a peripheral orexigenic and adipogenic peptide. Ghrelin-producing neurons are present in the arcuate nucleus of the hypothalamus, which is also a target for leptin. Several studies indicate that ghrelin is an upstream regulator of the orexigenic peptides NPY and AgRP and that it antagonizes leptin's effects in NPY/AgRP expressing neurons, resulting in an increase in feeding and

body weight. By activating the NPY/Y1 receptor-signalling pathway, ghrelin acts as a natural antagonist to leptin

Nevertheless, none of them has a role as important as the leptin in central metabolism. In addition, none of them including leptin has formed the basis of the possible therapeutics for weight control (Horvath and Diano, 2004)

Arguably, the most successful prescription medication for weight loss, so far, has been a combination therapy that targets serotonin and noradrenalin re-uptake. Most recently, the cannabinoid-1 receptor has a viable target for the development of a weight-loss drug. Although there is evidence that both of these therapies affect components of the melanocortin system, it is also logical that some of the appetite-reducing effects of these approaches lie outside the hypothalamic feeding circuits, for example in the cortex or reward circuitry. However, the leptin experience has provided an invaluable new approach to the understanding of central weight regulation by identifying the CNS, and neuronal communication in particular, as the site where the 'Holy Grail' of metabolism should be sought .Most recently, the cannabinoid-1 receptor has emerged as a viable target for the development of a weight-loss drug (Cota et al., 2003; Di Marzo et al., 2001; Fernandez and Allison, 2004).Although there is evidence that both of these therapies affect components of the melanocortin system(Heisler et al., 2002), it is also logical that some of the appetite-reducing effects of these approaches lie outside the hypothalamic feeding circuits, for example in the cortex or reward circuitry. However, the leptin experience has provided an invaluable new approach to the understanding of central weight regulation by identifying the CNS and neuronal communication in particular, as the site where the control of metabolism should be sought(Horvath and Diano, 2004)

Hypothalamic Signalling Pathways

The idea that the CNS and the hypothalamus in particular, are key in metabolism regulation was reinforced by the discovery of leptin. Before that time, research focused on various neuropeptides and classical neurotransmitters, including GABA (g-aminobutyric acid) glutamate, neuropeptide Y (NPY), galanin, serotonin and noradrenalin (Sommer et al., 1967), in relation to the regulation of feeding and energy expenditure. Interest in these neuromodulators stemmed, at least in part, from their presence in the hypothalamus. GABA, glutamate, NPY and galanin were found to be predominantly orexigenic (pro-feeding) when injected into the third ventricle or various hypothalamic regions, whereas serotonin and noradrenalin seemed to be anorexigenic (anti-feeding). However, some of these substances could trigger the opposite response, depending on the site of injection and dose.

A more recent breakthrough was the revelation that the melanocortin central system is a key mediator of energy balance(Fan et al., 1997; Huszar et al., 1997) and leptin-induce satiety(Seeley et al., 1997). In the arcuate nucleus, are of particular interest the counter balance relation between two sets of neurons- those that contain NPY/AGRP and those that contain Proopiomelanocortin (POMC), as the main regulator of appetite, satiety and energy expenditure regulation (Zigman and Elmquist, 2003). The POMC cells, which produce α-melanocyte stimulating hormone (α-MSH) maintain an anorexigenic tone, whereas NYP/ARGP neurons maintain an orexigenic tone in which AGRP antagonize α-MSH on the melanocortin 4 receptor(Ollmann et al., 1997). Melanocortin system represents the main center that gathers information for the integration of the peripheral signals in the hypothalamus for the final energy expenditure regulation. Increase

attention has been paid, therefore, to numerous hypothalamic peptides and the integration of their signalling in the melanocortin system(Horvath and Diano, 2004)

Transduction of Adiposity Signals into Neuronal Response

Situated adjacent to the floor of the third ventricle, the ***arcuate nucleus*** is an elongate ('arc-like') collection of neuronal cell bodies occupying approximately one-half of the length of the hypothalamus. NPY and AGRP are co-localized in arcuate nucleus neurons(Ollmann et al., 1997; van den Pol, 2003) demonstrating that a single neuronal cell type can contain multiple anabolic effectors molecules. The subsequent finding that POMC and CART are co-localized in a distinct, but adjacent, subset of arcuate nucleus neurons(Matsumoto and Arai, 1981)indicates that circuits originating in this brain area have highly specialized roles in energy homeostasis (Fig. 4).

The hypothesis that the ***arcuate nucleus*** transduces information related to signalling by leptin into a neuronal response is supported by the anorexic response to local microinjection of leptin into this area(Matsumoto and Arai, 1979), and the inability of ICV leptin to reduce food intake after the arcuate nucleus has been destroyed (Garcia-Segura et al., 1987; Naftolin et al., 1996) A majority of both NPY/AGRP and POMC/CART neurons have been found to co-express leptin receptors(Halaas et al., 1995; Zhang et al., 1994) and both types of neurons are regulated by leptin (as judged by changes in Neuropeptide gene expression), but in an opposing manner. Thus, NPY/AGRP neurons are inhibited by leptin, and consequently are activated in conditions where leptin levels are low (Ollmann et al., 1997) (van den Pol, 2003). Although less well studied, a deficiency of insulin also seems to activate these neurons (Leibel et al., 1997; Stanley and Leibowitz, 1984) and insulin receptors are highly concentrated in the

arcuate nucleus. Conversely, conditions characterized by reduced insulin or leptin inhibit POMC(Horvath et al., 1997; Horvath et al., 1992) and CART expression in the arcuate nucleus, and administration of these hormones can prevent or attenuate these neuropeptides responses. Moreover, involuntary overfeeding in rats, which potently inhibits spontaneous food intake once body weight has increased by more than 5%, elicits a threefold increase of POMC messenger RNA levels in the arcuate nucleus(Diano et al., 1998). The demonstration that anorexia induced either by leptin(Cowley et al., 2001)73 or by involuntary overfeeding(Diano et al., 1998)72 is reversed by central administration of a melanocortin-receptor antagonist (at a low dose that has no effect on food intake in control animals) indicates that melanocortin signalling is a mediator of the anorexic response induced by increased adiposity signalling to the brain. Taken together, these findings indicate that the ***arcuate nucleus*** is a major site for transducing afferent input from circulating leptin and insulin into a neuronal response.

Implicit in this hypothesis is the suggestion that brain areas innervated by arcuate nucleus neurons are sites where second order neurons involved in energy homeostasis are located. But the identification of such downstream neurons is just beginning, and energy homeostasis probably involves integrated and redundant pathways, rather than a discrete set of neurons in series to one another.

Integrative Model of Hypothalamic Peptidergic System Involved In Regulating Energy Balance and Food Intake

Models for understanding how arcuate nucleus neurons ultimately affects food intake provides a useful framework for study, but the identification of such downstream neurons is just beginning.

Hypothalamic areas including the Paraventricular nucleus (PVN), zona incerta, perifornical area (PFA) and LHA are richly supplied by axons from arcuate nucleus NPY/AGRP and POMC/CART neurons(Elmquist et al., 1999; Elmquist et al., 1998). Early stimulation and lesioning studies have demonstrated some of the features of these areas. PVN stimulation inhibits food intake, whereas the reverse is true for stimulation of LHA and adjacent PFA(Bray et al., 1989). Conversely, bilateral PVN Lesioning causes obesity and hyperphagia, whereas bilateral LHA lesioning (Stellar, 1954)produces anorexia and weight loss (Bray et al., 1989; Stanley et al., 1993; Stellar, 1954)). These observations indicate that anorexigenic and orexigenic molecules might be synthesized in the PVN and LHA respectively.

Several neuropeptides synthesized in PVN neurons reduce food intake and body weight when administered centrally. These include corticotrophin release hormone ***(CRH),*** which causes anorexia and also activates the sympathetic nervous system in addition to his role as a major regulator of the hypothalamic-pituitary-adrenal axis(Dallman et al., 1993); Thyrotropin-releasing hormone (TRH), which reduce food intake and stimulates the thyroid axis(Kow and Pfaff, 1991) and ***oxitocin*** which also reduce food intake in addition to regulating uterine function(Verbalis et al., 1995***)***. All of this is consistent with the predictions that PVN neurons act reducing food intake and body weight.

The hypothesis that second order neurons involved in anabolic signalling reside within the LHA/PFA is sustained by the presence of several orexigenic neuropeptides in the area. Melanin Concentrating Hormone (MCH) is an orexigenic peptide synthesize in the area which is elevated by energy restriction and leptin deficiency(Qu et al., 1996). Other evidence of the orexigenic effect comes from the MCH knockout mice which have reduce food intake and are excessively lean(Shimada et al., 1998). Two additional peptides are expressed exclusively in the LHA, zona incerta and PFA. Termed ***hypocretins 1*** and ***2***(de Lecea and Sutcliffe, 1999) or ***orexins A and B*** (Hagan et al., 1999; Sakurai et al., 1998a) these peptides increase food intake and cause generalized behavioral arousal when administer centrally. Reduced hypocretin/orexins signalling may contribute to sleep additionally to the control of food intake, as seen in the narcolepsy produced by deletion of the orexins/hypocretin gene(Chemelli et al., 1999). Integration of ***MCH*** and ***hypocretin/orexins neurons*** into a model of hypothalamic pathways controlling energy homeostasis predicts that they should be inhibited by melanocortin or CART input, and stimulated by NPY signalling, from neurons of the arcuate nucleus (Lopez et al., 2007; Schwartz et al., 2000)

Satiety Signals Control Meals Size

It is evident that either the amount of food consumed during individuals meals, the frequency of the meals or both must be regulated if energy balance has to be achieved. The major determinant of meal size is the onset of the satiety signals, a biological state induced by neurohumoral stimuli generated during food ingestion that leads to meal termination. In contrast to the timing of meal initiation which can be influence by a variety of factors (for example, emotional factors, time of the day, availability and

palatability of foods, threats from the environment), meal termination tend to be a more biological controlled process(Strubbe and Woods, 2004). Several findings indicate that control of meal size is a component of the feeding response induced by changes of body fuel store or adiposity signals. The hyperphagic response to central administration of NPY, for example arises predominantly from the consumption of larger meals(Leibowitz and Alexander, 1991). Conversely, ***leptin treated animals consume the same number of meals but smaller size (Flynn et al., 1998)***. One way that this could be achieved is modulating satiety signals in brain areas that process information.

Satiety information, in contrast with adiposity signals processed in the hypothalamus, is largely conveyed to the hindbrain by means of afferent fibers of the vagus nerve(Ritter et al., 1994) and by afferents passing into the spinal cord from the upper intestinal tract as well as taste information from the oral cavity(Travers et al., 1987). Satiety induced signal that reach the Nucleus of solitary tract(NTS) are initiated by mechanical or chemical stimulation of the stomach and small intestine during food ingestion, neural input related to energy metabolism in the liver(Friedman et al., 1999), and neurohumoral signals coming from secretory cells lining in the intestinal lumen cholecystokinin(CCK) (Moran and Schwartz, 1994)

In resume, Leptin and insulin are proposed to stimulate a catabolic pathway (***POMC/CART neurons***) and inhibit an anabolic pathway (***NPY/AGRP neurons)*** that originates in the arcuate nucleus (ARC). These pathways project to the PVN and LHA/PFA, where they make connection with central autonomic pathways that project to hindbrain autonomic centers that process satiety signals. Afferent input related to satiety from the liver, gastrointestinal tract, stomach and from peptides such CCK are transmitted through the vagus nerve and sympathetic fibers to the nucleus of the solitary

tract (NTS), where they are integrated with descending hypothalamic input. Net neuronal output from the NTS and other hindbrain regions leads to the termination of individual meals and is potentiated by catabolic projection from the PVN and inhibited by input from the LHA/PFA. Reduced input from adiposity signals (for example, during diet induced weight loss), therefore, increase meal size by reducing brainstem responses to satiety signals Ascending projection from the hindbrain to forebrain that may also contribute to adaptive changes in food intake (Schwartz et al., 2000)(

Figure 9)

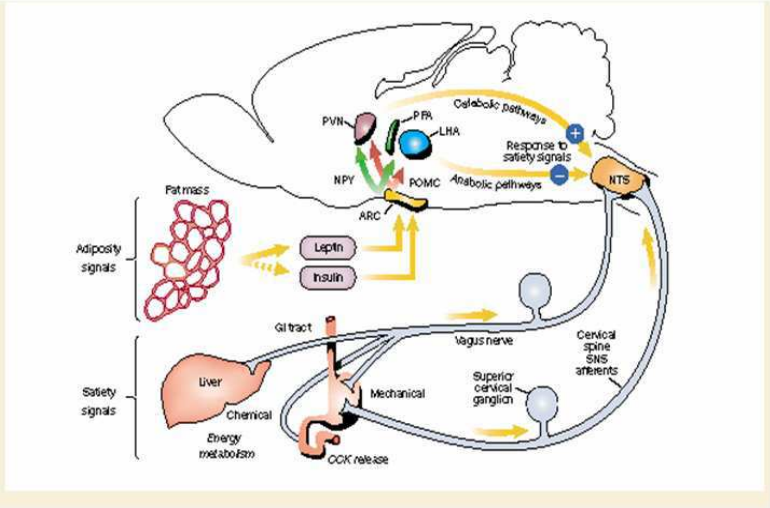


Figure 9: Model for hypothalamic control of energy balance and food intake

SUMMARY:

Three effects of eating limit the size of an ongoing meal:

1. Gastric distention
2. Postgastric detection of calories (via satiety signals such as Cholecystokinin(CCK)
3. Increased plasma osmolality.

These signals reach the brain through visceral afferents fibers (especially those from the vagus nerve) and the circulatory system. Meal size increases when these inhibitory signals are experimentally blocked. When the satiety signals disappear, hunger emerges and stimulates initiation of another meal.

Food intake is also normally linked closely with body weight. In experimental animals periods of starvation or forced feeding, are followed by compensatory changes in eating patterns until body mass is reestablished. Body weight and food intake can be mediated indirectly by altered gastrointestinal motility and absorption as well as by the centrally active hormones, leptin and insulin.

Although the details of the central control of food intake are incompletely understood, many neuropeptides have been implicated, either anabolic or catabolic. The amount in body fat is signaled to the brain by the leptin and insulin. Receptors for both peptides are located (among other sites) in the hypothalamic arcuate nucleus on two distinct groups of neurons. The first group synthesizes the neuropeptides α- melanocyte stimulating hormone (α-MSH) and cocaine amphetamine related transcript (CART) which produces a very powerful catabolic signal that increase energy expenditure and lose weight. The

other type of arcuate neurons influenced by adiposity signals synthesizes NPY and aglouti related protein (AgRP), both potent anabolic compounds which administration into the third ventricle results in hyperphagia, reduced energy expenditure and weight gain. The arcuate nucleus can be considered to be the brain's sense organ that detects body adiposity by monitoring the levels of leptin and insulin. In turn, axons from these two groups of arcuate neurons innervate many other hypothalamic nuclei as they modulate aspects of caloric homeostasis.

The central nervous system exerts control over eating at many levels. The spinal cord and brain stem influence all aspects of caloric homeostasis via the autonomic nervous system. The hypothalamus and limbic forebrain receive signals about ingested food and body adiposity and integrate them with information about memory of the food, taste, competition with other desires, environmental factors, and previous meals. Thus the central control involves many areas of the CNS in the collective maintenance of caloric homeostasis, the details of which remain to be fully understood.

CHAPTER III ANATOMY OF THE HYPOTHALAMICS NUCLEI

The brain regulates many aspects of energy homeostasis, along with the brain stem and the spinal cord, as we have seen previously. This process is highly complex and involves several brain regions, but most interest has focused on the hypothalamus. The human hypothalamus comprises only 4 cm^3 of neural tissue, or about 0.3% of the normal adult brain volume. Nevertheless it is critically involved in the coordination and integration of autonomic, endocrine, and behavioral responses necessary to maintenance of the homeostasis (Nieuwenhuys et al., 2008).

Our knowledge in the past years has increased dramatically and it has become evident that various neural circuits operate to different degrees and probably serve specific functions under particular conditions of altered energy balance.

Gross Anatomy

The hypothalamus lies directly above the pituitary gland (Figure 10) and occupies approximately 2 per cent of the brain volume. It is composed of a number of cell groups as well as fibre tracts that are symmetric about the third ventricle. In sagittal sections, the hypothalamus extends from the optic chiasm, lamina terminalis and anterior commisure rostrally to the cerebral peduncle and interpeduncular fossa caudally. The cavity of the third ventricle lies in the midline. In coronal section, each of the two walls of the hypothalamus can be divided into four surfaces: a lateral surface contiguous with the thalamus, subthalamus and internal capsule, the latter dividing the hypothalamus from the corpus striatum; a medial surface extending to the wall of the third ventricle, covered by ependymal cells; a superior surface corresponding to the hypothalamic sulcus that separates the hypothalamus from the central mass of the thalamus; and an inferior

surface that is in continuity with the floor of the third ventricle. The external surface of the hypothalamic floor gives rise to a median protuberance called the tuber cinereum (or gray swelling due to the pale bluish color of the blood vessels seen in the post-mortem human brain), whose central part extends anteriorly and downward into a funnel-like process, the infundibulum or median eminence. The infundibulum is in direct continuity with the infudibular stem of the posterior pituitary gland, and together with the pars tuberalis of the anterior pituitary, forms the pituitary stalk. Two additional symmetric eminences, the lateral eminences, corresponding to the most lateral portion of the hypothalamic wall and the post infundibular eminence, as well as the symmetric mammillary bodies, complete the macroscopic morphology of the hypothalamic floor (Lechan and Toni, 2000)Figure 10

Figure 10: Midsaggital section of the human brain (from the XIX century wax collection of human brains at the Museum of the Department of Human Anatomy of the University of Bologna, Italy). The hypothalamus (asterisk) lies above the pituitary gland (cross) (Lechan R.M. and Toni R, 2000)

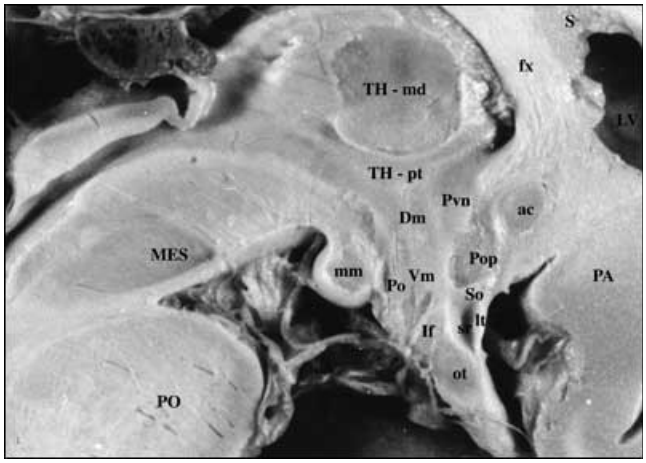


Figure 11 Magnified view of an immersed fixed human brain in Midsaggital orientation. The third ventricle makes up the core of the hypothalamus and extends into the pituitary (or infundibular) stalk, creating the infundibular recess. Many of the major cell groups are located near the midline. These include (from rostral to caudal) the preoptic nucleus (Pop), paraventricular nucleus (Pvn), dorsomedial nucleus (Dm), ventromedial nucleus (Vm), arcuate (or infundibular) nucleus (If), posterior hypothalamic nucleus (Po), and medial mammillary nucleus (mm). Ac = anterior commissure, fx = fornix, lt= lamina terminalis, ot = optic tract and chiasm, Lv = lateral ventricle, MB = midbrain, PN = pons, Sr = supraoptic recess, T = thalamus. (Lechan R.M. and Toni R, 2000)

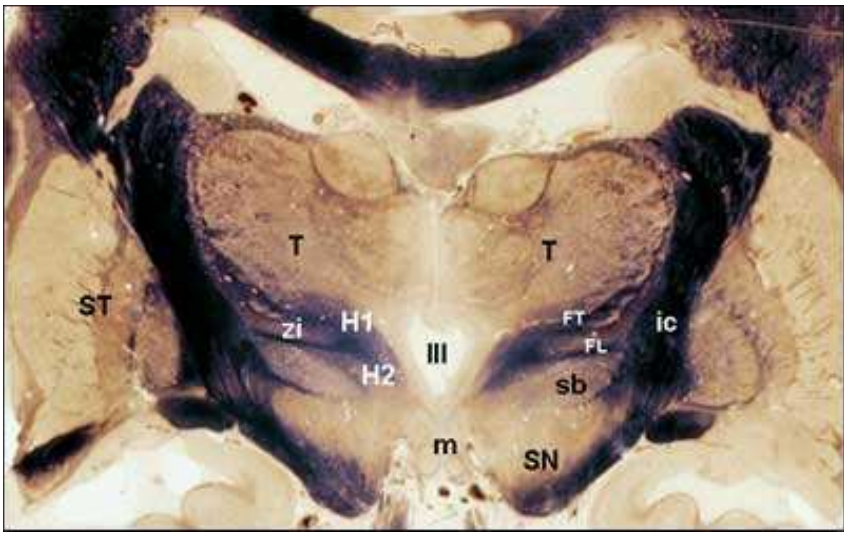


Figure 12. Coronal section of an immersed fixed human brain at the level of the posterior hypothalamus The third ventricle (III) lies in the midline directly above the mammillary bodies (m). The subthalamus (sb), zona incerta (zi) and thalamus (T) are located at the superior border of the hypothalamus, whereas the corpus striatum (ST) is located laterally. FL = fasciculus lenticularis, FT = fasciculus thalamicus, ic = internal capsule, SN = substantia nigra, H1 = field H1 of Forel; H2 = field H2 of Forel. (Toni et al., 2004)

Microscopic Anatomy

Boundaries and Organization of Neuronal Cell Groups

Using phylogenetic and cytoarchitectonic(Swanson, 1987)criteria, a number of nuclear groups and fiber tracts are recognized in the vertebrate hypothalamus. These are organized into three major regions including the ***lateral, medial*** and ***periventricular hypothalamus***, each having distinct morphological and functional features. In the human hypothalamus, the anterior column of the fornix that extends rostro-caudally through the substance of the hypothalamus to end in the mammillary bodies, and the mammillo-thalamic tract that projects from the mammillary bodies upward to the thalamus, create an anatomical boundary that divides the hypothalamus into medial and lateral subdivisions. Contained within the medial subdivision is the periventricular subdivision, a 5-6 cell layer thick nuclear group surrounding the third ventricle that is easily recognized in rodents using standard vital stains, but has less clear anatomical boundaries in the human brain(Toni et al., 2004). Figure 13

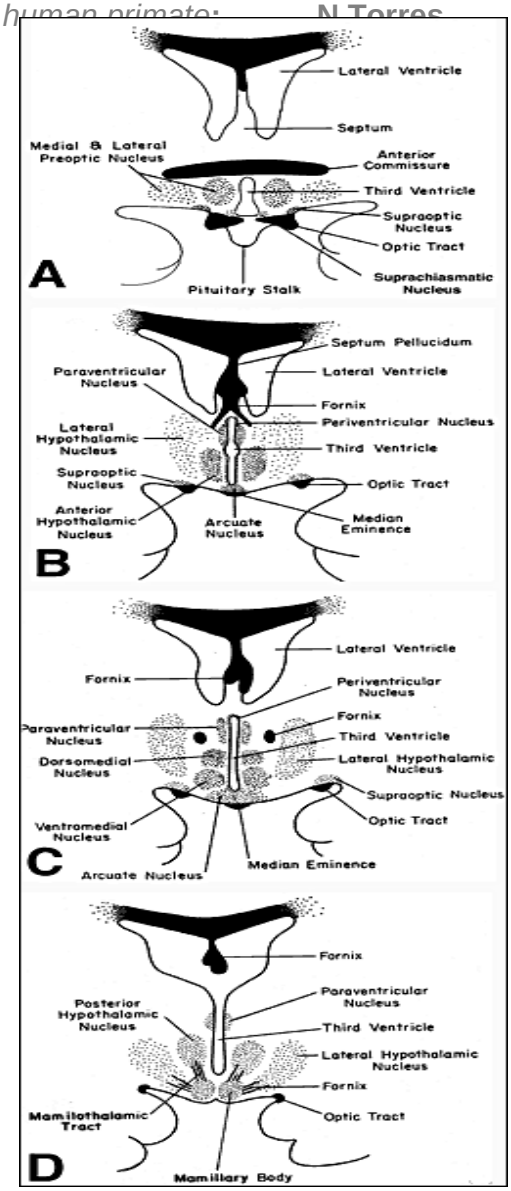


Figure 13: Schematic representation of the human hypothalamus in coronal orientation (A-D: rostral to caudal), demonstrating the location of major nuclear groups. Using the fornix (fx) as an anatomic landmark as it passes through the mid-portion of the hypothalamus on each side of the third ventricle, it is convenient to divide the hypothalamus into medial and periventricular zones (that lie largely medial to the fornix) and a lateral zone (that lies lateral to the fornix). The medial and periventricular zones contain most of the hypothalamic cell groups, and the lateral zone contains relatively fewer neurons. This is because the lateral zone is largely composed of a massive bidirectional fiber pathway - the medial forebrain bundle - that extends through the hypothalamus and interconnects it with the limbic system and brainstem autonomic centers.

Both the ***medial*** and ***periventricular*** subdivisions of the hypothalamus contain a high density of neuronal cell bodies organized into nuclear groups **(Figure 13)** and are crucial for the regulation of the anterior and posterior pituitary gland. The ***medial*** hypothalamus also contains nuclear groups that serve as relay centers for highly differentiated neural information coming from the limbic system and autonomic sensory centers in the brainstem involved in initiation phases of specific homeostatic behaviors such as thirst, hunger, thermoregulation, the sleep-wake cycle, and reproductive behavior(Swanson LW 1987) **(Table 2)**

Table 2 :Major Hypothalamic Cell Groups
PERIVENTRICULAR ZONE
ARCUATE NUCLEUS
PERIVENTRICULAR NUCLEUS
PARAVENTRICULAR NUCLEUS
SUPRACHIASMATIC NUCLEUS
MEDIAL ZONE
ANTERIOR HYPOTHALAMIC NUCLEUS
DORSOMEDIAL NUCLEUS
MAMMILLARY NUCLEUS
MEDIAL PREOPTIC NUCLEUS
POSTERIOR HYPOTHALAMIC NUCLEUS
PREMAMMILLARY NUCLEUS
VENTROMEDIAL NUCLEUS
LATERAL ZONE
LATERAL HYPOTHALAMIC NUCLEUS
LATERAL PREOPTIC NUCLEUS
SUPRAOPTIC NUCLEUS
(Nauta and W., 1969)

The ***lateral*** hypothalamus occupies the largest portion of the hypothalamus by volume. However, it has relatively fewer neurons compared to the medial hypothalamus, and only a limited number of nuclear groups intercalated within a massive fiber system, the ***medial forebrain bundle (MFB)***. It is through this fiber system that information from the medial forebrain (amygdala, hippocampus, septum, olfactory system) and the brainstem is carried to the medial and periventricular hypothalamic subdivisions, delegating an important role to the lateral hypothalamus to influence homeostatic control systems elaborated by the medial hypothalamus. Figure 14 schematically depicts the major interrelations between the periventricular, medial and lateral hypothalamic subdivisions and the rest of the brain.

Each of the three hypothalamic subdivisions can be further divided along the rostral-caudal axis into the: a) ***anterior or chiasmatic region***, extending between the lamina terminalis and the anterior limit of the infudibular recess; b) ***median or tuberal region***, extending between the infudibular recess and the surface of the anterior column of the fornix; and c) ***posterior or mammillary region,*** extending between the anterior column of the fornix and the caudal limit of the mammillary bodies (Lechan R.M. and Toni R, 2000).

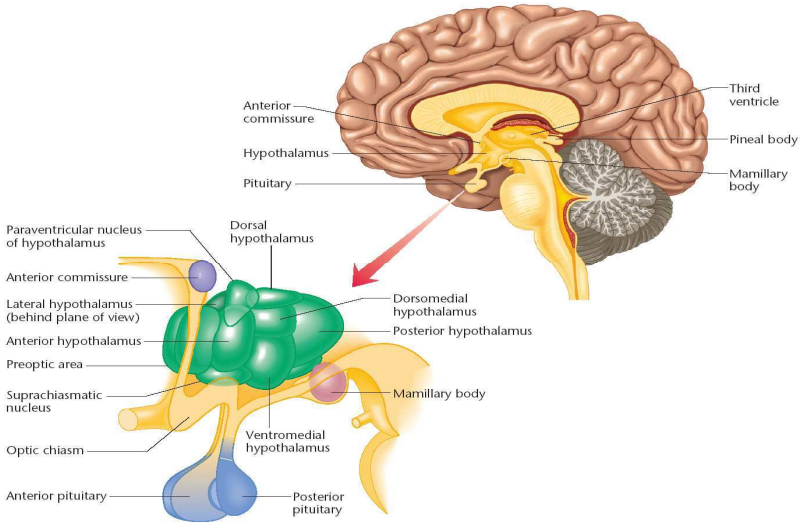


Figure 14: Anatomic distribution of nuclei in human hypothalamus: relative position to the third ventricle

Radiologic Anatomy

Magnetic resonance imaging (MRI) gives remarkable detail of the hypothalamus and thereby, has become the major radiologic tool to assess pathology in this region of the brain. While individual hypothalamic nuclear groups cannot be identified with this technique, some of the major fiber tracts that traverse the hypothalamus can be seen as high intensity signals, particularly in T-2 weighted images (Saeki et al., 2001)These tracts include the fornix and the mammillothalamic tract, shown in Figure 15B,D. Thus, using these fiber pathways as anatomical landmarks, it is possible to radiologically divide the hypothalamus into the two major subdivisions, the medial and lateral

hypothalamic areas. In addition, in the most rostral portions of the hypothalamus, the anterior commisure is readily seen by MRI Figure 15C and increased signal in the lateral hypothalamus is most likely due to the presence of the medial forebrain bundle (Figure 15)

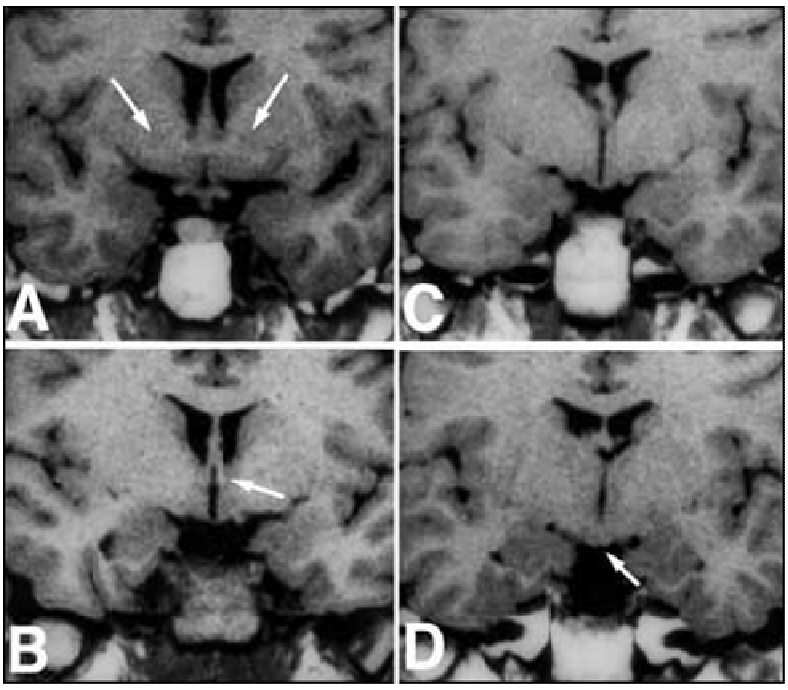


Figure 15 MRI of coronal sections through the hypothalamus. (A) Anterior hypothalamus corresponding to Fig. 10A showing location of the anterior commisure (arrows). (B) Mid hypothalamus corresponding to Fig. 10B showing location of the fornix (arrow). (C) Mid hypothalamus corresponding to Fig. 10C showing the optic tract. The fornix can sometimes also be visualized at this level. (D) Caudal hypothalamus corresponding to Fig. 10D at the level of the medial mammillary bodies (arrow). Sometimes the mammillothalamic tract can be visualized at this level.

Structures Involved In Energy Homeostasis

Several structures have been, from the early days of lesion studies in animals, implicated in energy homeostasis and food intake. Currently research abandoned the idea of "dual centers" limited to the ventromedian portion of the hypothalamus and lateral hypothalamic area Figure 16 **.** Instead several discreet neuronal populations that mediate different effects in food intake/energy homeostasis have been recognized and studied as a part of energy balance circuits:

The ***arcuate nucleus*** (ARC) is situated around the floor of the third ventricle, immediately above the median eminence. The ARC is an elongated collection of cells body covering nearly one half the lengths of the hypothalamus and is apparently divided into several functional domains. For instance, NPY(Ciofi et al., 1988; Leger et al., 1987) and Aglouti related peptide (AGRP), both potent stimulators of food intake, are colocalised in different neuronal population(Chen et al., 1999; Elias et al., 1998) and Melanocyte-stimulating hormone(MSH) and CART, which induce anorexic response are also an adjacent subset of ARC neurons(Baskin et al., 1999b; Vrang et al., 1999). The ARC also has extensive reciprocal connections with other hypothalamic regions, including Paraventricular nucleus (PVN)(Bell et al., 2000), dorsomedial (DMH) and ventromedian (VMH)(Segal et al., 2005; Sternson et al., 2005) hypothalamic nucleus, perifornical and lateral hypothalamic areas(Bai et al., 1985; Baker and Herkenham, 1995; Broberger et al., 1998; Elias et al., 1998; Sawchenko, 1998). Capillaries in the underlying median eminence lack tight junctions, allowing free access to circulating systemic messengers like leptin and insulin to this area(Dallman et al., 1993; Fei et al., 1997; Hakansson et al., 1998; Sipols et al., 1995). These and other signals (e.g glucose)

may also gain access to ARC via diffusion through the ependima layers from Cerebrospinal fluid (CSF) in the third ventricle(Peruzzo et al., 2000).

The ***periventricular nucleus*** (PVN) lies beside the top of the third ventricle in the periventricular zone. The PVN is an integrated centre in which various neural pathways that influence energy homeostasis converge and is supplied by axons projecting from the ARC NPY/ARGP and POMC/CART neurons(Elmquist et al., 1999; Elmquist et al., 1998) and from the orexins neurons from the lateral hypothalamus(Dube et al., 1999). Important neurotransmitters for energy homeostasis, including NPY, MSH, serotonin, galanin, and noradrenalin can be found and modulate the effects in terms of energy expenditure(Williams et al., 2001). The integration of signals within PVN initiates changes in other neuroendocrine systems. NPY/AGRP and melanocortin projections from ARC innervate thyrotropin releasing hormone (TRH) neurons in PVN(Legradi and Lechan, 1998), producing inhibition or stimulatory effects respectively over pro-TRH gene expression(Legradi et al., 1998). Also corticotrophin releasing factor (CRH) is expressed in neurons in the PVN that project to the median eminence and may act to inhibit the NPY neurons of the ARC PVN projection(Huang et al., 1998).

The ***ventromedian*** hypothalamic nucleus (VMH), one the largest nuclei of the hypothalamus, was long considered to be a satiety “center “(A. W. Hetherington, 1944). Stimulation of the VMH inhibits feeding(Beltt and Keesey, 1975; Ruffin and Nicolaidis, 1999), whereas a lesion in this region causes overweight and increases food intake (Hetherington and Ranson, 1940; Stellar, 1954). Recent studies have showed high abundance of leptin receptors (Ob-Rb) and the evidence indicates that this region may be important target for circulating leptin(Meister et al., 1989). The VMH receives

projections from arcuate NPY- , AgRP- and POMC-immunoreactive neurons and in turn VMH neurons project to other hypothalamic nuclei(Sternson et al., 2005) .The VMH has direct connection with PVN, DMV and lateral hypothalamus and to brain stem regions such as the NTS(Saper et al., 1976). Recent work has demonstrated that brain derived neurothophic factor (BDNF) is highly expressed in the VMH. These neurons may form another pathway through which melanocortin system regulates appetite and body weight (Unger et al., 2007; Wang et al., 2007; Xu et al., 2003).

The ***dorsomedian*** hypothalamic nucleus (DMH), located immediately dorsal to VMH, has intensive connections with other hypothalamic nucleus such as PVN, lateral hypothalamus and the brainstem. The VMH and the lateral hypothalamus have no direct connection but connect indirectly throughout the DMH and the PVN(Dai et al., 1998). The PVN and the DMH may function as a functional unit, involved in initiating and maintaining food intake(Christophe, 1998). The DMH has plentiful insulin receptors(Wilcox et al., 1989) as well as leptin receptors(Schwartz et al., 1996b). Some ARC NPY AGRP (orexigenic action) also connects with DMH(Kalra et al., 1999). Recent published data that have reviewed the function of DMH have signaled an important role of the nucleus in many processes that control both food intake and body weight regulation(Bellinger and Bernardis, 2002).

The ***lateral hypothalamic area (LHA)*** is a vaguely defined region and comprises a large, diffuse population of neurons including subpopulation that express orexins and melanin concentrating hormone (MCH) which produce an effect of stimulation food intake. NPY terminals are abundant in the LHA in contact with orexins and MCH

cells(Horvath et al., 1999). The LHA contains neurons expressing melanin concentrating hormone (MCH), which increase food intake and produce mild obesity in rats, while MCH 1 receptor antagonist produce reduction in food intake and sustained reduction in body weight(Elias et al., 1998). The Orexins A and B (or hypocretin 1 and 2) are also produced in LHA and the zone incerta by neurons distinct from those which produce MCH(Broberger et al., 1998). Orexins exert their effect via wide projections throughout the brain, for example PVN, ARC, NTS and dorsal motor nucleus of the vagus(Beck, 2000; Mondal et al., 2000). Orexins neurons project to the areas associated with arousal and attention as well as feeding (orexins knock out mice are thought to be a model of narcolepsy)(Baumann and Bassetti, 2005; Krahn et al., 2001). In circumstance of starvation, the orexins neuropeptides can mediate both arousal response and feeding response in order to initiate food seeking behavior(Tritos et al., 2001). Orexins also may play a role as a peripheral signal involved in energy homeostasis, because they have been identified in the gastrointestinal tract and appear to be activated during starvation(Karteris et al., 2005). The LHA was view classically as a feeding center: stimulation of this nucleus increases food intake, while its destruction attenuates feeding and causes weight loss. This nucleus contains also a large number of glucose sensitive neurons that respond to circulating glucose levels(Williams et al., 2001).

The mechanisms by which the MCH and orexins neurons exert their effect in energy homeostasis have not been fully elucidated. However is clear that major targets are the endocrine and autonomic nervous system, the cranial nerve motor nuclei and cortical structures.

The ***brainstem pathways*** involved in energy homeostasis and food intake modulation have extensive reciprocal connection with the hypothalamus, in particular the ***nucleus of the solitary tract*** (NTS). Like the ARC, the NTS is in close anatomical proximity to a circumventricular organ with an incomplete blood-brain barrier-the ***area postrema***- and is therefore in an ideal position to receiving vagal afferents from the gastrointestinal tract. The NTS has a high density of NPY binding sites and NPY neurons from this region project forward to PVN(Li et al., 1994; Vinuela and Larsen, 2001). There is also evidence that melanocortin system is present in the NTS separate from that of the ARC. POMC derived peptides are synthesized in the NTS and these neurons are activated by feeding(Grill et al., 1998; Hellstrom et al., 2004; Murphy and Bloom, 2005). Figure 17

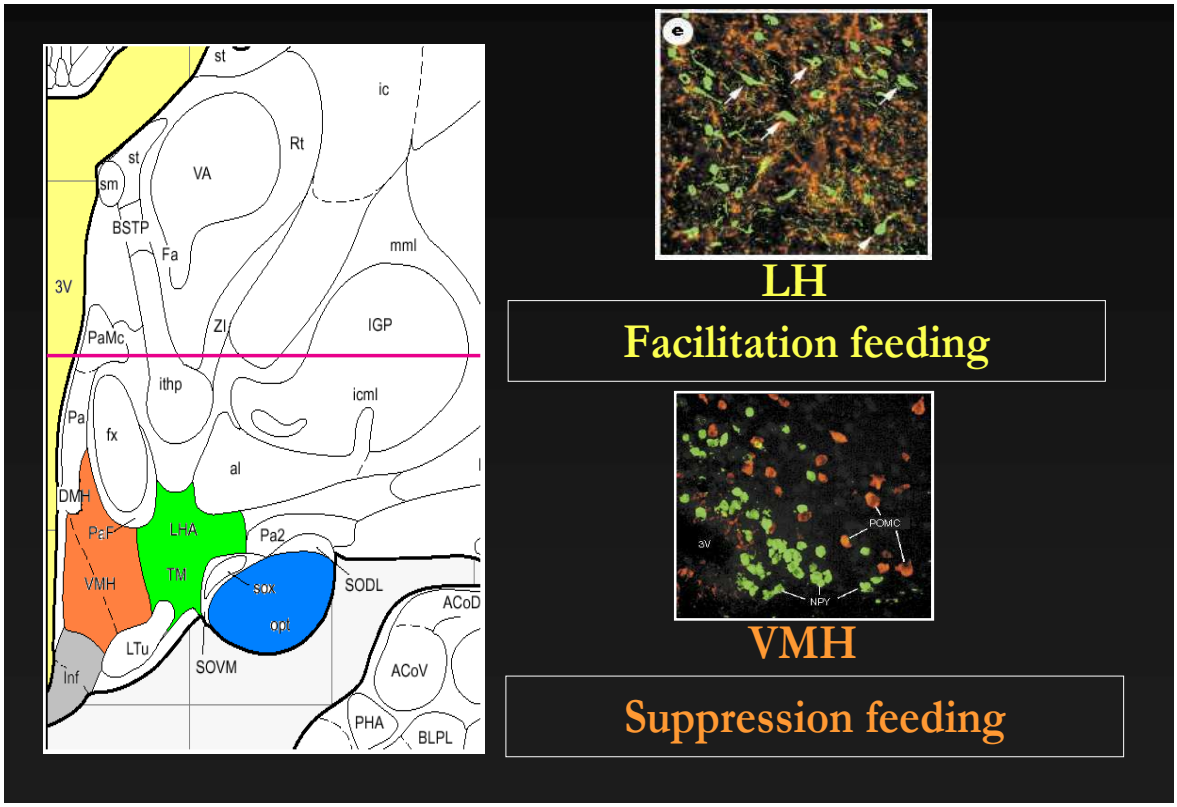


Figure 16: Dual center hypothesis: Hypothalamic ventromedian hypothalamus work as a satiety center whereas Lateral hypothalamus is implicated in hungry and drive to eat, and both nuclei are interconnected and function together. The hypothalamus in coronal orientation shows the location of medial and periventricular zones which contain most of the hypothalamic cell groups, and the lateral zone which contains relatively fewer neurons. This is because the lateral zone is largely composed of a massive bidirectional fiber pathway - the medial forebrain bundle - that extends through the hypothalamus and interconnects it with the limbic system and brainstem autonomic centers.

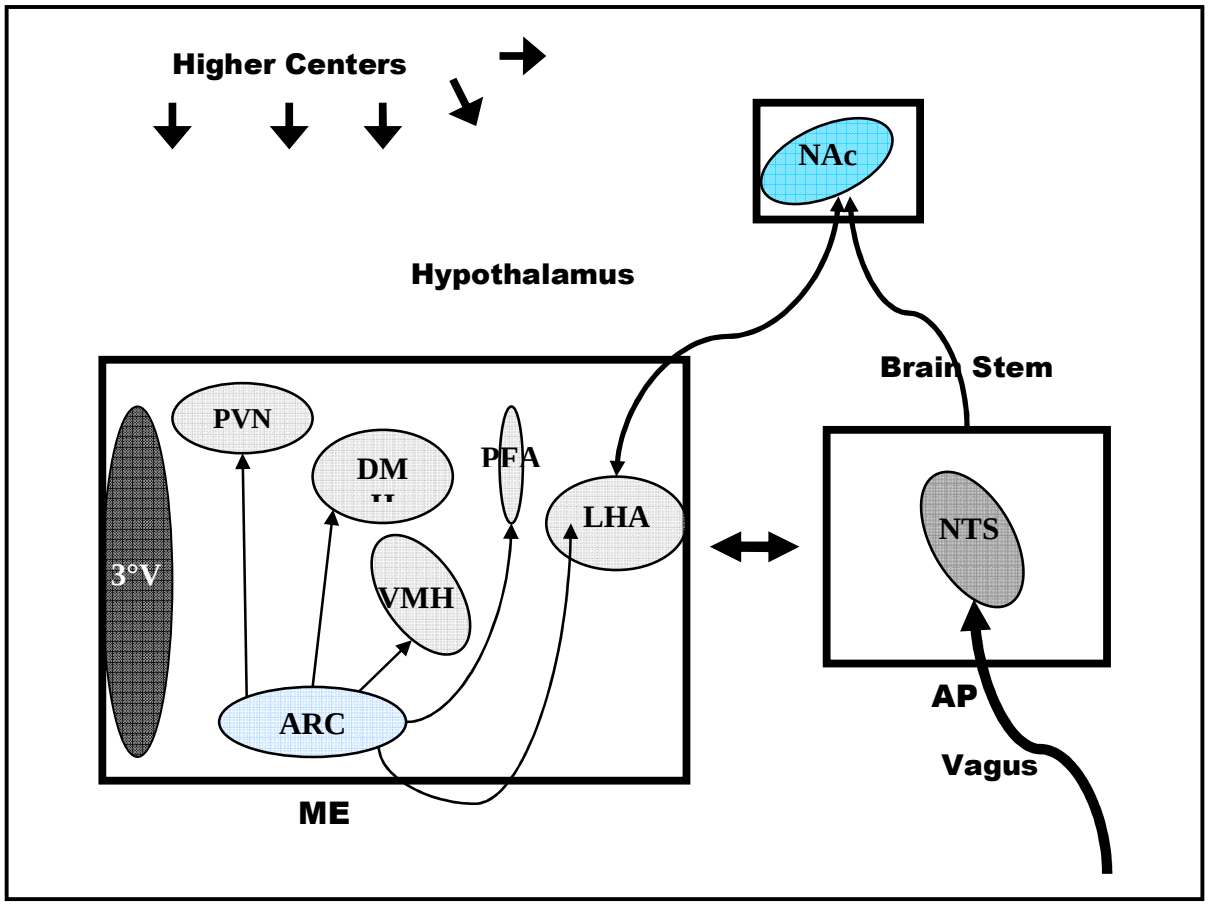


Figure 17: Schematic representation of the central control of the appetite and energy homeostasis. AP Area postrema, ME median eminence, NAc nucleus accumbens PFA prefornical area. There are extensive connections between the hypothalamus and brainstem. The reward circuitry is also connected and involves several signallingg systems. The nucleus accumbens is an important component of the reward circuitry; reciprocal connections between LHA and Nac may mediate hedonistic feeding by dishinibition of the LHA neurons. Peripheral signals contact the ARC and the NTS throughout incomplete brain-blood barrier in ME and AP, acting as a sensor of internal signals (insulin, glucose, leptin etc), which indicate energy state.

The Organization of the Ventromedial Hypothalamic Nucleus

The role of the Ventromedian hypothalamus has changed from the early lesion and stimulation studies, when it was the center of the mechanism in feeding behavior and body weight, to almost disappear from many feeding's researchers vocabulary. The VMH is barely mentioned in some reviews of central control of feeding behavior and in some anatomists' schema of the hypothalamus nuclei involved in feeding. But recently, the VMH area has regained an important place in the models for feeding and weight control in the hypothalamus circuitry(King, 2006)

Early description of the hypothalamus did not always differentiate among cells groups in the medial tuberal area. Malone and Morgan for example labeled the tuberal zone medial to the fornix as the substantia grisea ventriculi tertii. Continued examination of this region as limited some areas, being one of the best defined the VMH. Nissl stained sections show a very lightly staining zone entirely surrounding this nucleus, setting it off from the rest of the hypothalamus. This pale halo, called cell poor area was recognized by Ramon y Cajal to consist of an interweaving array of axons. He called it "the capsule of the nucleus".

VMH Dendrites are long and generally unramified and bear a number of spinous processes. They extend beyond the nucleus into adjacent hypothalamic regions.

Completely encircling the nucleus is a capsule formed partly by VMH afferents. Buried in this capsule are neurons whose dendrites curve along the periphery of the nucleus to form a dendritic grid.

Three general axonal patterns can be recognized. One type is characterized by having numerous collaterals that ramify extensively in VMH. Another variety has few collateral that ramify extensively in VMH. Another variety has little collateral. The third variety has no collaterals.

The numerous intranuclear collaterals with their *bouttons en passant* synaptic interconnect individual VMH units.

Most of the axons are beaded. None of the axonal systems has been found to be confined to VMH. They project to (1) lateral hypothalamus, especially ventral to the plane of the fornix, (2) anterior hypothalamus, (3) towards the zona incerta, perhaps into dorsolateral hypothalamus, (4) caudally, with the medial forebrain bundle, and (5) into the periventricular fiber system (Millhouse, 1978)

PHYSIOLOGY VENTROMEDIAL HYPOTHALAMUS FROHLICH'S SYNDROME AND EARLY EXPERIMENTAL STUDIES

An obesity syndrome in humans that was associated with abnormalities of the basomedial hypothalamus was reported as early as 1840(Mohr, 1840)This was initially believed to be a dysfunction of the pituitary gland and eventually came to be known as Frohlich's syndrome(Babinski, 1900; Fröhlich, 1902)The belief that obesity was due to pituitary dysfunction remained the prevailing view through the mid -1930The first challenge to the ideal that Frohlich's syndrome was due to pituitary abnormalities came from Erdheim who observed that obesity was often in people with tumors at the base of the brain near, but not extending into the pituitary(Erdheim, 1904)Later studies showed that hypophysectomy did not result in obesity unless there was additional damage to the basomedial hypothalamus;(Aschner, 1912; Camus and Roussy, 1913) (Camus and

Roussy, 1920; Camus and Roussy, 1922).Finally, Bailey and Bremer (Bailey and Bremer, 1921)in dogs and Hetherington (Hetherington and Ranson, 1942)using rats, similarly reported that basomedial hypothalamic lesions produced obesity, whereas hypophysectomy without additional hypothalamic damage did not. One of the first researchers to use the adaptation of the Horsley-Clarke Stereotaxic instrument for the use with rats was Albert Hetherington, who in a series of studies examined the effects on body weight of lesions placed throughout the hypothalamus(A. W. Hetherington, 1942). Obesity result when there was damage to the VMH, particularly when the lesion destroyed "the capsule of tissue immediately surrounding that nucleus, especially on its lateral and ventrolateral aspect". Brobeck (Brobeck, 1946)and Kennedy (Kennedy, 1950)particularly observed that small lesion placed on the ventrolateral borders of the VMH and extending to the base of the brain is singularly effective for evoking weight gain. Anand and Brobeck (Anand and Brobeck, 1951a)later reported that obesity could be produced by either VMH lesion or by small lesions just lateral to the VMH. In addition to the VMH lesions, Hetherington and Ramson also observed obesity with lesions posterior to the VMH dorsolateral to the mammillary body, which they attributed to an interruption of descending fibers(A. W. Hetherington, 1942). According to Stellar, the degree of hyperphagia as measured by overeating, rate of weight gain, and final weight gain was related to the amount of damage to the ventromedial nuclei, bilaterally(Graff and Stellar, 1962). Keesey and Ferguson found a direct correlation between size of VMH Lesion and post lesion weight gain(Ferguson and Keesey, 1971).

HYPERPHAGIA

Brobeck in 1943 attributed the obesity to hyperphagia. Rats with VMH lesions were describe as voracious and ravenous during the first several days after surgery, eating two or three times more food as normal(Brobeck, 1946; Brobeck et al., 1943) Brooks even noted that some rats would begin eating before they had fully recovery from the anesthesia (e.g. before they could stand up), often resulting in them choking to death (Brooks and Lambert, 1946). Rats with VMH lesions eat continuously for several hours after the lesion(Balagura and Devenport, 1970; Becker and Kissileff, 1974; Harrell and Remley, 1973) Studies that used chronically implanted electrodes or injection of procaine into the VMH in conscious animals generally found that the overeating began almost immediately after the lesion or injections (Balagura and Devenport, 1970; Becker and Kissileff, 1974; Berthoud and Jeanrenaud, 1979b; Epstein, 1960; Larkin, 1975; Maes, 1980).The feeding pattern is characterized not only by an increased frequency in meals, but by an even greater increase in meal size(King and Gaston, 1973; Teitelbaum and Campbell, 1958). However, when fed ad libitum, hyperphagic rats do not eat faster than normal animals(Sclafani, 1994). There are also large increases in food intake during the day, when normally they eat their daily food intake at night(Kakolewski et al., 1971). The hyperphagia and weight gained that follows complete bilateral VMH is quite dramatic. In just 30 days is not uncommon that the weight gains reach double of the body weight(King and Gaston, 1977). Brooks and Lambert coined the term of static and dynamic phases to describe the pattern of hyperphagia and weight gain observed after VMH lesions. They also noted that some animals did not reach a static phase and continued to gain weight for months (Brooks and Lambert, 1946).VMH lesion induced hyperphagia and obesity have been observed in a variety of species besides rats

[rabbits(Romaniuk, 1962), cats(Anand and Dua, 1955), dogs(Rozkowska and Fonberg, 1971) pigs(Khalaf, 1969), monkeys(Brooks et al., 1942), even ruminants(Baile et al., 1967)). Hypothalamic obesity has been well documented in human as well(Bray and Gallagher, 1975; Powley, 1977).

All of this evidence convinced Kennedy in 1950 to propose that the VMH was the brain's satiety center, that when activated, inhibited feeding behavior(Kennedy, 1950). When Anand and Brobeck reported 1951 that lesions of the lateral hypothalamus (LH) in rats and cats resulted in aphagia and weight loss they designated theLHA as the feeding center, activation of which causes hunger(Anand and Brobeck, 1951b). Anand and others reported that electrical stimulation of the VMH decreased food intake in food deprived animals(Anand and Dua, 1955; Oomura et al., 1967; Wyrwicka and Dobrzecka, 1960), while Brobeck et al observed that intravenous injection of the appetite-depressant drugs Benzedrine or Dexedrine resulted in an increased frequency and amplitude of the EEG in the VMH (the EEG in other parts of the hypothalamus resemble that usually observed during barbiturate anesthesia)(Brobeck et al., 1956). Later studies with cats reported that brief stimulation of the VMH resulted in a decrease in single neuronal spike frequency in the ipsilateralLHA and vice versa when theLHA was stimulated(Oomura et al., 1964; Oomura et al., 1967). In cats, about 73% of the individual VMH Neurons display a frequency related to feeding behavior, as do 63% ofLHA neurons(Oomura et al., 1967). By 1954, the evidence was so strong that led Stellar to conceive the dual-center hypothesis for motivated behavior, where all motivated behaviors (like hunger, sex, thirst etc) were controlled by an excitatory and an inhibitory brain center(Stellar, 1954).

CONTROVERSY REGARDING THE ROLE OF VMH

Serious questions regarding whether or not the VMH was the most effective anatomical site for producing lesion-induced hyperphagia and obesity began to arise in the late 1960s. Several groups failed to produce hyperphagia with lesion limited to the VMH nuclei (Joseph and Knigge, 1968). Studies of the changes incurred in the hypothalamus of mice injected with goldthioglucose found that the changes first occurred ventral to the VMH (including the ARC) and then spread to the VMH(Arees et al., 1969; Caffyn, 1972). When radioactive isotopes were injected intravenously, radioactivity was observed not only in the VMH, but also very heavily in the lateral part of the ARC and in the area between ARC and VMH(Debons et al., 1974). These data demonstrated a clear role for ARC in the lesion induced hypothalamic obesity. The major blow was struck by Gold. Using female rats, he examined the effects on body weight of electrolytic lesions in and around the VMH nuclei. He concluded that: "lesions restricted to VMH produced neither overeating nor obesity. The VMH lesions cause obesity only when they overflow the VMN and the amount of the obesity is proportional to the amount of overflow". Gold claimed that the most effective lesions for producing obesity were rostral to the VMH, and the larger the lesion, the greater the weight gain (as long as the damage does not involve the nigrostriatal dopamine pathway at the lateral border of the hypothalamus). He attributed the obesity observed in rats with lesions just rostral to the VMH to damage to the ***Ventral noradrenergic bundle*** (VNAB)(Gold, 1973). The VNAB originates in the brainstem just caudal to the locus coeruleus and supplies noradrenergic terminals to the midbrain, hypothalamus and a variety of forebrain structures(Ahlskog and Hoebel, 1973; Jacobowitz and Palkovits, 1974; Maeda and Shimizu, 1972; Ungerstedt, 1971). Lesions of the VNAB at the level of the midbrain substantially reduce hypothalamic

norepinephrine (NE) and results in hyperphagia and obesity(Ahlskog, 1974). Parasagittal knife cuts were most effective in producing obesity when they were long and included an area rostral to VMH, the area where he said fibers of the VNAB turn medially to supply the hypothalamus. He then reported that a unilateral lesion of the VNAB in the midbrain or in the mammillary area combined with contralateral parasagittal knife cut rostrolateral to the VMH resulted in hyperphagia and obesity equal to bilateral mammillary lesions or long bilateral parasagittal knife cuts(Gold, 1973; Kapatos and Gold, 1973).

In the early 1980, Gold and other also determined that the optimal site for parasagittal knife cuts to produce hyperphagia and obesity was at the coronal level of the PVN(Aravich and Sclafani, 1983; Leibowitz et al., 1981). The PVN was also found to be the optimal site for electrical stimulation induced eating and also for NE-induced feeding(Atrens and Von, 1972). Finally, Aravich and Sclafani found that lesion in PVN without damage in VMH, resulted in hyperphagia and obesity in rats(Aravich and Sclafani, 1983). These studies replicated earlier reports by Heinbecker that reported PVN lesion-induced obesity in dogs (Heinbecker et al., 1944)

REEVALUATION OF THE ROLE OF THE VMH

Many researchers accepted Gold's conclusion that VMH has nothing to do with feeding behavior. However, the fact of the matter is that Gold's conclusion flew in the face of many previous, equally diligent anatomical studies that compared the effects of lesions in and around the VMH. Hetherington, for instance came to a conclusion that was completely the opposite than that made by Gold: "obesity is not produced by medium size symmetrical lesions of the caudal half of the anterior hypothalamic area and rostral ends of the ventromedial hypothalamic nuclei"(A. W. Hetherington, 1942). According to

Anand and Brobeck, lesion just anterior to the VMH did not lead to hyperphagia and obesity(Anand and Brobeck, 1951a). In seven studies that have examined the effects of bilateral coronal knife cuts anterior to the VMH, five observed only modest weight gains and two observed none(Grossman, 1971; Palka et al., 1969; Paxinos and Bindra, 1972; Rollins et al., 2006; Sclafani, 1971; Storlien and Albert, 1972).

Hetherington initially found that maximum obesity was produced by lesion of the caudal half of the VMH which includes part of the dorsomedial hypothalamic nuclei and premammillary nuclei (Hetherington, 1941)Obesity could be produced by bilateral lesions anywhere within a large longitudinal zone in the hypothalamus(A. W. Hetherington, 1942). Their lesions had not doubt transected the VNAB. But recent data has shown that isolated lesion in VNAB produced hyperphagia and obesity syndromes different than the VMH syndrome(Ahlskog et al., 1975) ((Ahlskog, 1974; Ahlskog et al., 1974; Hoebel, 1976). And lesion in VMH could produce and addictive effect to lesion in VNAB(Ahlskog et al., 1974; Hoebel, 1976)

Several non lesional studies seem to support the role play by VMH in feeding behavior. SF-1 knockout mice, with an abnormal VMH became obese and eventually double their normal body weight(Davis et al., 2004; Majdic et al., 2002; Parker et al., 2002). Fetal VMH transplanted in third ventricle, not only diminish weight gain in VMH lesion rats(Mickley et al., 1987), but diminished weight gain in Zucker obese rats as well(Fukagawa et al., 1996). Studies using Positron emission tomography have found an increase in regional blood flow to the hypothalamus during hunger and a decrease after food intake(Morris and Dolan, 2001; Tataranni et al., 1999). Using functional magnetic resonance imaging, Matsuda et al were able to differentiate among the hypothalamic nuclei. They observed a decrease in both VMH and PVN activity after subjects drank a

glucose solution and the response was smaller and delayed in obese subjects(Liu et al., 2000; Matsuda et al., 1999). More important brain activity recorded in VMH nucleus were specifically reduced by glucose ingestion whereas the response in PVN was non specific (activity also decrease when the subjects drank water(Matsuda et al., 1999)).

Several recent studies have revealed the nature and mechanism of VMH glucoresponsive neurons(de Vries et al., 2003; Kang et al., 2004; Routh, 2003; Song et al., 2001; Song and Routh, 2005). Five types have been identified(Song et al., 2001), which very dynamically respond to hypoglycemia and lactate levels(Routh, 2003) and ghrelin(Chen et al., 2005). Microinjections of orexins into the hypothalamus decrease cytosolic Ca levels in glucosensitive neurons(Muroya et al., 2004). Only VMH, nucleus of the solitary tract, and cortex of the amygdala showed permanent changes in Fos expression when rats were switched from normal diet to high protein diet(Darcel et al., 2005). The PVN and ARC showed changes only during the initial transition period (Darcel et al., 2005. Glucose responsive neurons in VMH might have an integrative function in glucose homeostasis(Routh, 2003).

The role of VMH in feeding behavior could be assessed by injection of a variety of agents into the VMH. Several lines of evidence suggest that histamine decrease food intake via H1 receptors at least in the VMH or the PVN(Morimoto et al., 2001). Intrahypothalamic injection of nicotine reduces food intake and is associated with an increase in leptin biding in the VMH, ARC and PVN(Li and Kane, 2003). Food intake increase 4 fold by injection of thyroid hormone T3 directly into VMH(Kong et al., 2004) , while intra VMH injection of glucagon-like peptide 1(GLP-1), significantly decrease food intake(Schick et al., 2003). Gamma-aminobutyric acid (GABA) is also heavily involved in VMH function. Electrophoretic injections of GABA depress the firing rate of almost all

VMH neurons(Dreifuss and Matthews, 1972), and injections of GABA agonist like muscimol, into VMH markedly elevates food intake in dose dependent manner(Kelly et al., 1979).

POSSIBLE PATHWAYS INVOLVED IN VMH LESION INDUCED HYPERPHAGIA AND OBESITY

Researchers in the middle of the 80s established that PVN has major connection with key gustatory relay stations, including the dorsal vagal complex, the nucleus of solitary tract, and parabrachial area(Luiten et al., 1987; Moga et al., 1990; Sawchenko and Swanson, 1981). The discovery of the importance of ***the connection between ARC and PVN*** in feeding behavior produced a profound shift and revision of the role of VMH in the feeding behavior physiology(Broberger and Hokfelt, 2001; Schwartz et al., 2000). Even more specific, the apparent lack of significant connections of VMH(Chi, 1970) contributed to many researchers became skeptical about VMH playing any role in feeding behavior. Early studies conducted by Millhouse gave the impression that VMH was an isolated structure (Millhouse, 1973b).It was Millhouse who first described the fiber capsule that surrounds the VMH nucleus; This ***capsule*** is made up of axons originating from the stria terminalis, mammillary peduncle, medial forebrain bundle,LHA, zona incerta, supraoptic decussation and periventricular neurons. Although the afferent terminate in the capsule, some collaterals penetrate into the nucleus itself. The capsule contains also some neurons that along with their dendrites form a “dendritic grid” surrounding the nucleus. These dendrites extend beyond the nucleus into adjacent hypothalamic regions. The axons projects to theLHA, anterior hypothalamus, zona incerta, the medial forebrain bundle caudally and the periventricular fiber system(Millhouse, 1973a; Millhouse, 1973b)

It was with the use of the autoradiographic methods, that Saper et al. provided detailed description of VMH efferents (Saper et al., 1976). They found that VMH axons projects extensively to surrounding hypothalamic nucleus (but not median eminence). Studies using Phaseolus vulgaris leucoagglutinin, later confirmed that the VMH established massive intrahypothalamic terminal fields in other parts of the medial zone but not the nuclei of the periventricular and lateral zones(Canteras et al., 1994). Most of the ***VMH efferents*** originate from the ventrolateral part of the VMH(Saper et al., 1976). Anterior targets of long VMH efferents include the preoptic areas, ventral part of the lateral septum, bed nucleus of the stria terminalis and amygdala (dorsal part of the medial nucleus and the capsule of the central nucleus). VMH also sends dense projections to the subparafascicular nucleus of the thalamus, a target for nociceptive, visceral and accessory gustatory stimuli from the parabrachial nucleus(Bester et al., 1999; Canteras et al., 1994; Cechetto and Saper, 1987; Yasui et al., 1991). Kita an Oomura used HRP injections into VMH and reported labeled cells in the amygdala (medial and basolateral nuclei), subiculum peripeduncular nucleus and parabrachial area(Kita and Oomura, 1982).

Targets of ***descending VMH axons*** include (a) the mammillary complex, supramamillary nucleus and ventral tegmental area via the medial hypothalamus and ventral tegmental bundle; (b) posterior hypothalamic area and central gray (including anterior pole of the locus ceruleus) via a periventricular route; and (c) contralateral amygdala and zona incerta, central tegmental fields and peripeduncular nucleus via the supraoptic commissure. The ***VMH afferents*** has been studied by Fahrbach using HRP technique.(Fahrbach et al., 1989). The ***septal area***, medial preoptic area, rostralLHA,

and ventral subiculum project mainly to the ventrolateral VMH, whereas the ***median amygdala*** projects to the VMH as well as to the area ventral to the VMH.

Several studies have shown that the VMH-input from the amygdala and bed nucleus of the stria terminalis (particularly the posterior division) is extensive(Dong and Swanson, 2004; Krettek and Price, 1978; Petrovich et al., 2001). Lesion in of the medial amigdaloid nucleus results in extensive degeneration throughout the capsule of the VMH(Krettek and Price, 1978). Small bilateral lesions limited to the most posterodorsal aspects of the medial amygdala results in hyperphagia and obesity in female rats, with weights gains of as much as 100g in 20 days(King et al., 1993a; King et al., 1993b; King et al., 2003b). Abundant literature exists that demonstrate that medial amygdala and VMH are part of an ipsilateral pathway regulating feeding behavior and body weight regulation(Grundmann et al., 2005; King et al., 2003a; Rollins et al., 2006). It remains to be determined however what type of information is transported from the median amygdala to the VMH to affect feeding behavior. It is most certainly different from the information conveyed by the vagus nerve and blood to the ARC and VMH. The pattern of anterograde degeneration observed after amigdaloid lesions (King et al., 2003a)is remarkably parallel to pathways involved in sexual behavior. The VMH, in addition to its role in feeding behavior/metabolism, has numerous terminals mediating sexual behavior(Canteras et al., 1994). Similar to the VMH, the amygdala has numerous estrogen receptors (Li et al., 1993; Pfaff and Keiner, 1973)and the fos inmunoreactivity in the posterodorsal amygdala is highly correlated with satiation sexual activity(Coolen et al., 1996; Coolen et al., 1997). Olfactory input is thought to be critical in this VMH-median amygdala connection, regulating sexual behavior and modulator of food intake

as well. Olfactory bulbectomy in static phase VMH lesioned rats results in an additional stage of hyperphagia and excessive weight gain(Larue and Le Magnen, 1970).

VMH has a large number of glucoresponsive neurons and neurons responsive to neurotransmitters involved in feeding behavior. VMH has numerous leptin receptors, particularly in the dorsomedial portion(Baskin et al., 1999a; Elmquist et al., 1997; Elmquist et al., 1998; Hakansson et al., 1998; Schwartz et al., 1996b). Many of these neurons are also glucoresponsive(Shiraishi et al., 1999). Infusion of leptin in PVN or VMH markedly decreases food intake and body weight and this effect can be countered by pretreatment with corticotrophin releasing hormone antagonist(Masaki et al., 2003). NPY, when injected in VMH, produce a strong feeding response(Stanley et al., 1985). Cholecystokinine afferents from parabrachial nucleus to the VMH are abundant(Bester et al., 1997; Fulwiler and Saper, 1985)and has been linked with the central inhibition of food intake by this element(Takaki et al., 1990). By far, however, the best understood of the neuropeptides with regards to their role in the VMH are the Melanocortins. Receptors MC 3 and MC 4 of the anorexigenic POMC derivative α-MSH are found in VMH(Gantz et al., 1993; Lindblom et al., 1998; Mountjoy et al., 1994; Roselli-Rehfuss et al., 1993). The Melanocortins effectiveness on neuronal activity of the VMH is reduced during starvation, reducing sympathetic stimulation and the satiety signal(Li and Davidowa, 2004). The question remained as to how MC4 receptors regulated energy balance. And the answer appears to be brain derived neurothophic factor (BDNF) a neurotrophin that is critically important for brain development and neuronal plasticity(Hofer and Barde, 1988) but which is involved in energy homeostasis in mammals postnatally. Removal of the BDNF gene (Rios et al., 2001)or marked

reduction in the BDNF receptor in mice(Xu et al., 2003) results in hyperphagia and obesity. BDNF mRNA is expressed at high levels in the VMH and decrease during fasting. This was not found in other hypothalamic or extrahypothalamic nuclei(Xu et al., 2003). Obesity model SF 1 knockout mice have a complete loss of VMH BDNF(Tran et al., 2003). MC4 receptor antagonist injected intracerebroventriculary reversed the effects of fasting on VMH BDNF m RNA Levels, therefore demonstrating that BDNF operates via melanocortin signallingg(Butler and Cone, 2002). Wisse and Schwartz conclude that POMC neurons reduce food intake via activation of BDNF neurons in the VMH (Figure 18), integrating definitively VMH into food ingestion and weigh regulation mechanism.(Wisse and Schwartz, 2003)

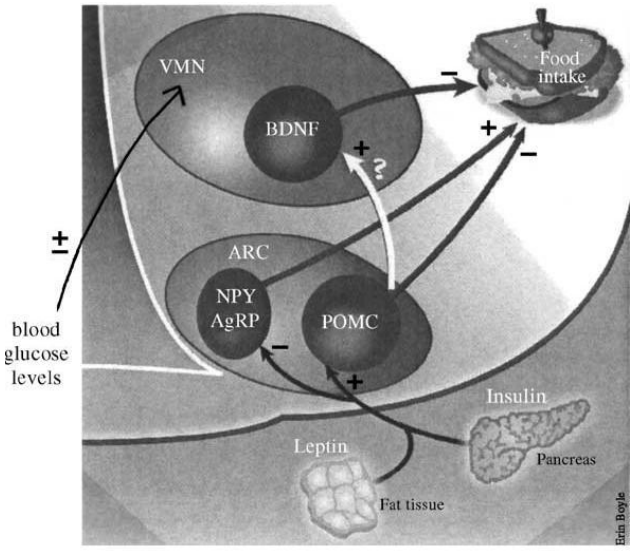


Figure 18: Diagram showing the interactions between VMN (or VMH) and alimentary behavior. Arcuate nucleus has apparently an influence over VMN via BDNF. Humoral factors have "sensors" in both nuclei, indicating metabolical states. Please note the close relationship with the ventricle (modified from Schwartz)

CONCLUSIONS REGARDING THE ROLE OF VMH IN FOOD INTAKE AND WEIGHT CONTROL

There is abundant evidence that VMH helps regulate autonomic responses, both parasympathetic and sympathetic and that lesions of the VMH results, at least in part, in a "metabolic obesity" independent of hyperphagia, as originally proposed by Powley(Powley, 1977). This is supported by studies in pair fed tube rats with VMH lesions(Han, 1968) and more recently with knockout mice that are deficient in the orphan nuclear receptor SF-1(Davis et al., 2004). Changes in autonomic responses can be detected within 20 minutes of lesioning the VMH in anesthetized rats (Berthoud and Jeanrenaud, 1979a).

Many studies have also demonstrated that VMH lesions induced obesity, particularly in adult female rats, cannot be due entirely to altered autonomic responses(Sakaguchi et al., 1988). Recent neuroimages studies have demonstrated that neurons in the immediate areas of both the VMH and PVN are activated during feeding, but are different in function(Matsuda et al., 1999). VMH has many receptors that respond to dopamine(Davidowa et al., 2002), serotonin(Hikiji et al., 2004), GABA(Dellovade et al., 2001), histamine(Magrani et al., 2004) and estrogen(Pfaff and Keiner, 1973; Wade and Zucker, 1970) to affect feeding behavior. The response to these receptors has been found to be abnormal in obese animals that were overfed since birth(Davidowa et al., 2002; Huang et al., 2004a).(Huang et al., 2004b; Huang et al., 2005)

VMH has neural connection with many other areas of the brain implicated in feeding behavior. The dorsomedial portion of the nucleus is particularly dense with neurons that

respond to feeding related stimuli, while the capsule is critical in receiving afferent information. Most efferent exits from ventrolateral part of VMH to extrahypothalamic location(Saper et al., 1976). VMH also has a massive terminal field throughout medial hypothalamus(Canteras et al., 1994; Millhouse, 1973b).

In recent years an inhibitory feeding pathway between the medial amygdala and VMH possibly carrying olfactory information has been identified and extensively studied(Coscina et al., 2000; Grundmann et al., 2005; King et al., 2003a; King et al., 1993b; Rollins and King, 2000). Melanocortins receptors within VMH have been found to play a critical role in the regulation of feeding behavior(Bagnol et al., 1999; Elias et al., 1998; Gantz et al., 1993; Harrold et al., 1999; Haskell-Luevano et al., 1999). Food intake decrease when ARC POMC neurons activate VMH BDNF neurons(Wisse et al., 2006; Wisse and Schwartz, 2001).

In summary, the dual centre hypothesis(Stellar, 1954) has give place to a hypothesis of discreet neuronal pathways that generates integrated responses to afferent input related to changing body fuel stores.

The causes of hypothalamic obesity are certainly numerous, but today, we can identify a few of the major causes:

1. A primary metabolic effect of VMH damage due to alterations in both parasympathetic and sympathetic functional
2. A primary effect on feeding behavior due to damage to ARC POMC neurons to the VMH and or damage to VMH BDNF neurons

CHAPTER IV NEUROSTIMULATION OF CENTRAL NUCLEI IN THE BRAIN

Electrical stimulation of the nervous system for therapeutics purposes dates back to the 18th century. Floyer used electrical shocks in an attempt to reverse blindness, Jallabert used spark to treat arm paresis and Kite reported revived the drowned with electrical shock. During the last 15 years, neurosurgery has been greatly influenced by deep brain stimulation .More recently, with the development of new hardware and electrophysiological techniques, deep brain electrical stimulation(DBS) have been routinely used for treating movement disorder(Benabid et al., 1998)), pain(Levy, 2003) and lately obsessive compulsive disorders (Gabriels et al., 2003) and clinical refractory epilepsy (Benabid et al., 2002b) With this tool is now possible to explore new targets and extend therapeutically available options.

Physiological Principles of Neurostimulation

Neuropharmacology limitations have lead to the development of alternative ways of dealing with brain circuits. Clinicians commonly use pharmaceutical agents that modulate neural processes in order to treat disorders of the central nervous system (CNS). Most drugs used to treat neurological disorders do so by affecting synaptic transmission. However, they may not be specific in their action and can cause modulation in other neural circuits not involved in the initial disorder. Often this leads to unintended neural side effects. Electrical stimulation of the nervous system is an alternative way to modulate the subcircuits of CNS with the possibility of greater specificity (Rise, 2004)

Nerve cells convey information from one part of the CNS to another through electrical phenomena. An action potential is a transient reversal of the transmembrane voltage potential of a nerve cell axon. The action potential propagates along the nerve cell membrane. Neurostimulation affects the CNS through the creation of a voltage in the neighborhood of a specific circuit of the nervous system artificially manipulating membrane voltages. Manipulation of the membrane potential through the use of the neurostimulator can cause nerve cells to propagate action potentials orthodromically and antidromically along the axon. Alternatively appropriately applied voltages can block the propagation of action potentials. Thus, the use of implantable extracellular electrodes connected to neurostimulators to modulate the activity in selected pathways of the CNS can be palliative treatment for central controlled disorders (Rise, 2004)

Biophysics of Neurostimulation

Neurostimulation can be thought of as being a tool for treating neurological dysfunction. The particular therapeutic application will determine which part of the nervous system is activated or deactivated by stimulation. There are some basic principles associated with the use of neurostimulation to aid the clinician in predicting the effect of using different kinds of stimulation settings in a safe manner with the desired outcome.

Reduced to its simplest form, a neurostimulator consist of a power supply (i.e. battery), a pair of electrodes in contact with the tissue, extension wires to connect the electrodes to the battery and a switch that enables the power to be intermittently connected to the electrodes. Ohm's law governs the relationship between voltage and current. Much of the basic understanding of the nerve cell electrophysiology was discovered as results of experiences carried out with intracellular electrodes referenced to electrodes in the

extracellular space. Neurostimulators used as a neuromodulators, however, make use of extracellular electrodes to generate voltage/current fields.

By convention, there are two types of electrode configuration, referred as monopolar and bipolar stimulation. Of course, for electricity to flow is necessary that there be two electrodes, a positive anode and a negative cathode. Monopolar stimulation then refers to an electrode configuration that includes an electrode of relatively small surface area located near or in the nervous tissue to be stimulated. This electrode is typically the negative electrode or cathode, for reasons described below. The positive electrode has a larger surface area and is located remote to the stimulation target. Typically, the outside surface area or 'case' of the neurostimulator is used as a positive anode when performing monopolar stimulation. When performing bipolar stimulation, both positive and negative electrodes are in or near the nervous tissue targeted for stimulation and have the same or similar surface areas (Rowbottom and C., 1984).

Neuromodulation is performed by applying intermittent, electrical stimulation to surrounding neural tissue. The electrical fields generated by DBS electrodes, using therapeutic stimulation parameters, is capable of directly activating a large volume of tissue. Extracellular stimulation generates a complex electric field in the tissue medium that is applied to the underlying neural process as a distribution of extracellular potentials. For that motive, analysis of the effects of DBS is complicated by our limited understanding of the response of neurons surrounding the electrode to the applied fields. The Extracellular potentials along with each stimulating electrode will produce both transmembrane and axial currents that will be distributed throughout the neuron. Each neuron exposed to the applied fields will experience both inward and outward transmembrane currents and regions of depolarization and hyperpolarization. These

theoretical predictions have been verified in numerous experimental preparations demonstration the difference between anodic, cathodic, and bipolar stimulation on the ability to both activate and block neural activity with Extracellular stimulation (McIntyre and Grill, 2002).Despite the difficulties explaining the mechanism of action of Extracellular potential in DBS some general rules or guidelines can be summarized: a) nerve cells further away from the electrode will be less likely to be stimulated, b) axons will be stimulated at lower stimulation amplitudes than nerve cells bodies, c) larger axons will respond to lower stimulus amplitudes than smaller axons, d) axons with branching processes will be more easily stimulated than those without branching. Also a key point is the orientation of nerve cells relative to voltage fields. The important parameter of the voltage determining whether a nerve cell is stimulated is the second spatial derivative of voltage. The value of the second derivative falls off rapidly with distance from the surface of the electrode. Also nerve cells that are oriented along isopotential lines may not be activated while nerve axons in the direction of the voltage gradient will be preferentially stimulated. Orientation of cell body and axons with respect to current flow is important. For an axon it is the component of the voltage gradient parallel to the fibers that is important. The pia has a significant resistance and capacitance. Gray matter, white matter, and cerebrospinal fluid have different resistivities, which affect patterns of current flow. Finally, the nerve cells near the cathode (negative) will be more easily activated than those near the anode (positive) (Ranck, 1975).

The effect of DBS on the various neural elements depends on the nonlinear relationship between the stimulus duration (pulse width) and the amplitude (voltage or current) that is necessary to stimulate the neural element.

DEEP BRAIN STIMULATION

DEFINITION

Excitability due to electrical stimulation was well known and applied in clinical settings as far as 1950. However, the term 'deep brain stimulation' (DBS) dates back to the 1970s.The original therapy that lead to the development of DBS technology was electrical stimulation of sensory thalamus to treat chronic pain (Hosobuchi, 1986). Low frequency stimulation (LFS) (30-60 hertz) was applied in pain patients producing excitation in target areas (Hosobuchi et al., 1977; Richardson and Akil, 1977b; Richardson and Akil, 1977c). Patients with certain motors deficits (Liberson and Pavasars, 1960) neurogenic sphincter disorders(Brindley, 1977), spasticity and epilepsy (Cooper et al., 1973) were also successfully treated with LFS . But brain lesioning procedures were irreplaceable until the emerging concept of high frequency stimulation as a producer of functional neuronal inhibition was not recognized.

High frequency DBS provides functional inhibition of target structures producing significant improvement in Parkinson symptoms in both animal and human studies (Benabid et al., 1998; Fang et al., 2006)and mimicking the clinical effects of tissue lesioning (Benabid et al., 1987; Bergman et al., 1990). As a consequence, well known neurosurgical or basic research lesional targets can now be easily explored and studied. There is also a recent exciting return to LFS due to a better understanding of electrical stimulation, better available equipment and medical team expertise as shown with the promising results in the treatment of non dopaminergic symptoms of Parkinson disease using DBS LFS in the pedunculo pontine nucleus (Mazzone et al., 2005; Stefani et al., 2007)The success of DBS in multiple neurological conditions as well as its capacity of

producing diverse effects in target structures made it the ideal tool to consider for application in morbid obesity. Some of the landmarks in DBS history are shown in Table 3 .

Some characteristics of this method explain its popularity. DBS parameters can be adjusted to a proper threshold avoiding secondary effects from surrounding structures (Blomstedt and Hariz, 2006). In contrast to Stereotaxic lesion, DBS provides the added benefit of reversibility and titratability (Deuschl et al., 2006) diminishing the risk of causing major permanent neurological deficits. DBS is versatile; because while the underlying mechanisms of electrical brain stimulation are only scarcely known (Benabid et al., 2005a; Fraix et al., 2004)when appropriate parameters are chosen, DBS may either excite or inhibit neural structures, providing a wide range of potential clinical applications. The recognition of the frequency as a key factor for modulation of CNS structures allowed the apparition of a large subset of applications and predicts an even larger emerging field of DBS in the future (Benabid et al., 2005b). Modulation of hypothalamic structures represents an exciting new objective in translational research of DBS.

DEEP BRAIN STIMULATION OF THE HYPOTHALAMUS

The imbalance between food intake and energy expenditure leads to weight gain in individuals with weak central (hypothalamic) recognition of peripheral signals (Leptin, ghrelin, glucose or insulin)(Heini et al., 1998) Activation of these centers throughout LFS DBS or inhibition (HFS DBS) of overacting areas may change the body weigh “set point” in intractable morbid obese patients (Benabid et al., 2005a; Benabid et al., 2005b). Here we review the potential targets for DBS in the hypothalamus.

PUTATIVE TARGETS

LATERAL HYPOTHALAMUS

The LHA has long been implicated in feeding behavior and energy expenditure (Bernardis and Bellinger, 1996).Its role in appetite regulation is well described in early studies of LHA lesions, which induced leanness). In 1951, Anad and Brobeck, shown a diencephalic center for food intake in the hypothalamus: Bilateral lesions of the lateral hypothalamus provoked weight lose with aphagia (Anand and Brobeck, 1951b)This impact on appetite can be partially explained by peptides expressed predominantly in the LHA such as melanin concentrating hormone (MCH) and orexins. Indeed, MCH -/- mice are lean and hyperphagic, while mice with over-expression of MCH are obese and insulin resistant (Ludwig et al., 2001)Chronic administration of an orally active selective MCH 1 receptor antagonist decrease food intake, body weight and adiposity in rodent obesity models(McBriar et al., 2006)Injections of orexins into LHA increases feeding behavior and enhances arousal, and up regulated expression of the orexins gene occurs in fasting rats (Kotz et al., 2002). Electrical stimulation of the LHA induced a pronounced bout of eating in previously satiated cats, and electrical stimulation VMH caused food deprived cats to stop eating(Anand et al., 1955). Here also a metabolic effect is suggested by Teitelbaum and Stellar, because theirs rats were able to keep a low weight and regain normal food intake after bilateral lesion of LHA(Teitelbaum and Epstein, 1962). Low frequency stimulation of LHA produced ingesting behavior. Several biochemical and neurophysiological studies have shown activation of LHA neurons during fast period .Recently, Ruffin and Nicolaidis have confirmed that the metabolic response is previous to behavioral changes in food intake(Ruffin et al., 1995).

Given this evidence, it was proposed that chronic bilateral DBS of LHA would mimic the results of lesion studies, just as STN-DBS mimics the effects of subthalamotomy (Andy et al., 1963)providing significant and sustained weight loss in obese rats (Sani et al., 2007).In the study, on post operative day 24, 13 % of total body weight was lost in rats maintained on seven day high fat diet undergoing LHA-DBS, a difference that was significant compared to controls.

VENTROMEDIAL HYPOTHALAMUS

Lesions of the VMH induce obesity (Brobeck, 1963) In 1940, Hetherington and Ramson published that the bilateral lesion of the ventro-median region of the hypothalamus produced hyperphagia followed by obesity with normophagia (A. W. Hetherington, 1944; Kennedy, 1950). Like LHA VMH has also been implicated in appetite regulation, as well as maintaining energy homeostasis VMH lesions results in substantially more carcass lipid and hyperinsulinemia in rats even if pair-fed with ham-lesion controls, suggesting a metabolic bias towards obesity (Cox and Powley, 1981)In contrast to electrolytic lesioning, electrical stimulation of VMH at slight intensities (20-25 uA) suppresses feeding in rats and increases their metabolic rate (Ruffin 1999). Beltt and Keesey had shown earlier (1975) that VMH low frequency-stimulation was susceptible of inhibit food intake (Beltt and Keesey, 1975). Pauwson in 1988 have also suggested that a metabolic effect is probable in low frequency stimulation of VMH, leading to a chronic decrease in weight without change in food intake behavior (Pauwson 1988). This increase in energy expenditure was associated with increased fat oxidation given a concomitant drop in the respiratory quotient. Thus, heightened metabolism induced by VMH stimulation is sustained by utilization of fat stores via the lipolytic pathway and is most likely due to noradrenergic turnover(Saito et al., 1987). Recently, VMH DBS in rat at four different

frequencies (25, 50, 75 and 100 Hz) replicated this effect on energy expenditure, demonstrating an increase in metabolism indicated by indirect calorimetry (Covalin et al., 2005) There was a trend towards an indirect relationship between energy expenditure and increase frequencies, suggesting lower frequencies have stimulating effects (Benabid 2006 Personal communication) (McIntyre and Grill, 2002)

In brief, VMH/LHA critical involvement in feeding regulation can be summarized by:

1) The suppression and facilitation of feeding regulation by electrical stimulation of the VMH and the LHA respectively,

2) The hyperphagia and hypophagia after bilateral electrolytic ablations as well as bilateral chemical lesions of the VMH and LHA respectively.

3) The presence of neurons in both nucleuses that sense the metabolic signals such a glucose, free fatty acid and leptin.(Dhillon et al., 2006).

4) A metabolic effect plays a role in weight loss following electrical stimulation and it is most likely to be related to an increase in autonomic sympathetic tone.

It is clear that hypothalamic nucleuses play an important role in weight regulation, food intake and motivation for feeding.

Attempts have been made to use this knowledge in preclinical settings. In 1984 Brown and cols using implantable chronic platinum-tipped electrodes in the ventromedial hypothalamic area (VMH) showed changes in food intake in fasted dogs. Dogs that received 1 hour of VMH stimulation every 12 hours for 3 consecutive days maintained an average daily food intake of 65 % of normal baseline levels (Brown et al., 1984).In the light of their results and the availability of this new technology, the authors recommended deep brain stimulation as a potential mean of regulating food intake and

therefore a possible therapeutic modality for human morbid obesity. Feeding suppression was also elicited by electrical and chemical stimulation in non human primate's hypothalamus. Ventromedian, dorsomedian and ventromedian part of lateral hypothalamus produced prolonged suppression in food intake suggesting neuronal inhibitory mechanism of feeding in these centers in awake monkeys(Takaki et al., 1992).

HUMAN STUDIES

In the early 70, Sano and colleges, explored and selective destroy posteromedial part of the hypothalamus in men in cases of violent, aggressive behavior. This approach also allowed them to stimulate discreet zone and to observe autonomic, somatomotor and other responses. In a series of 51 lesioned patients, a tendency to gain weight after posteromedial hypothalamic destruction was seen Also during acute electrical stimulation (100 pps, 1 msec, 5 - 10 Volts) of the medial hypothalamus, autonomic sympathetic response were obtained, probably related with stress and energy expenditure mechanisms (Sano et al., 1970). The first human study specifically centered in treating human obesity using lesion and stimulation of the specific brain centers was carried out by Quaade in 1974. In that study, five patients with morbid obesity were subject to an electro stimulatory exploration of the lateral hypothalamic area. In three cases a convincing transoperatory hunger response was elicited. Two of theses patients received unilateral electro coagulatory lesion, and in a third a contralateral coagulation was performed. The patients with lesions showed a statistically significant, but transient decrease in caloric intake and a slight and transient decrease of body weight(Quaade, 1974). Although no direct DBS of the hypothalamus has been attempted for weight control, indirect evidence of weight modulation can be found as a secondary effect in the

established or experimental indication of DBS in the hypothalamic or near hypothalamic area. For instance, several studies have reported increased body weight and body mass index after high frequency stimulation in the subthalamic nucleus. While the mechanism is still unknown, possible explanations of body weight gain after DBS STN might include reduction of energy output related to elimination of dyskinesias, improved alimentation or direct influence on function of lateral hypothalamus by DBS STN. (Maschke et al., 2005; Novakova et al., 2007; Tuite et al., 2005)

Chronic unremitting cluster headache refractory to medical treatment is a developing indication of DBS in the posterior hypothalamus. Stimulation in this region in a previous hyperphagic and hypersexual patient has produced pain relief and 25 kg. Weight reduction(Franzini et al., 2007).Indirect observations of the effect of DBS in weight in the hypothalamic regions have been also made in our center. A patient with intractable gelastic epilepsy due to a hypothalamic hamartoma was implanted with 3 DBS quadripolar electrodes. While her number and severity of crises were greatly reduced, a 15 kg weight gain associated to menstrual cycle disturbance appeared following high frequency stimulation of the medial hypothalamus (Figure **19**).(Kahane P et al., 2003)

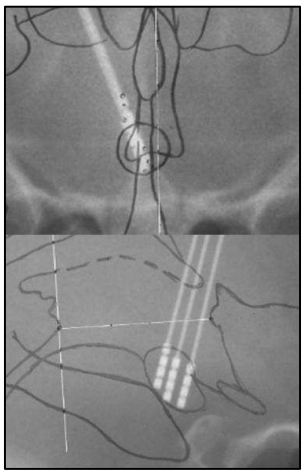


Figure 19: Hypothalamic Hamartoma: Postoperative x ray of 3 DBS Electrodes implanted in a Case of drug resistant gelastic epilepsy. Acute stimulation at a frequency of 130 Hz-100 µs – 0.4 mA, reduced Interictal spikes; but chronic high frequency stimulation (130 Hz/90 µs/0.5V, and then 185 Hz/60 µs/0.1V) produced 10Kg weight gain, which returned to baseline after turning stimulation off(Kahane P et al., 2003).

Table 3: History of Deep Brain Stimulation

The first direct electrical stimulation of the cortex of the animals, (Fritsch and Hitzig, 1870)5} **1870**
The first direct stimulation of the human cortex((Bartholow, 1874; Zimmermann, 1982)5} **1874**
A Stereotaxic frame that provides safe and efficient access to deep brain structures in animals is developed(al-Rodhan and Kelly, 1992; Horsley, 1908)6} **1908**
A Stereotaxic frame is adapted for use in human neurosurgery(Spiegel et al., 1947)3} **1947**
Stimulation of frontal tracts is used in psychiatric surgery for the treatment of, among other things, chronic pain **1948**
Rats are implanted with self stimulation electrodes(Olds and Milner, 1954)4} **1954**
Brain stimulation is used for the treatment of neuropsychiatry disorders(Heath, 1954)5} **1954**
Direct thalamic stimulation is used to reduce tremor(Hassler et al., 1960)2} **1960**
Intermittent chronic basal ganglia stimulation is used for the treatment of tremor in Parkinson disease(Bechtereva et al., 1975)3} **1968**
Stimulation of the somatosensory thalamus is used for the treatment of chronic pain **1973(Hosobuchi et al., 1973)6}**
Stimulation is used for the treatment of movement disorders(Cooper et al., 1974)6} and epilepsy(Cooper et al., 1976)1} **1973**
Stimulation of the PVG/PAG is used for the treatment of chronic pain(Richardson and Akil, 1977a)1} **1977**
Thalamic stimulation is used for the treatment of tremor(Brice and McLellan, 1980)3} **1980**
Thalamic stimulation is used for the treatment of dyskinesia(Merienne and Mazars, 1982)4} **1980**
Thalamic stimulation is used for the treatment of depression(Andy and Jurko, 1987)7} **1987**
Stimulation of the Vim is used for the treatment of tremor and Parkinson's disease(Benabid et al., 1987)9} **1987**
The usefulness of implantable battery driven DBS pacemakers is demonstrated(Benabid et al., 1996; Siegfried and Lippitz, 1994)1} **1990**
The efficacy of STN lesion in MPTP monkey is demonstrated(Aziz et al., 1991; Bergman et al., 1990)8} **1990**
Stimulation of the STN is used for the treatment of Parkinson's disease in human(Benabid et al., 1994)1} **1994**
he efficacy of PPN stimulation in an MPTP treated primate is demonstrated **2003(Jenkinson et al., 2004)7}**

(Based on Kringelbach M, Jenkinson N, Owen S, Aziz T, Translational principles DBS Nature Neurosciences(Kringelbach et al., 2007)6})

CHAPTER VI SCIENTIFIC RATIONALE FOR THE STUDY

With the aim of extend therapeutical available options, new targets are currently been tested. The hypothalamic nuclei are involved in the alimentary disorders and they could provide excellent new strategies for treating pathological behavioral alimentary disorders like morbid obesity and malignant anorexia. In principle, several basic statements can be established as the basic rationale for the study.

Statement N°1: Hypothalamic Stimulation or Lesion in Ventromedian Hypothalamic Area modulates Food Intake and Total Body Weight

The hypothalamic nucleuses implicated in feeding behavior have been the object of several studies in mice. In 1940, Hetherington and Ramson published that the bilateral lesion of the ventro-median region of the hypothalamus produced hyperphagia followed by obesity with normophagia(A. W. Hetherington, 1942). These centers, according with Cox and Powley 1981 were not only producing the obesity due to a hyperphagia, but weight gains also follow even in the rats with restricted food intake(Cox and Powley, 1981). Beltt and Keesey had shown earlier (1975) that low frequency stimulation de VMH was susceptible of inhibit food intake (Beltt and Keesey, 1975). Others also have suggested that a metabolic effect is probable in low frequency stimulation of VMH, leading to a chronic decrease in weight without change in food intake behavior(King, 2006). In 1951, Anad and Brobeck, shown a second diencephalic center for food intake in the hypothalamus: the bilateral lesions of the lateral hypothalamus provoked weight lose with aphagia(Anand and Brobeck, 1951b). Anand and Dua then reported that electrical stimulation of the LHA induced a pronounced bout of eating in previously

satiated cats, and electrical stimulation VMH caused food deprived cats to stop eating(Anand et al., 1955; Delgado and Anand, 1953). Here also a metabolic effect is suggested by Teitelbaum and Stellar, because theirs rats were able to keep a low weight and regain normal food intake after bilateral lesion of LHA (Teitelbaum and Epstein, 1962; Teitelbaum and Stellar, 1954). Low frequency stimulation of LHA produced ingesting behavior. Several biochemical and neurophysiological studies have shown activation of LHA neurons during fast periods .Recently, Ruffin and Nicolaidis have confirmed that the metabolic response is previous to behavioral changes in food intake (Ruffin et al., 1995). In brief, VMH/LHA critical involvement in feeding regulation can be summarized by:

1) The suppression and facilitation of feeding regulation by electrical stimulation of the VMH and the LHA respectively

2) The hyperphagia and hypophagia after bilateral electrolytic ablations as well as bilateral chemical lesions of the VMH and LHA respectively.

3) The presence of neurons in both nuclei that sense the metabolic signals such a glucose, free fatty acid and leptin (Dhillon et al., 2006).It is clear that hypothalamic nuclei play an important role in weight regulation, food intake and motivation for feeding.

Statement N°2 High Frequency DBS inhibits and conversely, Low Frequency (LFS) DBS activates Central Brain Structures.

Low frequency stimulation LFS (30-60 hertz) was applied in pain patients producing excitation in target areas (Hosobuchi et al., 1977; Richardson and Akil, 1977b; Richardson and Akil, 1977c). Patients with certain motors deficits (Liberson and Pavasars, 1960) neurogenic sphincter disorders(Brindley, 1977), spasticity and epilepsy

(Cooper et al., 1973)were also successfully treated with LFS . But brain lesioning procedures were irreplaceable until the emerging concept of high frequency stimulation (HFS) as a producer of functional neuronal inhibition was not recognized.

High frequency DBS provides functional inhibition of target structures producing significant improvement in Parkinson symptoms in both animal and human studies (Fang et al., 2006)and mimicking the clinical effects of tissue lesioning (Benabid et al., 1987; Bergman et al., 1990). As a consequence, well known neurosurgical or basic research lesional targets can now be easily explored and studied. There is also a recent exciting return to LFS due to a better understanding of electrical stimulation, better available equipment and medical team expertise as shown with the promising results in the treatment of non dopaminergic symptoms of Parkinson disease using DBS LFS in the pedunculo pontine nucleus (Mazzone et al., 2005; Stefani et al., 2007). The success of DBS in multiple neurological conditions as well as its capacity of producing diverse effects in target structures made it the ideal tool to consider for application in morbid obesity where discreet brain centers direct energy homeostasis.

Statement N°3 DBS can modulate hypothalamic structures

Hypothalamic nuclei have been targeted for several conditions in the past, either for lesioning and more recently, for electrical stimulation. Pioneering works by Sano have opened the way to this very complex and potentially dangerous region. In a classical article, they presented a series of 51 patients with pathologically aggressive behavior, in which they performed a series of electrical stimulation of the hypothalamic area. In the points of the posteromedial hypothalamus where sympathetic responses were elicited, lesions were made bilaterally (performed with an interval of 7-10 Days). A tendency to

gain weight after posteromedial hypothalamic destruction was seen. Also, during acute electrical stimulation (100 pps, 1 msec, 5 - 10 V) of the medial hypothalamus, autonomic sympathetic response were obtained, probably related with stress and energy expenditure mechanisms (Sano et al., 1970) Authors classified their results as excellent and good in 40 cases, with one importantly postoperative death after one week of the procedure. Most importantly, intraoperative electrical stimulation elicited several autonomic and somatomotor responses. The stimulated area caused rise in the blood pressure, tachycardia and pupillary dilatation, defining an area that is now known as the ergotopic triangle of Sano(Sano et al., 1970).

Other clinical lesion study aimed the lateral hypothalamus in morbid obesity. In 1974 Quaade et al. performed electrostimulatory exploration of the lateral hypothalamus and electrocoagulation in obese humans. Vegetative response, feeling of fear or euphoria and alterations in the pulse rate and respiration were all seen. An important hunger sensation was produced in 3 out 5 patients, but no lasting effect either in food intake or weight was obtained (Quaade et al., 1974).

There are also other direct indications that electrical stimulation in the hypothalamus can modulate their functioning. Leone et al, after extensive work with functional neuroimaging (PET), defined a region in the posterior hypothalamus thought to be implicated in the mechanism of the cluster headache. The hypothesis has lead to the implantation of bilateral DBS in these region with very interesting preliminary results(Leone et al., 2005). A recent multicentric study have found similar good results, with 4 out six patients pain free in a 6 month follow up (Bartsch et al., 2008). In contrast, others have warned over transoperative risks in this area and only temporary results

which should not justify surgery until larger multicentric studies were performed (Pinsker et al., 2008).

Indirect evidence of modulation of the weight and food intake in patients implanted with neurostimulators comes incidentally from long term outcome analysis of STN DBS in Parkinson's disease(PD)(Krack et al., 2003; Novakova et al., 2007). Using retrospective survey, authors intended to evaluate weight changes in patients with advanced PD treated with DBS STN. 25 PD patients (16 men, 9 women), of mean age 55 (42-65) years, mean PD duration 15 (9-21) years, who previously received bilateral DBS STN were evaluated. In the first survey, 1 to 45 months after DBS, weight gain was found in all patients comparing to pre-DBS period. The mean increase was 9.4 kg (from 1 to 25 kg). The patients' mean body mass index (BMI) increased from 23.7 to 27.0 kg/m2, i.e. by 3.3 kg/m2 (+2 to +6.1 kg/m2). In the repeated survey one year later, in 12 of the patients body weight moderately decreased, 3 did not change, and 6 patients further increased their weight. (Novakova et al., 2007). Others have shown acute increase in body weight after few weeks of HFS DBS STN in patients slightly overweight, and reduction in energy expenditure in parkinsonian patients treated in STN(Montaurier et al., 2007). In conclusion, DBS-STN implantation leads to body weight gain in both male and female patients with Parkinson's disease. The risk of body weight gain is highly variable among patients and differs between genders. As men gained primarily fat free mass, a reasonable weight gain may be tolerated, in contrast with women who gained only fat. Parkinson's disease is associated with profound alterations in energy metabolism that are normalized after DBS-STN implantation (Montaurier et al., 2007). Possible explanations of body weight gain after DBS STN include a reduction of energy

output related to elimination of dyskinesias, improved alimentation or ***direct influence on function of lateral hypothalamus by DBS STN*** (Novakova et al., 2007)

Clinical and Preclinical studies have shown that hypothalamic structures can be accessible to DBS and hence used as a powerful tool for a variety of disorders. That has led some to try DBS in morbid obesity. Until now, there have been no published reports of patients been implanted for morbid obesity. But translational research has created animal models in order to mimic clinical settings. Sani et al have implanted rats in the lateral hypothalamic nucleus bilaterally and observed changes in food intake and body weight after 24 days of continuous stimulation at HFS; unstimulated group has a mean weight gain of 13.8 % whereas stimulated groups have a mean weight loss of 2.3% (Sani et al., 2007). Similar results were seen in a study conducted in our group, which found reduced weight gain when stimulating VMH and increase weight gain when HFS LHA without permanent changes in food intake (Chabardes in preparation). Strong evidence of functional modulation of the hypothalamic function as seen in those aforementioned studies has naturally led to use non human primates and DBS leads to try to reproduce more efficaciously a clinical conditions. Lacan et al assessed the feasibility of ventromedial hypothalamus (VMH) DBS in freely moving vervet monkeys to modulate food intake as a model for the potential treatment of eating disorders, showing interesting results in terms of reduction in food intake and procedure safety (Lacan et al., 2008). In summary, DBS has shown efficacy in modulating subcortical central structures like the basal ganglia and have also shown promise when applied specifically to hypothalamus in either preclinical and human studies. We hope that our study can contribute to support the possible role of DBS in modulation of hypothalamic structures and morbid obesity.

Statement N°4 Intraventricular Approach is a safe and effective method to reach intracranial medial Structures

A critical issue in functional neurosurgery is the acceptable overall amount of risk that a determined therapy could bring to an individual patient whose condition, even though severe or invalidating, is not life-threating. Morbid obesity, a severe condition with a major morbidity, is a chronic disease that has to be managed with the least risk for the patients. Some ethical concerns, some of them very well funded, could arise .Risks that could be acceptable for other condition like dystonia(Pillon et al., 2006) or Parkinson disease(Constantoyannis et al., 2005; Vesper et al., 2007) might be unacceptable for Morbid obesity or anorexia nervosa. Keeping these arguments in mind and in view to a possible clinical application, our team has developed a new, less invasive trajectory for placement stimulating electrodes, without directing disrupting sensitive brain structure like the median hypothalamus. Ventromedian and periventricular hypothalamic nucleus are projecting to the lateral walls of the third ventricle while the anabolic areas of the lateral hypothalamus are far from the stimulating leads in this location.

Reaching central structures like the third ventricle using CSF pathways is a common neurosurgical practice extensively used in hydrocephalus (Cardia et al., 1986; Pople et al., 1990), ventricular tumors (Buxton et al., 2001) and others procedures involving shunts or endoscopic procedures (Hellwig et al., 2003). Instruments like flexible endoscopes and ventricular catheters of different diameters can easily travel trough the ventricular system and through Monro's foramen decreasing the risk of complication when compared to intraparenchymal trajectories. For instance, third ventriculostomy endoscopy has shown advantage in terms of safety and reduction of morbidity when

compare to standard stereotaxic procedure for third ventricle tumor's biopsies (O'Brien et al., 2006). In a large multicentric study, endoscopic catheterization of third ventricle showed safety and provide good outcome in obstructive hydrocephalus (Gangemi et al., 2007). Dusick found 6 surgical complications in a series of 180 adult patients undergoing 3V endoscopy for noncommunicating hydrocephalus, none of them directly related to endoscopic procedure per se (Dusick et al., 2008). Although it is clearly exaggerated to make a parallelism between endoscopic ventriculostomy and electrode 3V placement, fiberscope studies using primarily Monro's foramen approach can give an idea of the type of complication that could be found using this pathway.

Intraventricular stimulation resembles the cortical stimulation in the sense that the effective current should be directed toward a surface, in contrast to the intra-nucleus stimulation where the stimulation is directed to a spherical volume. The lead position in contact with the ependima layer and in direct relation with the medial structure situated at less than 2 mm might effectively activate the tissue of the medial hypothalamus. Conductive elements like CSF and the ependymal layer provide adequate impedance to the passing current which can reach medial structures without altering surrounding more lateral areas. Moreover, the use of DBS for stimulation subcortical structures using the ventricular system has proven successful in the past for somatic pain. Lead placement in the ventricular system has been attempted for modulation of the periventricular grey (PVG) matter in chronic pain syndromes. Richardson review his own series of PVG leads found good to excellent results in pain relief with no reported neurological complication and few side effects (Richardson, 1982; Richardson, 1983; Richardson, 1995). Also, Hosobuchi has evaluated a series of 122 patients who underwent electrode implantation for the control of severe chronic pain over a follow-up period of 2 to 14

years. Of the 65 patients with pain of peripheral origin, who were treated with stimulation of the PAG, 50 obtained successful pain control. The electrical stimulation technique appears to provide in those series long-term pain control safely, with few side effects or complications(Hosobuchi, 1986; Hosobuchi, 1987). These reports support the idea of electrical stimulation could modulate central grey nuclei surrounding the ventricular system.

Technological advances in DBS materials made now possible the use of electrical brain stimulation in more flexible way for the study over longer period of time in nearly normal condition the behavior in non human primates. Advances in DBS technology and in Stereotaxic techniques make now possible to reach areas of difficult access with a better security margin. This exploration could hopefully serve as a pre-clinical study for future human application of this approach in diverse types of eating disorders.

Experimental Design and Methods

CHAPTER VII EXPERIMENTAL DESIGN AND METHODS FOR OUTCOME ANALYSIS

OBJECTIVES

The purpose of the present study is to assess the effects of chronic ventromedian hypothalamic (VMH)/inferior dorsomedial hypothalamic (DMH) stimulation on feeding behavior, metabolism, and global body weight in the primate model. The study was conducted to replicate previously reported acute effect of VMH stimulation on food intake (Takaki et al., 1992), to determine whether Intraventricular electrode positioning can exert modulation in the third ventricle walls (where VMH lies), to observe the effects of VMH stimulation at different frequencies on gross behavior in non human primate and to evaluate the safety of the procedure using deep brain stimulation electrode technology already available for human application. Until now investigation of the effects of chronic deep brain stimulation of the medial hypothalamus in higher animals has never been performed. We have reviewed the efficacy and safety associated with stimulation in the chronic settings. Results of these experiments will be used to assess the feasibility of using such stimulation as a potential, future therapy for cases of morbid obesity.

EXPERIMENTAL DESIGN

Five Normal Weight *Macaca Fascicularis* monkeys (H4, H5, H7, H8 and H10) will be acclimated to two daily meals. Two monkeys were implanted with one quadripolar electrode (of 1.5 mm long in contact surface) in the third ventricle at the level of the VMH/DMH nucleus. Two others monkeys were implanted with one unipolar electrode

(3.5 mm long of active contact surface) at the same location. The last monkey was implanted in an extrahypothalamic site, with the 1.5mm quadripolar electrode Figure 32.

Phase 1: of the study identified electrode contacts that are effective in reducing the amount of food intake and lack of unacceptable side effects using different stimulation parameters. The experiment was performed as follow: After a period of fast of 24hrs, the animal received 8 hr biphasic wave stimulation at different frequencies and voltage intensities and a full day standard meal was presented (17:00 to 18:00 hrs). Latency of feeding initiation, time expends during feeding and total amount of food consumed was recorded. Overall motor activity during stimulation was also recorded and video taped for further behavioral analysis. Each animal underwent one or more control sessions in which meals were presented in the same conditions but off-stimulation. The combination of parameters settings that reduce meal size with longer latency feeding initiation and without unacceptable side effects (best parameter settings or bps) were chosen to be in use for the phase 2 or the chronic Stimulation period. Figure 20

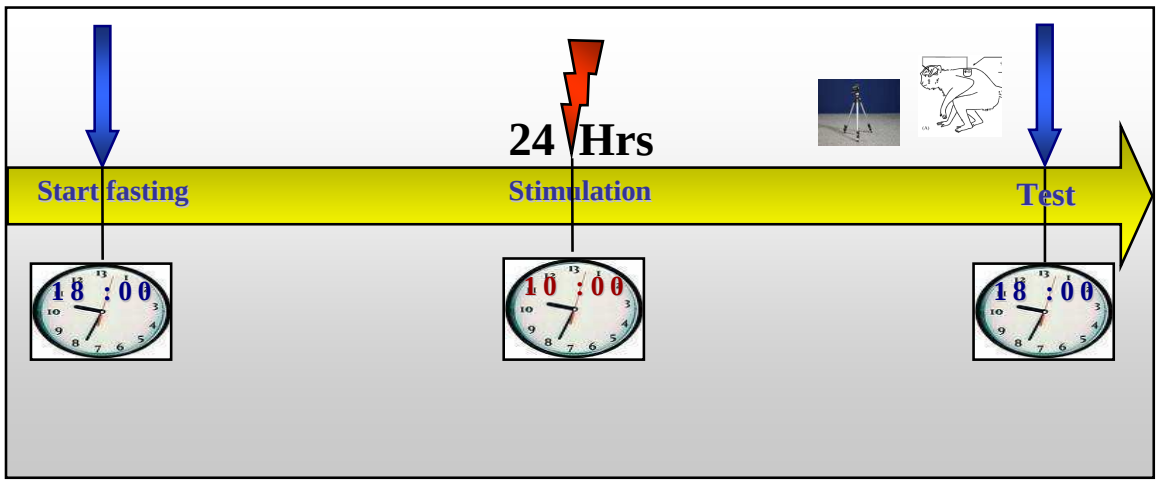


Figure 20: Diagram showing the acute stimulation trial protocol: fasting for 24 hrs, stimulation for 6-8 hrs and standard meal during which meal size, time eating and locomotion are measured. Each monkey was stimulated several times at different frequencies in order to establish the set of parameters able to reduced more effectively FI

Phase 2: Assessed the effects of Intraventricular VMH/DMH continuous stimulation on the feeding behavior and global body weight: **Paradigm 1:** Monkey H5 and H7 received the best or more effective frequency stimulation (BPS) during an 8 week period. Monkey H8 and H10 received simultaneously High frequency stimulation (HFS) at the parameter in use for subthalamic DBS for Parkinson's disease (130 Hz). Monkey H4 was kept off stimulation continuously during 8 weeks trial. **Paradigm 2:** Monkey H8 and H10 received BPS stimulation, for an 8 week period. Monkey H5 and H7 were given HFS for the same period and monkey H4 received no stimulation. **Paradigm 3:** monkeys H5, H7, H8, 10 all received stimulation considered as Low frequency stimulation (LFS) (30 Hz) currently in use in clinical settings (pain, gait freezing etc). Sham monkey H4 remained off stimulation. The animals were weighed and food intake, hormonal/electrolyte levels were periodically monitored. (Figure 21)

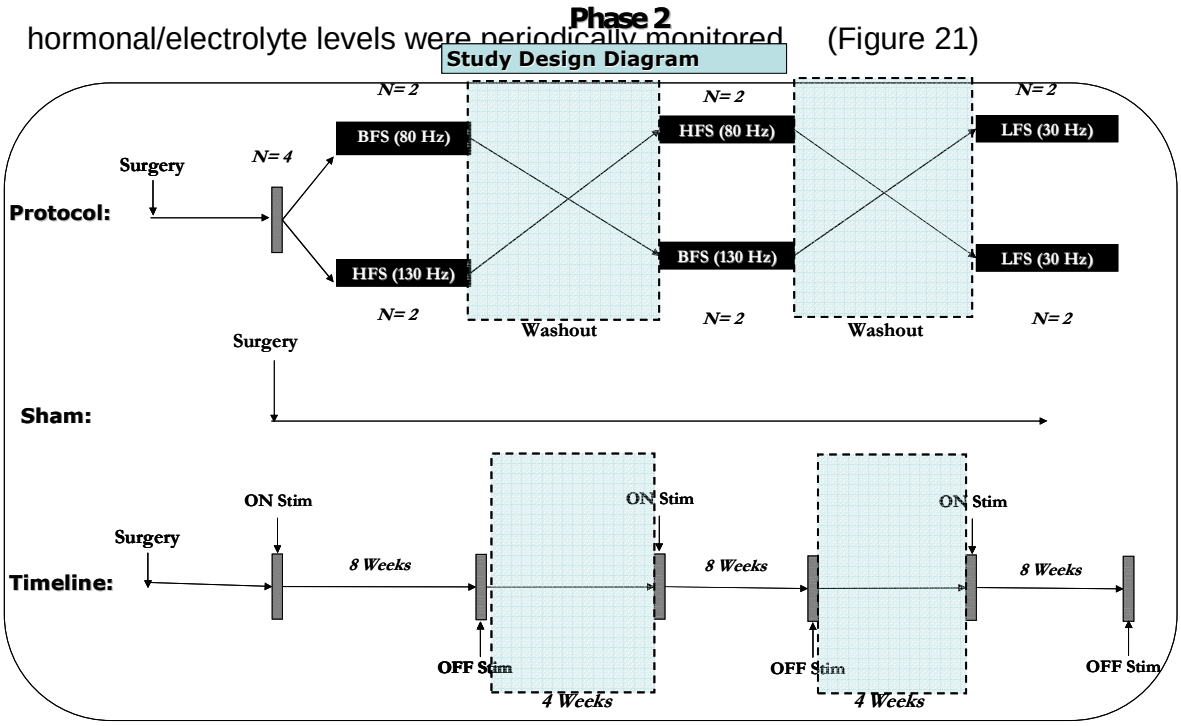


Figure 21: Protocol for chronic DBS at different frequencies. Between each paradigm, a washout period of 4 weeks was allowed to avoid 'carry on' effects. BFS or best frequency stimulation is the stimulation frequency which produced the most important effect over FI in the acute trials. HFS or High frequency was set at 130Hz as used in DBS for Movement disorders. LFS or Low frequency was 30 Hz, the frequency used in pain and other pathologies.

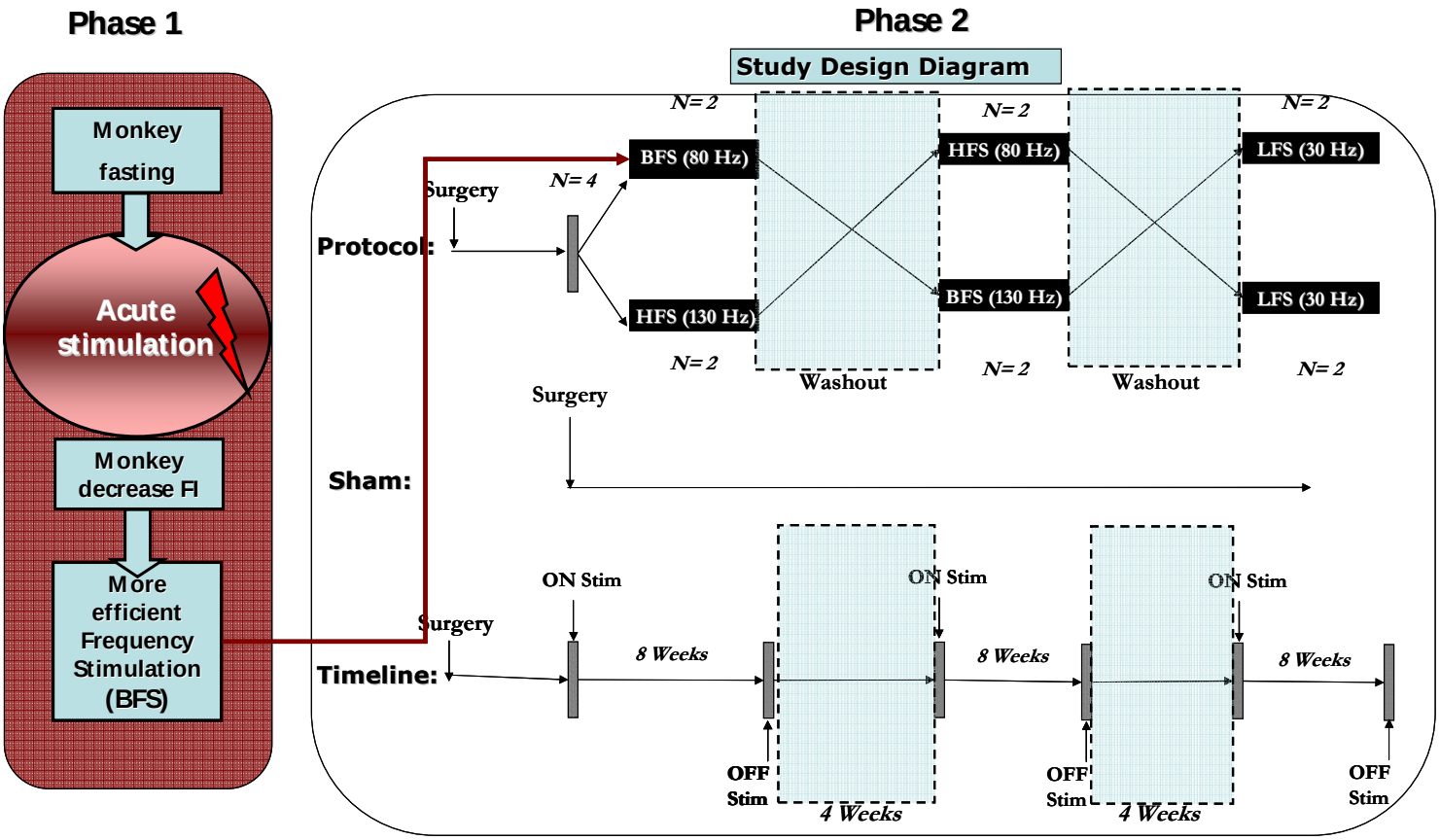


Figure 22: Summary of phase 1 and 2 protocol for acute and chronic DBS at different frequencies. Results in acute test were used in the crossover study for determining the best frequency stimulation parameter able of producing reduction in FI. This settings were test against other settings used in the clinical environment(HFS at 130 and LFS at 30Hz)

STATISTICAL ANALYSIS

Statistical analysis of the data presented in this work was done using the following tests:

- The test non parametric of Kruskal-Wallis for multiple comparisons between groups non-paired, was done for the analysis of the *acute trials*. This was followed by a post hoc Dunn's Test. For each monkey, we transformed the

variables in ratios (using off stimulation as baseline). For each *acute trial,* at a given frequency, the variation in the variable was analyzed for each animal.

- For multiple comparisons between groups, paired non parametric one way ANOVA, Friedman's test was performed. This was followed by a post hoc Dunn's Test. For comparison, all measures were transformed in ratios, representing % of variation from baseline. The test was used to compare the influence of the stimulation parameters or most specifically, the influence of the frequency in the different variables (weight, fat, food intake etc.). To do that, all the subjects were paired, and compared using the variation of each variable (or ratio) in response to the stimulation of the electrode at different frequencies (Figure 23)

Figure 23: Statistical test used and analysis of the differences.

Kruskal-Wallis test
The Kruskal-Wallis test is used to test:
*** The null hypothesis H0 according to which k independent samples were drawn from the same population (or identical populations),**
*** Against the alternative hypothesis H1 according to which these samples were drawn from populations sharing the same shape but with different central tendencies (medians).**
The observations must be on a numeric or ordinal scale (not just categorical).
The samples **do not need to have the same number of observations.**
The Kruskal-Wallis test may be perceived as a generalization of the (Wilcoxon)-Mann-Whitney test to more than two samples.
The Kruskal-Wallis test is non parametric, that is, it does not make any assumption on the nature of the underlying distributions (As many other non parametric tests, it will not use the values of the observations directly, but will first convert these values into ranks once these observations are merged into a single sample.
The statistic of the Kruskal-Wallis test is built from the means of the ranks of the observations across the samples. This approach is similar to that of one-way ANOVA: * ANOVA compares the sample means. But it also assumes the populations to be normal with equal variances, so in fact, it tests whether these populations are identical.
* The Kruskal-Wallis test does not assume normality or equal variances, and instead of comparing sample means, it compares sample means of ranks.
This similarity is the reason why the Kruskal-Wallis test is sometimes called "one-way ANOVA on ranks".
The Kruskal-Wallis test should not be confused with the Friedman test. This test also tests the hypothesis according to which several samples originated from the same distribution, but these samples must then **be matched,** that is be made of identical (or very simular) individuals that were submitted to different conditions. The Friedman test then attempts to detect differences in the effects of these conditions.

Friedman test
The Friedman test addresses the issue of deciding whether k matched samples were drawn from the same population. It is therefore an identity test.

The observations must be measured on an numerical or ordinal scale (i.e. not categorical).
The samples **need to have the same number of observations.**
The Friedman test more than two samples.
The test is non parametric, that is, it does not make any assumption on the nature of the underlying distributions (except continuity). As many other non parametric tests, it will not use the values of the observations directly, but will first convert these values into ranks once these observations are merged into a single sample.
Test does not assume normality or equal variances, and instead of comparing sample means, it compares sample means of ranks.
The Friedman test is a non-parametric statistical test developed by the U.S. economist Milton Friedman. Similar to the parametric repeated measures ANOVA, it is used to detect differences in treatments across multiple test attempts. The procedure involves ranking each row (or block) together, then considering the values of ranks by columns.

CHAPTER VIII IMPLANTATION SURGERY

MATERIALS AND METHODS

A pilot study was done, before starting our work, for evaluate the feasibility of the hypothalamic monkey surgery and the efficacy of a direct intraparenchymatous implantation VMH bilateral implantation.

PILOT STUDY: HYPOTHALAMIC BILATERAL DEEP BRAIN STIMULATION USING INTRAPARENCHYMAL ELECTRODES

In order to evaluate the direct effect of DBS over medial hypothalamic structures, a non human primate was implanted bilaterally intraparenchymal in the ventral hypothalamus.

Objectives

1. Analyze the technical feasibility of bilateral intraparenchymal implantation of DBS electrodes in the ventromedial hypothalamic region in monkeys
2. Evaluate tolerance and safety of ventromedial and lateral (VMH/LHA) Hypothalamic region DBS in the non human primate.
3. Assess the efficacy in terms of weight and food intake of bilateral VMH/LHA DBS electrodes stimulation at different parameters in this model

Materials and Methods(pilot study)

Animal

Study was performed on a male macaque monkeys (Macaca fascicularis, CRP, Port Louis, Mauritius) weighing 7.8 kg. Monkey was implanted bilaterally (March 2004) with two 3389 DBS electrodes (Medtronic Corp. Richmond Minnesota).The exact age was not known as they were captured in the wild. Animal was maintained in individual primate cages under controlled conditions of temperature (25±1℃) and light (12 hour

light/dark cycles, light on at 8 am), were fed regularly on a diet of fresh fruit and biscuits, and had free access to water. The laboratory is authorized by the French Ministry of Environment and all experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) for care of laboratory animals. Every effort was made to minimize the suffering of the animals while maximizing the data obtained.

Surgical Technique

1. Anesthesia and Intraoperative Care

Anesthesia was a combination of ketamine (20 mg/kg loading dose, 5 mg/kg maintenance im.) and diazepam (0.2 mg/kg iv.) in addition to 1% lidocaine with epinephrine for local anesthesia of the scalp and muscles. 0.9% NaCl was continuously infused intravenously during the operation for drug access and hydration. Figure 24

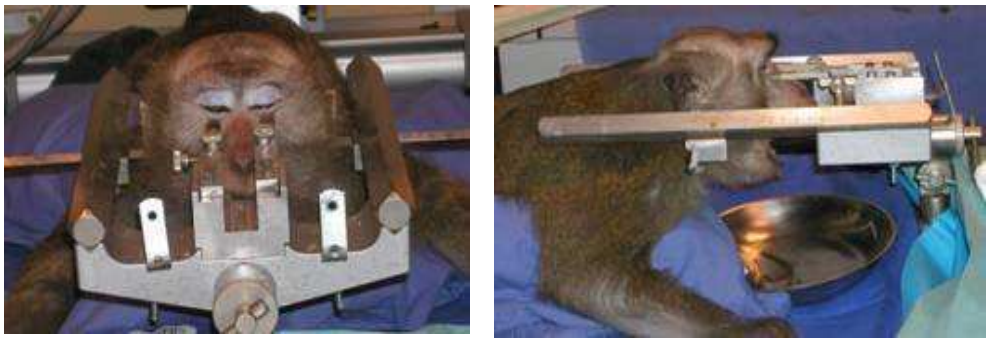


Figure 24: AP and lateral views of a monkey installed on the KOPF® (David Kopf Instruments Tujunga, CA USA) stereotaxic frame

2. Stereotaxy

Stereotaxy was performed using a KOPF® frame with a primate adapter kit (KOPF® David Kopf Instruments Tujunga, CA USA Instruments, USA). As shown in Figure 24, the frame was modified by removing a central block from the piece holding the mouth

hook and orbital bars. This was done to better visualize the third ventricle on anteroposterior (AP) ventriculography.

3. Ventriculography and Targeting

AP and lateral ventriculography was performed**(Figure 25)** through a stereotactically placed right lateral ventricular puncture through which 2 mL of ventricular contrast (Iopamiron® 200, iodine 200mg/mL, Bracco, Italy) was injected after 0.5 mL of confirmatory air. All films were processed in a darkroom adjoining our animal operating room. The trajectory was chosen taking into account Paxinos and Huang atlas (Paxinos et al., 1999)corrected by actual animal Ventriculography (reflecting internal nervous systems landmarks as anterior/posterior commisure line and thalamus height). The coordinates for VMH: The anteroposterior coordinate (y) was 2.8 12^{ths} of the ac pc length posterior to the ac. The dorsoventral coordinate (z) was 6.12 8^{ths} of the thalamic height below the ACPC plane. The lateral coordinate (x) was 2 mm from the midline of the third ventricular.

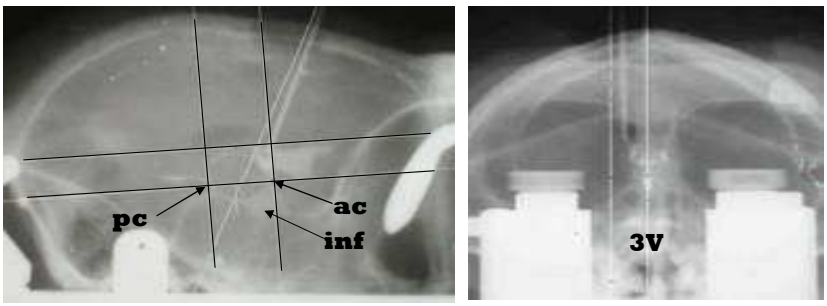


Figure 25: Lateral (left) and AP (right) ventriculograms with planning. Lines were drawn between ac to pc and a parallel line passing through the dorsal thalamus. Central line passed throughout the middle of the 3rd Ventricle. ac: anterior commisure, pc: posterior commisure, ht: thalamus height, inf: infundibulum, 3V: third ventricle. Paxinos Atlas showed cuts from a macaca mulatta, a larger animal than fascicularis, with an ac pc distance of 14.4mm (our monkeys had between 8-11 mm ac pc line distances). Normalization (dividing all distances for animals internal landmarks) allowed proper targeting(Paxinos et al., 1999).

4. Electrode Implantation

After fixing the head to the KOPF® frame with a primate adapter kit (David Kopf Instruments Tujunga, CA USA) as shown in Figure, a midline incision was made. The skull was accessed bilaterally and two burr holes of 2 mm over coronal suture were drilled for the approach to the hypothalamic targets. Using the ventriculograms previously performed and with the coordinates from Paxinos atlas(Paxinos et al., 1999), a guide tube fitted with a stainless steel stylet was stereotactically inserted into the brain bilaterally by using micromanipulators. Several twist drill holes were made for electrode fixation. A four contact electrode Medtronic 3389 was introduced after withdrawal of the stylet until the tip reached the target VMH area. AP and Lateral X rays were performed and compared to previous ventriculograms. In this way, the accuracy of the implantation was controlled and verified intra-operatively. After carefully checking final position, the electrode was secure in place using ethicon sutures 4-0 and acrylic cement. This method describe for human DBS procedure, allows effective anchoring the lead in place.Short Medtronic extensions were then tunneled subcutaneously to the back of the animal. Same procedure was repeated for the other side. Left and Right IPG were subcutaneously implanted below the scapula, allowing unrestricted and free movement. Non absorbable sutures were used to secure the extension leads and the IPGs.

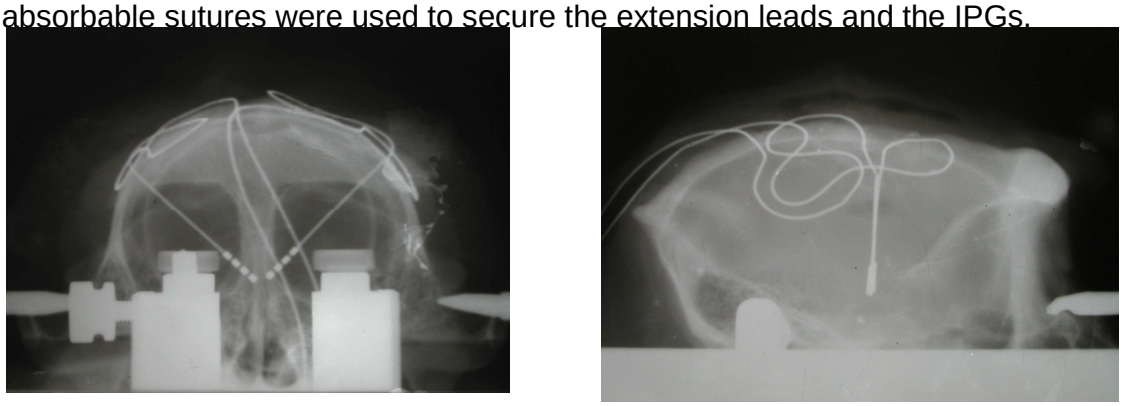


Figure 26: AP and lateral views at the end of the procedure. Final position of bilateral electrodes

5. Postoperative Testing and DBS Programming

After two months of observation, animals were anesthetized briefly with ketamine (20 mg/kg loading dose, 5 mg/kg maintenance im.) and put in an operative table. Side effects were tested for a wide arrangement of parameters setting. Using monopolar stimulation, all contacts (0-3) in both electrodes were tested (1-5 V, 60µs, 25-130 Hz). All effects were recorded and the stimulation intensities that evoked minimal adverse effect were used for programming IPG in the subsequent experiments.

6. Feeding Regimen and Weight Measurements

Animal received two daily meals compose by 150 g primate chow biscuits and 350 g of fruits (carrots, apples, orange and bananas). Water supply was taken ad libitum by the monkey from a bottle in the frontal part of the cage. Food intake was difficult to measure; we did a subtraction from the amount of food delivered and the amount of food left in cage. The daily food intake was between a range 390grs-500grs a day. Water intake was not possible to measure due to problems with the bottle (monkey was able to manipulate the bottle, some liquid was lost). The implanted pulse generator (IPG), programming was made transdermally on the animal back using Medtronic N-vision programmer, after temporarily restraining it with the sliding bottom of the cage. Two to four weeks stimulation was then made using first unilateral and then bilateral monopolar stimulation at different frequencies

7. Histological Analysis

Animals were anesthetized by intravenous injection of sodium pentobarbital (50mg/kg) and perfused transcardially with 0.9% saline followed by 4% buffered formaldehyde.

Brain was removed, blocked, immersed in the same fixative for 24hrs, and then placed in saline with the addition of 30% sucrose until the block sank. They were then sectioned coronally on a cryostat at a thickness of 40µm. Every section was collected in sequence onto gelatinized slides and processed for routine cresyl violet staining.

Results(pilot study)

Post Operative Period

Animal tolerated the surgical procedure without negative incidents. After recovery from anesthesia, monkey was able to perform self maintenance in the cage. No appreciable changes in behavior were seen. Wound healing was fast and uneventful.

The initial weight was 7.8 kg. After surgery, there was an important weight lost without stimulation, reaching 6.8 kg in two weeks. The following weeks, the weight was stabilized at 6.9 kg. Unable to reach the departing preoperative weight; we started the chronic stimulation in the left electrode after two month stabilization.

Acute Side Effects during Postoperative DBS Testing

The stimulation parameters were estimated in the postoperative period, according to the results of DBS testing at different amplitudes and frequencies in all contacts. Animal was lightly anesthetized and put in an isolated mattress and several intensities were tested. At 4.5 V in contact 0, a flutter in upper eyelid was elicited in left side and in the right side, and a unilateral midriasis compared with the contralateral side was seen when stimulating in contacts 0, 1, 2 at 4.5 V. To avoid any possible effect due to current spread beyond the target area, 2 volts were chosen as maximal stimulation intensity.

Behavioral Observations

Several important observations were made during this experiment. Behavioral changes were occasionally seen when stimulating lateral hypothalamic region at 50 Hz. Penile

erections, fear reaction, lip smacking piloerection were seen in left and right side that last few hours-days. Also in lateral hypothalamus, adipsia was found once when stimulating in VMH left side at 50 Hz, disappearing also after 48 hrs period. After the change in the frequency (from 25 to 50 Hz), during 48 hrs the monkey stop drinking water without change in solid feeding behavior. The animal wasn't presenting any sign of dehydration, with its body weight remaining stable at 7.5 kg. Blood samples did not showed any change in electrolyte concentration. This effect disappeared spontaneously in few hours. Also the contact projecting to lateral hypothalamic area produced acute responses over several behavioral. For instance , after starting stimulation, penile erection were observed, sensible to intramuscular ketamine and a clear increase in chewing movements, dorsal region piloerection and fear reaction when we approach the animal (he was jumping to the back in the cage). All this changes were decreasing after a period of adaptation, when the monkey regained it usual behavior

Weight and Hormonal Changes

The initial weight was 7.8 kg and immediately after surgery, there was a decrease in body weight up to 6.9 Kgs. After two month stabilization period, stimulation was started in the left side using 25Hz, 1.5 V, 0.6 ms and the deepest contact, projected to the VMH nucleus. Using continuous chronic stimulation, we evaluated weight gain or loss, and hormones levels related with basal metabolism. Weight and hormones levels were measured after a period of continuous stimulation between 2-4 weeks, during the same hour (The weight and the blood sample were obtained between 10:00-12:00 hours in a fasting animal). The stimulation was monopolar with the positive polarity in the case. Pulse width and intensity were kept constant during the duration of the whole experience

at 60 us and 3v (after an initial short essay with 1.5V). Blood samples were analyzed in an associated laboratory using standard human reactive and techniques. Results were summarized in the (

Table 4), (Table 5), and (Table 6)

Stimulation from unilateral left electrode in the LHA area at 50 Hz produced a drop in weight from the baseline of 5% (We corrected this value with the postoperative baseline weight of 6.91 Kg). This was effect did not last long time and animal returned to baseline weight by the end of the period. Changes in the total body weight in general ranged between 5% increase to 5% decrease. We did not obtain a clear tendency towards a reduction or augmentation of weight related to the contact placement or the fixed frequencies explored. Acute stimulation effects were seen when stimulating lateral hypothalamic area: piloerection, penile erection, fear reaction (jumping back of the cage when approached) that were decreasing after a period of adaptation. Bilateral stimulation to the VMH produced a slight reduction in total body weight of <2% and in LHA produced an increase in the total body weight of 3.94% at low frequency and 5.92% at 130 Hz (Table 6). No change in food intake was seen in this trial

Table 4: Weight and hormones levels related to Frequency and Active Contact in monkey H3 Unilateral (left) chronic stimulation.

Contact	FQ	W (Kgs)	Variation (%)	Cortisol	TSH	T3L	T4
VMH	25	7,21211	0,046	614	<0.005	3,1	9,6
VMH	50	7,4389	0,0489	1111	<0.005	2,7	9,2
VMH	130	6,87191	-0.30%	633	<0.005	4,3	9,9
LHA	50	6,50904	-0,056				
LHA	130	6,89459	0.00%	633	<0.005	4,3	9,9

Table 5: Weight and hormones levels related to Frequency and Active Contact in monkey H3 Unilateral (Right) chronic stimulation.

Contact	FQ	W (Kgs)	Variation (%)	Cortisol	TSH	T3L	T4
VMH	130	6,667802	-0,0329	561	0.015	3	11,2
VMH	50	6,985316	0,0132	650	0.013	3,5	11,8
LHA	130	7,189433	0,0427				
LHA	50	6,690482	-0,026	704	0.019	4	11,4

Table 6 Weight and hormones levels related to Frequency and Active Contact in monkey H3 Bilateral chronic stimulation.

Contact	FQ	W (Kgs)	Variation (%)	Cortisol	TSH	T3L	T4
VMH	130	6,7812	-0,0164	868	<0.005	3,4	10.7
VMH	50	6,871918	-0.30%	540	0.013	3,5	14,1
LHA	130	7,302831	0,0592				
LHA	50	7,166753	0,0394	551	<0.005	3,4	10.7

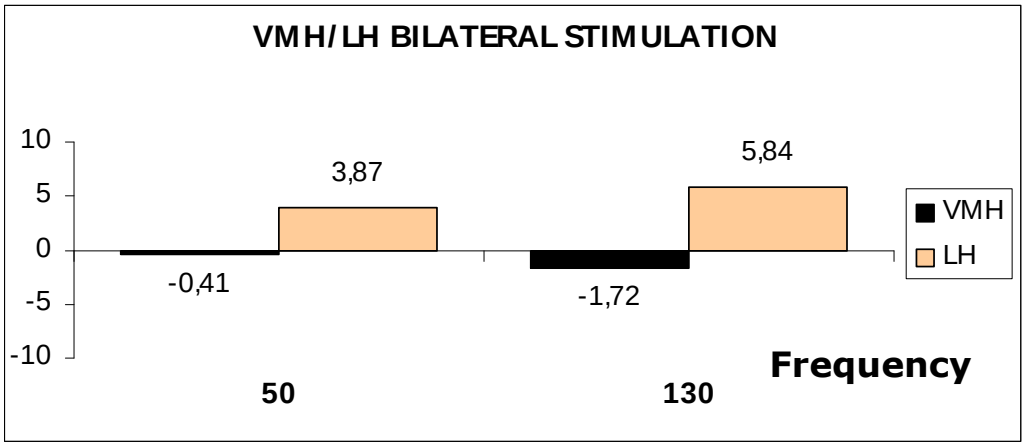


Figure 27: Variation in weight during bilateral DBS of VMH and LHA at 50 and 130 Hz (2v, 0.60 msec). A tendency to increase weight during bilateral LHA was seen regardless the frequency employed

Histological Changes

Macroscopic findings:

Brain with cortical damaged due to electrode passage and some inflammatory reaction over the entry point in the right side. Macroscopic cuts showed small hemorrhagic zone in temporal lobe and lesion in the floor of the third ventricle.

Microscopic changes: Third ventricle floor was partially missing and probably damaged. No other changes in the surrounding tissue were remarked.

Discussion (pilot study)

Chronic electrical stimulation was delivered continuously in a normal weight bilateral implanted non human primate in hypothalamic area. Variation from the post surgical body weight (6.9kg) ranged between 5% increase to 5% decrease in different parameters settings. Remarkably however, is the fact that monkey never regain initial

pre-surgical body weight (7.8 kg) during the two month off-period before start stimulation or afterwards(two weeks off stimulation the weight remained almost constant between 6,44-6,71 Kgs). Hormonal changes were not significative: circulating T3L and T4 remained at the same level, with no sign of induced secondary hyperthyroidism (TSH is released in the Paraventricular region). We have not found in our study changes in the levels of sexual hormones (diffusely distributed in the preoptical region) nor the cortisol (corticotrophin-release hormone also located in the paraventricular region).

Several important observations were made during this experiment. Behavioral changes were occasionally seen when stimulating lateral hypothalamic region at 50 Hz. Penile erections, fear reaction, lip smacking piloerection were seen in left and right side that last few hours-days. Also in lateral hypothalamus, adipsia was found once when stimulating in VMH left side at 50 Hz, disappearing also after 48 hrs period. No objective signs of dehydration were found from the blood laboratory electrolytes.

It has been well established that ventromedian hypothalamus and lateral hypothalamic area play a cardinal role in the control of food intake and in weight control. There has been, however some controversy regarding the results of studies on the monkey. Although an electrical lesion placed in the VMH in monkey resulted in hyperphagia, Robinson *et al* found that electrical stimulation of the monkey VMH did not constantly suppress the ad libitum feeding even in partially satiated state. In fact VMH stimulation at 50 Hz with high current up to 1ma induced food intake (Robinson and Mishkin, 1968), effect been similar to a lesion. Lateral hypothalamic area also failed to shown which specifical areas were able to facilitates or suppress food intake. More recent work for Takaki (Takaki et al., 1992) revisited those issues and made very pertinent observation that might be useful in our actual settings: One important aspect is the current spread

and intensities. Strong electrical stimulation might electrotonically stimulate both feeding inhibitory and facilitatory sites. Areas that induce feeding behavior are located immediately lateral to the inhibitory nucleus VMH. In that study, when carefully control electrical parameter and applied to smaller areas, they were able to obtain feeding suppression in hungry monkeys. In this pilot study, we failed to show changes in food intake and we obtained non significant changes in weight when stimulating either VMH or LHA. A recent experience in vervet monkeys has found that during VMH DBS, total food consumption increased. The 3-month bilateral implant of electrodes and subsequent periods of high-frequency (185 Hz) VMH stimulation did not result in significant changes in body weight, in concordance with our results(Lacan et al., 2008).

One possible explanation is electrode size and contact size. Human's electrodes with a big surface area might easily spread electrical stimulation beyond the borders of the nucleus of interest, and reach areas with opposite actions. Other issues that remains to be solved in this Series is the effective parameters (frequencies, pulse width) that induce changes in food intake and weight gain and whether continuous stimulation is better than stimulation given in shorter periods during the day.

Methodological Consideration and Technical Problems

There were several technical difficulties that might help explain some results. The first difficulties was related to the used of electrodes of big surface usually employed in clinical practice. While there are more reliable for being tested for human use, the contact surface is probably big enough for delivering energy to adjacent nuclei and areas with opposite functions. In previous experiences we tested customized DBS electrodes for non human primates of approximately a third the size of the 3389

Medtronic lead. But the system was not strong enough and connections with the extensions were easily damaged.

The external magnet activation of IPGs in the conscious and freely moving animal allowed ready access for initiating DBS periods. Initially, the animal was anesthetized in order to changes parameters, representing a possible change in behavior during the post anesthesia periods. The cage was adapted for the passage of the magnet through the bars and with the sliding cage bottom and some training, it was possible to change the stimulation parameters without putting the animal to sleep.

The targeting in the Macaca fascicularis ventromedian hypothalamic region was an adaptation from the methods used to localize structures in basal ganglia(Percheron, 1975; Percheron et al., 1986a), like the subthalamic nucleus in the MPTP primate model(Benazzouz et al., 1996). The accuracy of the methods coupling Ventriculography and atlas superposition was already assessed in previous studies, either when doing lesions or when implanting DBS Leads(Wallace et al., 2007). Final electrode position was evaluated using Ventriculography and indirect measures, like the relative position of the contact to the AC PC line and the atlas coordinates for VMH and LHA. The area was further explored using the leads to set appropriate stimulation parameters and the side effects also provide us with information about final position. But the histological analysis only revealed a lesion in the third ventricle floor, which does not allow us to confirm the final position of the stimulating leads.

Other critical elements in the evaluation and interpretation of the results are the stimulation parameters considered for chronic stimulation. Different parameters used were obtained from current human stimulation protocols and clinical experience in the movement disorder functional practice, like high frequency stimulation in Parkinson

disease (Benabid 2003; Benabid 2003(Benabid, 2003a; Benabid, 2003b) and dystonia(Coubes et al., 2000). Low frequency parameters were set from pain studies found in the literature and clinical experience in chronic pain and cluster headache accumulated in our center. The intensity in general was set under threshold for side effects using also pulse width 0.60 msec as used in patients and monopolar cathodic stimulation. This way of setting electrical parameters could be inadequate to stimulate or inhibit this region. The anatomical complexity of the area composed of various nuclei surrounding the III ventricle and a large area with mixed axons and neuronal body accounts for the relative difficulty to reproduce an exclusively inhibitory effect when using 130 Hz or avoid current spread and hence stimulation of surrounding areas when using low frequency stimulation. It could have been interesting to test different frequencies and different intensities and possible find a particular setting able to yield a valuable response.

Conclusions (pilot study)

The pilot work has served to establish the possibility of the modulation of the hypothalamic medial/lateral area in the study of possible therapies for obesity and eating disorders. Monkey tolerated well the procedure without any significant life threatening side effect. Eating behavior and weight are quantitative measurable variables which can be obtained in a freely moving animal without learning task or using neurotoxins to mimic neurological conditions. Stereotaxic DBS methodology can be applied to this particular setting and the use of Ventriculography coupled with specific monkey stereotaxic brain atlas wrapped to individual internal landmark provided good accuracy in targeting hypothalamic structures as we have already seen in basal ganglia structures(STN, GPi etc.). But many question remained unsolved and unanswered. The

results obtained were not conclusive and significant weight changes were not seen. Some results were even contradictory (weight gain during LFS of VMH) but in general, the changes were short lived. Some of the results may be due to factors involved in the anatomy of the structure and the geometry of the electrode. The confounding effects might also be related to the current spread beyond nucleus boundaries, involving remote structures. In summary, these results have shown that VMH DBS can be applied a might represent a new interventional strategy, but new ways of modulate this medial structures exclusively with minimal current spreading to antagonistic structures has to be found. We establish a chronic non human primate model applicable to eating disorders. For this motive we have chosen to try a new procedure for reaching medial structures: the intraventricular deep brain electrode in the ventrobasal hypothalamic region for weight modulation.

HYPOTHALAMIC DEEP BRAIN STIMULATION USING THIRD VENTRICLE ELECTRODES (3V)

Animals

After a period of observation of behavioral parameters and stable base line food intake, Five normal Weight *Macaca Fascicularis* monkeys (Macaca fascicularis, CRP, Port Louis, Mauritius)(H4, H5, H7, H8 and H10) weighing 5.05 - 6.18 kg, were operated in this experiment. The exact age of the animals was not known as they were captured in the wild. Animals were maintained in primate cages (Genestil®, Royaucourt France) under controlled conditions of temperature (25±1℃) and l ight. Each monkey was housed individually before and during these experiments, and had complete veterinarians supervision throughout this period. They were acclimated to two daily meals on standard

laboratory chow biscuits (150 grs) (Scientific Animal Food products SAFE, France, 15-21 biscuits a day) and fruits. Lighting was maintained on a 12 h light-dark cycle (light 8:00 -20:00 h). The laboratory is authorized by the French Ministry of Environment and all experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) for care of laboratory animals. Every effort was made to minimize the suffering of the animals while maximizing the data obtained.

SURGERY FOR HYPOTHALAMIC INTRAVENTRICULAR DBS ELECTRODES

Anesthesia and Intraoperative Care

Each animal was initially anesthetized using ketamine (Imalgene®, MERIAL Lyon France) *(20 mg/kg loading dose, 5 mg/kg maintenance im.)* followed by the administration of diazepam (0.25 mg/kg IV, IM) *Anesthesia was a combination of ketamine and diazepam in addition to 1% lidocaine with epinephrine for local anesthesia of the scalp and muscles. 0.9% NaCl was continuously infused intravenously during the operation for drug access and hydration.* Intubation and assisted ventilation were not required.

Stereotaxic Procedure

A Stereotaxic Kopf® large animals frame was used. The procedure was performed using a Kopf frame with a primate adapter kit (KOPF® David Kopf Instruments Tujunga, CA USA). The frame was modified as previously explained. The frame is attached to a fix base placed in a special room designed to use transoperative teleradiography. AP and Lateral views Fluoroscopy can be obtained in this special set up that place monkey

head distant from X rays sources, diminishing distortion. Our surgical methods are based on Ventriculography and direct transsurgical visualization of internal landmarks (anterior commisure, posterior commisure, thalamus height, infundibulum third ventricle) designed mainly for targeting central structures in the brain(like the basal ganglia). This approach, widely used in humans ((Talairach and Szikla, 1980)) and adapted for monkeys (Percheron et al., 1986a) is quite valuable when targeting periventricular areas (like in human's periacueductal grey matter). After fixing monkeys head in the frame, AP and lateral X-ray's views were obtained to avoid rotation in either sagittal or coronal plane.

Ventriculography was performed using Iopamiron contrast medium *(Iopamiron® 200, iodine 200mg/mL, Bracco, Italy)* and introducing a ventricular rigid trocar (0.8 mm diameter) at 70° to the horizontal 2mm lateral from sagittal suture coupled with a Electrode holder (KOPF® David Kopf Instruments Tujunga, CA USA).

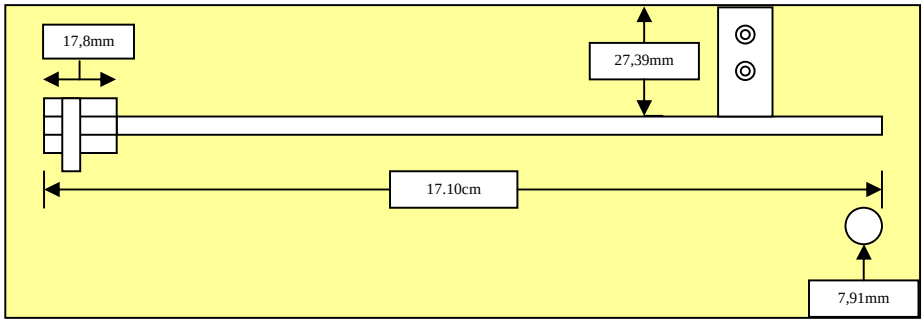


Figure 28: KOPF® electrode holder used to perform Ventriculography and direct a guide tube into the Monro's Foramen allowing DBS lead to slide into the Third ventricle

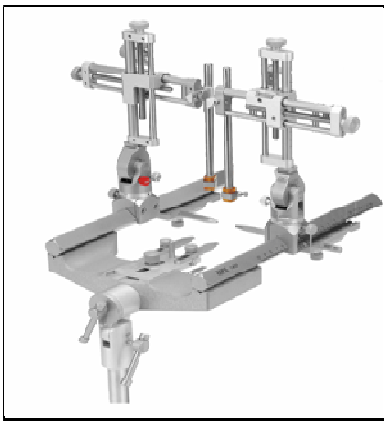
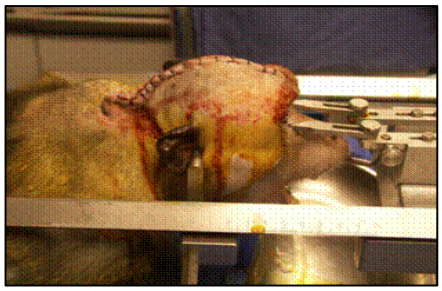


Figure 29: Stereotaxic KOPF® (David Kopf Instruments Tujunga, CA USA) frame used to introduce DBS electrode into the third ventricle. Two electrode holders are needed: one for Ventriculography and the other for secure the DBS lead into place.

All films were processed in a darkroom adjoining our animal operating room. Between 16 to 20 mm from cortex we usually found the lateral ventricles. In seated animals CSF pressure is neutral. There are no resistant when introducing the catheter, so the best way of finding the ventricles is withdrawing the guide after reaching appropriate depth .Then water level inside the trocar must drop when traversing the CSF Ventricular area. About 2 ml was *injected* swiftly after 0.5 mL of confirmatory air and radiography was obtained immediately, as the product is rapidly evacuated. Monro's foramen is then visualized in the two projections and a second catheter is introduced directed under fluoroscopy reaching directly into the foramen. A DBS electrode is then advanced through the trocar and wire guide is withdrawn when reaching third ventricle entrance. Without wire guide, DBS electrode easily slips to anterior third ventricle near mamilaries bodies. Coronal, and sagittal X rays views were obtained and fusion with previous initial Ventriculography.



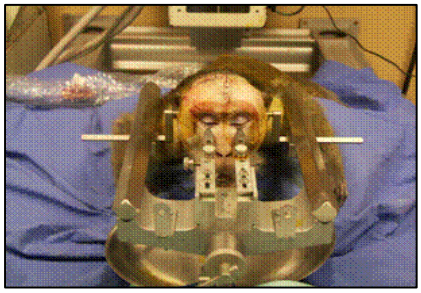


Figure 30: AP and Lateral view of Macaca fascicularis after surgery. Through the same incision, a pocket is made in the back of the animal to put the extension cables and the IPG (Soletra). No Intubation was required.

Target Determination

The VMH targets were identified in a *Macaca Mulatta* Paxinos monkey Atlas. The VMH target was identified on coronal sections between 16.95 13.80 mm- anterior to interaural plane. Based on these reference images, the VMH was localized: 0 to 2.3 mm posterior to the ac image in Ventriculography, and 2 mm superior to the ventral tip of the rostral portion of the nucleus corporis mamillaris. We also used the normalized coordinates calculated during the first implantation in the pilot study (see targeting section for pilot study or Figure 25)

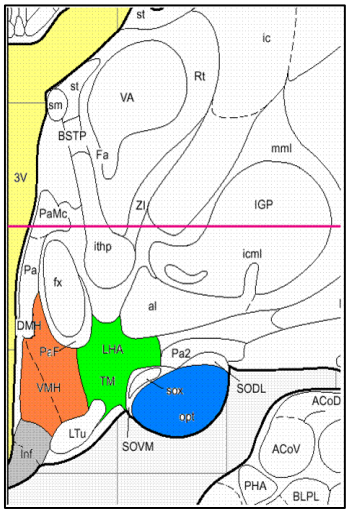
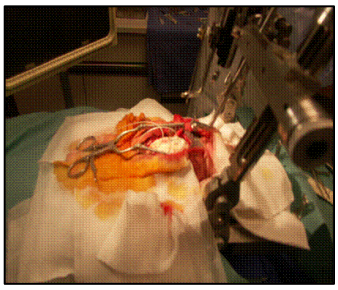
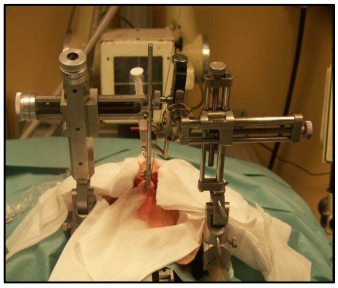


Figure 31: Paxinos and Huang Brain Monkey Atlas. VMH is in close relationship with the third ventricle in both sides, causing that only one electrode is needed for stimulation both sides. Intraventricular electrode is in the midline, or slightly in contact with one of the ventricle walls. Nucleus and nucleus capsule are between 1 to 2 mm from ependymal ventricular layer(Paxinos et al., 1999).

Implantation of the Electrode and implantable pulse generator





After making a midline incision, the skull was accessed bilaterally and 2 mm-diameter holes were drilled for the approach to the hypothalamic targets. A second rigid trocar tube [outer diameter(OD) 1.04 mm, inner diameter (ID) 0.89] mm; Phelps Dodge® High Performance Conductors HPC, Trenton GA USA) fitted with a stainless steel stylet was stereotactically inserted into the left brain hemisphere (right hemisphere were still holding the ventricular catheter to further contrast injection) by using a micromanipulator SM-15 Stereotaxic Micromanipulator coupled with IMS-3 Microinjector (NARISHIGE SCIENTIFIC INSTRUMENT LAB, Setagaya-ku, Tokyo 157-0062, Japan). The distal tip of the guide tube was placed at the Monro's foramen entrance. Several anchoring stainless steel screws (OD 1 mm, length 3 mm) were placed around the burr hole, and the guide tube was secured to Stereotaxic frame with Narishige electrode holder and micromanipulator (NARISHIGE SCIENTIFIC INSTRUMENT LAB, Setagaya-ku, Tokyo 157-0062, Japan). The stylet was removed, and the stimulating 4-polar DBS lead was stereotactically inserted through the guide tube using the micromanipulator. The distal tip of the inserted electrode protruded 4–5 mm from the tip of the guide tube. The Medtronic®, 3389 and 3388 DBS lead (Medtronic®, Richmond MN)) had at its distal end 4 90% platinum/10% iridium contacts. The 3388 has one contact of 3.5 mm long. The 3389 has 4 contacts, each one of them of 1.5 mm long separated by 0.5 mm. At the proximal end, the metallic contacts fit into a Medtronic

Extension #7495 that was connected to the IPG [Soletra® and Itrel I® (Medtronic®, Richmond MN, USA)] The lead was secured to the skull with acrylic and medical-grade silicone cements (DePuy CMW 3 Bone Cement, Johnson & Johnson Gateway Piscataway, New Jersey, USA.). Some days afterwards, a second surgery were made for battery implantation in the back of the monkey [Soletra® or Itrel I® (Medtronic®, Richmond MN USA)] allowing us to easy stimulate through the cage without the need of using primate chair or training, reproducing more clearly normal condition. The lead was connected to the extension cord tunneled subcutaneously to the back of the animal. IPGs were subcutaneously implanted between the scapula, tolerating unrestricted and free movement of the animal. Nonabsorbable sutures were used to secure the extension leads and IPGs. Observations were design to video tape feeding behavior, locomotion, eating time, and amount of regular and reward food ingested during trials. Monkeys were allowed to recover full strength and appetite after surgery for 3 to 4 weeks. After that period, they were put in observation cages and stimulated for periods of 5-6 hrs.

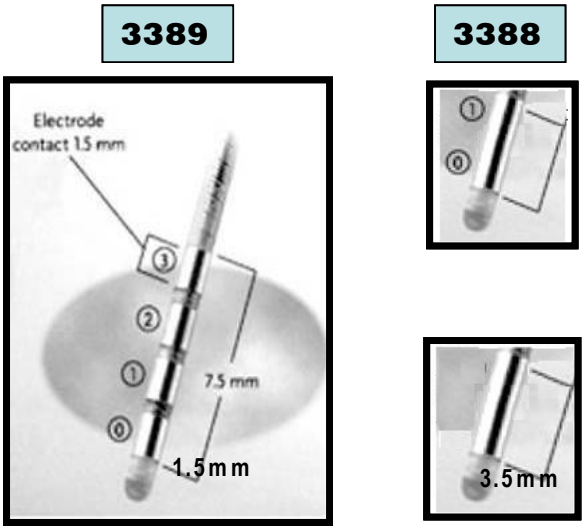


Figure 32: Electrodes 3389 and 3388 showing the actual active contact surface. 3389 has contacts of 1.5mm and 3388 has only one contact of 3.5mm, which is the equivalent to two contacts of 1.5 + 0.5mm space inter-contacts

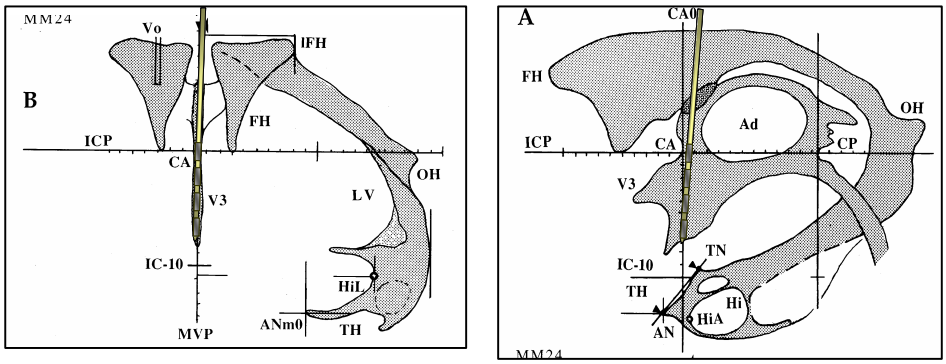


Figure 33: AP and Lateral views of Monkey Ventriculography showing actual electrode position. Ventricular walls are in close relationship with lead contacts. The antero posterior coordinate (y) was 2.3 mm posterior to AC. The dorsoventral coordinate (z) was determined by third ventricle floor. The lateral coordinate(x) was the midline of the third ventricle[modified from (Percheron, 1997)].

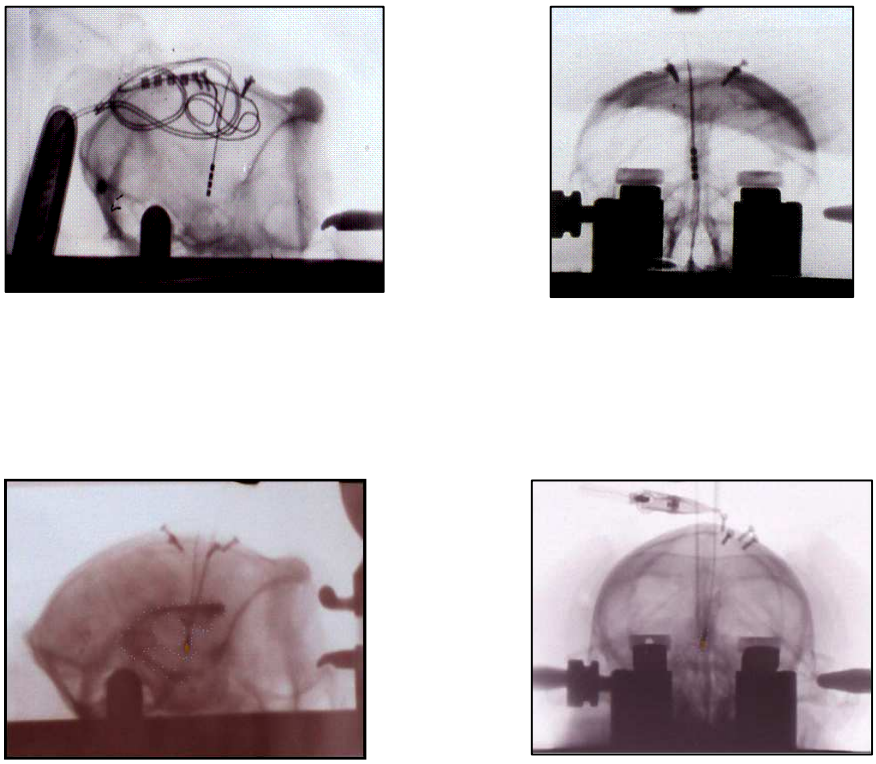


Figure 34: Frontal (a) and Lateral (b) view of the monkey head after implantation of the DBS Electrode with the head still fixed in the Stereotaxic frame. The four electrode tips can clearly been seen and the battery is lying in the back of the head to allow easy access to manipulation. The deepest contact is projecting to the VMH area. Monkey H5 and H10 were implanted with one quadripolar electrode Intraventricular at the level of the VMH/DMH nucleus (Medtronic 3389 1.5 mm long contact). Monkeys H7 and H8 were also implanted in the third ventricle at the level of VMH, but using a larger contact electrode (Medtronic 3388 3.5 mm long).

MRI SCANING, FINALS X RAY AND HISTOLOGICAL PREPARATIONS

Animals were anesthetized by intravenous injection of sodium pentobarbital (50mg/kg) and perfused transcardially with 0.9% saline followed by 4% buffered formaldehyde. After adequate fixation was attained, the head was careful removed. All the metallic elements were removed from the head in order to perform 3Tesla MRI before brain removal and histological analysis. The main goal of this procedure was to obtain a clear image of the DBS electrode before cutting the brain. The position of the DBS Lead in the ventricle makes it difficult to find in the histological anatomical cuts.

Before MRI scanning, AP and lateral x rays views for each animal's head were obtained in order to measure final contact position in relationship to Ventriculography landmarks and also to make sure there was no fixation screws in monkey head. The animal's heads were then placed in the 3 Tesla MRI (Drucker® Biospin).Whole brains were placed in a plastic bag filled with 4% PAF or neutral formalin and maintained in the scanner with foam pieces. Images were acquired in two positions: with the head parallel to the magnetic field and with the head perpendicular to the magnetic field. The head was placed inside the MRI such that the DBS lead was approximately parallel to the static magnetic field in order to reduce susceptibility artifacts in the images. All data were acquired on a Bruker Medspec 3-Tesla whole-body horizontal MRI scanner. We used a quadrate birdcage head coil (designed for humans) for RF transmission and signal reception.

High-resolution images were acquired with a RARE sequence (Rapid Acquisition with Relaxation Enhancement), see: Hennig J, Nauerth A, Friedburg H, "RARE imaging: a fast imaging method for clinical MR." Magnetic Resonance in Medicine, Dec; 3(6):823-33 (1986))(Hennig et al., 1986). Two image volumes were acquired with two different slice orientations: one transversal and one coronal in a plane parallel to the DBS lead.

Except for the slice orientation, acquisition parameters for both acquisitions were identical. We used: a field of view of 160x160mm, a matrix size of 320x320, (in-plane voxel size 0.5x0.5mm), 30 slices of 1.5mm thickness, echo time (TE) 13.5ms, RARE factor 8, effective echo time 54ms, repetition time 3500ms, number of averages (NA) 8, total acquisition time for one orientation 18min40s.

The relatively short effective echo time and the long repetition time result in images that are weighted by transversal relaxation time (T2) and proton density (PD). The T2

contrast allows distinguishing between tissue and cerebro-spinal fluid in order to visualize the ventricles. The PD weighting provides contrast between brain tissues, such as gray and white matter. Differences in T2 between tissues are weak in formalin-fixed brain, and T2 images with little PD weighting do therefore not provide satisfactory image contrast (Pfefferbaum et al., 2004)

After images acquisition, brains were removed, blocked, immersed in the 4% buffered formaldehyde for 24 hrs, and then placed in saline with the addition of 30% sucrose until the block sank. They were then sectioned coronally on a cryostat at a thickness of 50µm. Every section was collected and processed for routine cresyl violet staining and for lugol staining. Coronal cuts were reviewed and compared with Paxinos atlas for macaca mulatta (Paxinos et al., 1999). Using all those methods, we were able to localize the electrode position in relationship to the hypothalamus.

IMAGES ANALYSIS

The Images obtained through these methods (X-rays and MRI) were compared to each other and placed in a macaca atlas. We used macaca mulatta atlas of Paxinos and Huang during surgery and for the determination of the final positioning done using Ventriculography(Paxinos et al., 1999). Knowing ac-pc line and thalamus height values for the brain fixed in the atlas, we were able to normalized this atlas and then easily compare to the measures obtained in the monkey Ventriculography. In this way, it was easily positioned the active contact in relationship with coordinates of VMH.

Identifying electrodes contacts in the autopsy brain piece was done using the 3 Tesla MRI imaging. The study produced an easily identifiable artifact. The MRI image of the active contact was projected into the atlas also using MRI-visible landmarks.

With these two methods (MRI and Ventriculography) is possible to determine the actual final position of the active stimulating contact.

CHAPTER IX PHASE 1: ACUTE STIMULATION PROTOCOL

Phase 1 of the study was performed according to the experimental design in order to establish the most appropriate stimulation parameters. The principal goal was to determine the influence of different frequencies over food intake during acute stimulation using Intraventricular electrodes. The variability and complexity of the area requires ample testing of different stimulation settings in order to establish parameters that can be used during chronic stimulation and produce the desired effect. Other variables were also measured, including velocity, to assess the primate energy expenditure.

MATERIALS AND METHODS

Before acute trials were performed, adequate voltage intensities were tested in animals lightly anesthetized (Imalgene®, MERIAL Lyon France) *(20 mg/kg loading dose, 5 mg/kg maintenance im.)* Side effects at different intensities and frequencies were registered and voltage threshold for acute stimulation were established. After that, trials started as explained in chapter VII. Briefly, monkeys were kept in fasting for a period of 22-24 hrs. Stimulation started in the morning around 8:30-9:30 and was kept on during 6-8 hours prior to the trial. A fix meal, representing approximately total daily food intake was given at a fixed hour (1700-1800 hrs).

Using a Plexiglas transparent window, 150 g of primate laboratory chow biscuits along with fruits were given. A video camera (WV-CP470 Panasonic® Matsushita Electric

Industrial Co) was placed and connected to a desktop computer(Dell® Desktop Computers; Dell Products; One Dell Way; Round Rock Texas US) equipped with a special software(Mediacruised® Canopus) for video stock and digitization in order to conduct posterior analysis. An hour meal was register. Using these settings, different stimulations parameters were tested: A large spectrum of frequencies (between 0 up to 185 Hz) and voltage (0.1 to 2.5 V) were studied along with two different contacts(0 and 1 in electrode 3389 quadripolar and one contact in electrode 3388 unipolar) keeping fix pulse width at 0.6.

Time expend eating complete meal, meals size and Locomotion (cm/s) during stimulation were the parameters measured. 87 hours videos were stored and analyzed using special software for movement recognition (Ethovision ® Noldus Information technology)

Noldus® Ethovision® Software for Movement Recognition and Behavioral Assessment:

Image analysis was made using Noldus ® software. Identification was completed using grey scale and image subtraction. With a reference first image, the animal is identified and followed by a marker which measures the total distance and the velocity of the movement during the experience. Software took a reference central point in the body of the animal as an identification marker. One hour trial during meal was measured and total distance and velocity were calculated.

Figure 35: Schematic representation of experimental set up. Monkeys were video recorded during one hour during meal after 24 hrs fasting. Different frequencies and parameters were measured.

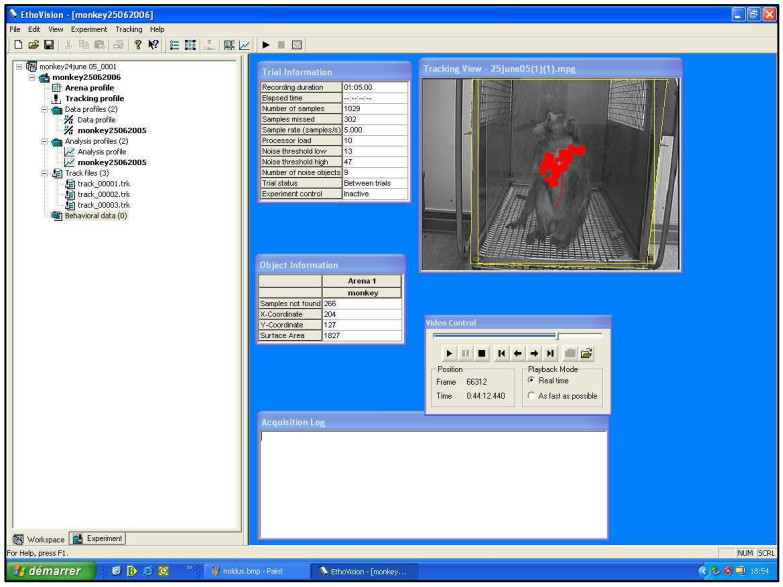


Figure 36: Off line analysis were conducted to determine locomotion as an indirect measure of energy expenditure. Changes in behavior were also close monitored

Animals (H5, H7, H8 and H10) were kept in observations cages and were free to move in his normal environment. Baseline food intake was reached after 2 to three weeks post operatively. Adverse effects connected to acute stimulation were observed under light anesthesia (ketamine) and recorded for all range of frequencies and voltages (0-5V), keeping pulse width constant (0.60 ms). Quadripolar 3389 electrode contacts 0 and 1 were tested for monkey H5 and safe voltage were set below 3v and 3.5v respectively. In

monkeys H7 and H8, using a single contact electrode, a range of voltage and frequencies were tested. Side effects are summarized in Table 7. H5 showed eyes opening and bilateral internal eye deviation in contact 0, 1 and 2 and neck contraction in contact 3. Monkeys H7 and H8 showed similar eye lids opening and binocular internal conversion along with neck-shoulder contraction at different frequencies and intensities.

RESULTS

Side Effect: During Acute Stimulation Of Intraventricular Electrode In The VMH Region In Macaca Fascicularis

We summarize in Table 7 the results of the acute side effects due to stimulation in Intraventricular VMH implanted monkeys. Two animals received quadripolar electrodes (H5 and H10) and the others were implanted with the 3388 Medtronic unipolar lead of 3.5 mm. Stimulation of this region generated motor ocular symptoms and muscular contraction of the neck and shoulder. The intensity threshold for side effects was different for each animal: ocular signs like blinking and bilateral eyes opening were seen at 3 to 3.5 V setting an appropriate level of voltage at to 2V

Table 7: Table shows the acute side effects for each monkey at each contact during testing. Animals were observed under light anesthesia

MONKEYS	CONTACTS	FREQUENCIES	VOLTS	SIDE EFFECTS
	0	50	3.5	Eyes opening
	1	50	4.0	Eyes opening
	2	50	4.5	Right eye opening
H5	3	50	4.5	Vertical nystagmus
		50	5.0	Neck & shoulder contraction
	0	10	3.0	Eyes opening
			3.25	Eyes opening , blinking.
		30	3.5	Neck contraction
		50	3.5	Neck & shoulder contraction
H7		80	2.50	Eyes opening , bilateral midriasis
				Bilateral inward eye deviation
			3.0	Bilateral inward deviation
				Eyes Opening
		130	2.00	Bilateral inward deviation and eye open
	0	10	3.0	Eye lids tremor and slow eyes opening
			3.5	Neck clonic contraction(frequency 10Hz
		50	3.0	Eyes opening,tremor in the shoulder
H8		80	2.5	Bilateral eyes opening
			3.0	Left Shoulder contraction
		130	2.5	Bilateral inward eye deviation
	0	30	5.0	Left side midriasis
				Eye lids tremor
		50	4.0	left eye lid elevation
				left eye aduction
		80	3.5	left eye lid elevation
				Left/right eyes aduction
		130	3.5	left eye lid elevation
				Left/right eyes aduction
	1	30	5.5	Left Shoulder contraction
H10		50		Left shoulder contraction
		80	5.3	Left shoulder contraction
		130	4.5	Left shoulder contraction
	2	30	5.9	Left shoulder contraction
		50	5.8	Left shoulder contraction
		80	5.6	Left shoulder contraction and omoplate
		130	5.0	Left shoulder contraction and neck mus
	3	30	6.1	Left shoulder contraction and neck mus
		50	6.1	Left shoulder contraction and neck mus
		80	6.0	Left shoulder contraction and neck mus
		130	3.8	Left shoulder contraction and neck mus

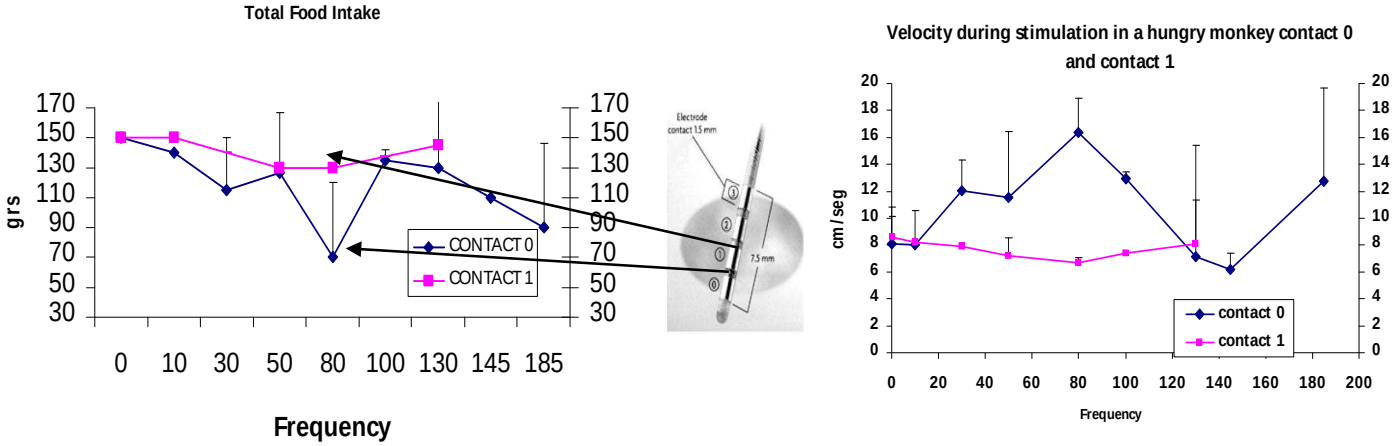
ACUTE RESPONSES TO VMH STIMULATION VIA INTRAVENTRICULAR

MONKEY H5:

Stimulation in contact 0 (located in the third ventricle floor adjacent to VMH) elicited a reduction in food intake in all frequencies with a maximum effect in meal size reduction at 80 Hz, 2V, 0.6 ms pulse width (reduction in meal size in off stimulation from 150 grs to mean 70 grs ± 50 grs). At a frequency of 185 Hz, additional reductions in food intake were seen (mean meal size was 90±56 grs from baseline of 150grs) (see Graphic 1). Eating time was between 14.5 to 20.77 min (Baseline at 15.07 ± 10 min) with no identifiable trend in frequencies. Locomotion expressed in velocity (cm/s) was recorded during meals using Noldus movement recognition software. There was, between frequencies 30 to 100 Hz, a significant increase in velocity during meals with a peak at 80 Hz of 16.37 ± 2.56 cm/s (baseline 8.06 cm/s). Another increase in velocity during trial was seen at 185 Hz to 12.75 ± 6.7 cm/s (see Graphic 1).

Contact 1, located dorsal to VMH and whose center is at 1.75 cm to contact 0, was explored, showing discrete reduction in meal size at 50 and 80 Hz (13.3% from the baseline). Eating time was longer at 80 Hz and velocity during meals did not change significantly. (Graphic 1)

Graphic 1 Total Food Intake and velocity after 24hrs fasting in VMH 3V stimulated Monkey H5. Comparaison between different frequencies and different contacts



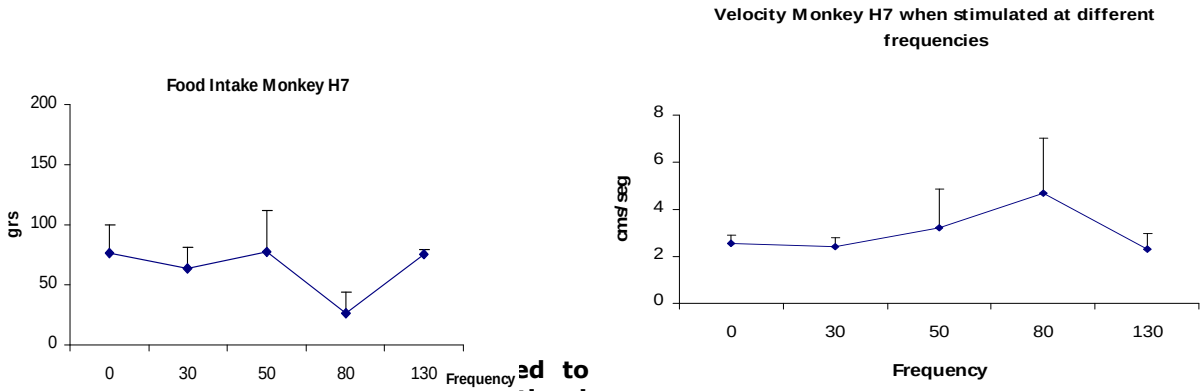
Graphic 1: Several trials were conducted to determine the more effective parameters settings for Intraventricular VMH stimulation. The first Monkey had an electrode 3389 with two plots in the hypothalamic basal area, being 0 the more ventral and 1 the more dorsal. In the graphic, important reductions in FI during monopolar stimulation of plot 0 were seen at 80Hz. Some effect was also elicited at the same frequency in the dorsal contact n°1 (13% reduction in FI). Velocity augmented during stimulation at different frequencies in contact 0 but remained constant when stimulating the more dorsal contact 1.

MONKEY H7:

Using a monopolar electrode 3388 Medtronic (3.5mm in height instead of 1.5 mm), several acute stimulation trials were completed in Monkey H7. Food intake was reduced at 80 Hz in relation with baseline (26.33 ±17.6 grs of FI in comparison with baseline 76.5 ±23.73 grs)Additional reduction was seen at 30 Hz (see Graphic 2). Variability in baseline food intake makes difficult to set an adequate “off” state level. Eating time, as in the previous Macaca H5, did not represent a clear trend varying between 39.12 to 48.88

min. Increasing speed in movements were found at 80 Hz (4.7±2.32 cm/s related to baseline 2.55±0.36 cm/s).

Graphic 2 Total Food Intake and velocity after 24hrs fasting in VMH 3V stimulated Monkey H7. Comparaison between different frequencies

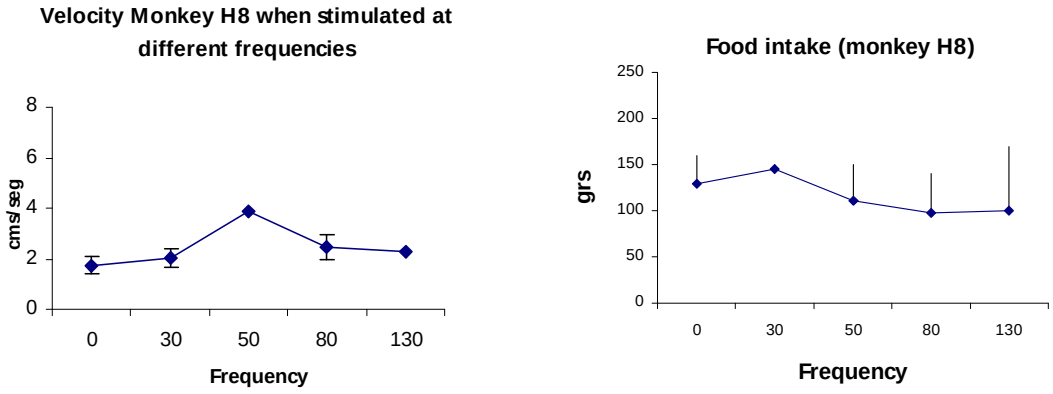


Gr ed to settings for intraventricular VMH stimulation ir
one contact-electrode of 3.5mm of height (Medtronic 3388). Figures show FI and velocity during each trial.

MONKEY H8:

Stimulation using monopolar one contact-electrode (3.5 mm Medtronic 3388) elicited a reduction in food intake at 80 Hz(97.5 ± 42.72grs from a baseline of 129 ± 31.30 grs) .Eating time remained between 51 and 61 min and velocity was increased at 80 Hz (2.46 cm/s from 1.75 cm/s when stimulation was off).

Graphic 3 Total Food Intake and velocity after 24hrs fasting in VMH 3V stimulated Monkey H8. Comparaison between different frequencies



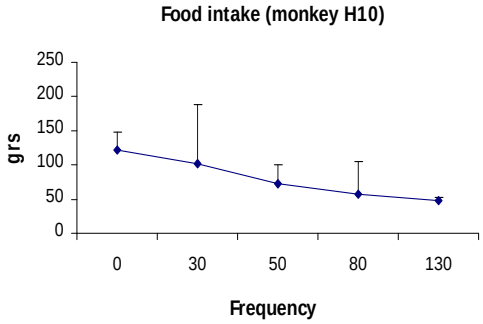
Graphic 3: Several trials were conducted to determine the more effective parameters settings for Intraventricular VMH stimulation in Monkey H8. This animal was implanted with a one contact-electrode of 3.5mm of height (Medtronic 3388). Figures show FI and velocity during each trial.

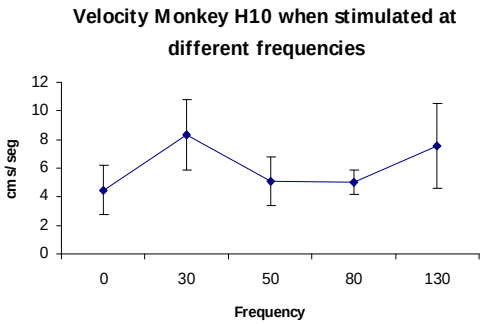
MONKEY H10:

In Monkey H10, quadripolar 3389 electrode was again used (contact surface 1.5mm). Stimulation in contact 0 (located in the third ventricle floor adjacent to VMH) elicited a reduction in food intake in all frequencies with a maximum effect in meal size reduction at 80 Hz (FI of 56.33 ± 49.09 grs) and at 130 Hz (FI of 47.6 ± 5.5) with a baseline FI of 122.55grs. Eating time was between 52 to 65.5 min (Baseline at 58.75 ± 5min) with no identifiable trend in frequencies.

Locomotion expressed in velocity (cm/s) was recorded during meals using Noldus movement recognition software. There was, between 30 to 130 Hz, a significant increase in velocity during meals with a pick at 30 Hz of 8.3 ± 3 cm/s and increases at 130 (7.54±3.59 cm/s). The baseline velocity during this monkey trial was 3.9 cm/s.

Graphic 4 Total Food Intake and velocity after 24hrs fasting in VMH 3V stimulated Monkey H10. Comparaison between different frequencies





Graphic 4: Several trials were conducted to determine the more effective parameters settings for Intraventricular VMH stimulation in Monkey H10. This animal was implanted with a quadripolar electrode, each active contact of 1.5mm. The contact used was the deepest one or contact 0. (Medtronic 3389). The contact 1 was not explored in this animal. Figures show FI and velocity during each trial

Effects of VMH Stimulation at Different Frequencies in Food Intake Expressed In Ratio (n/Baseline Fi)

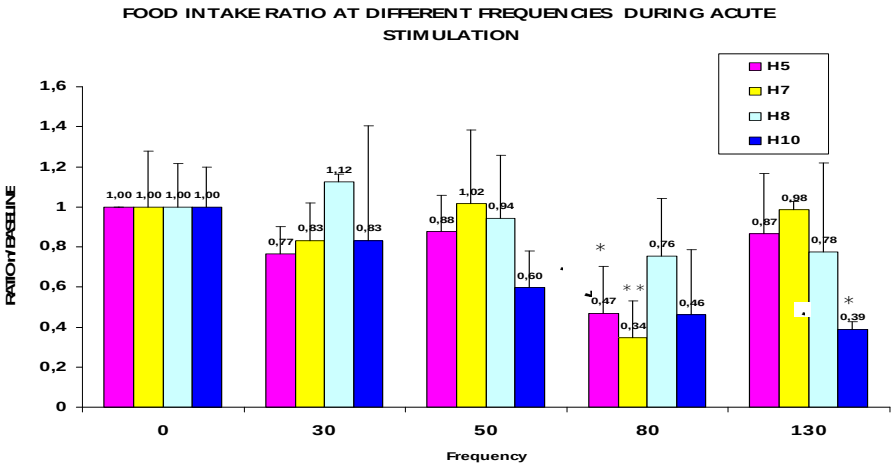


Figure 37: Food intake ratio (n/baseline) during acute trial after 24 hrs fast in monkeys implanted with an Intraventricular electrode. Baseline food intake was obtained after several tests in off stimulation. Different animals have different daily food ingestion. Monkeys H5, H7 reduced significatively the meal size when stimulated at 80Hz and H10 when stimulated at 130Hz **(** p< *0,01* *p< *0,05* **Kruskal-Wallis non parametric test with Dunn post test comparisons)**

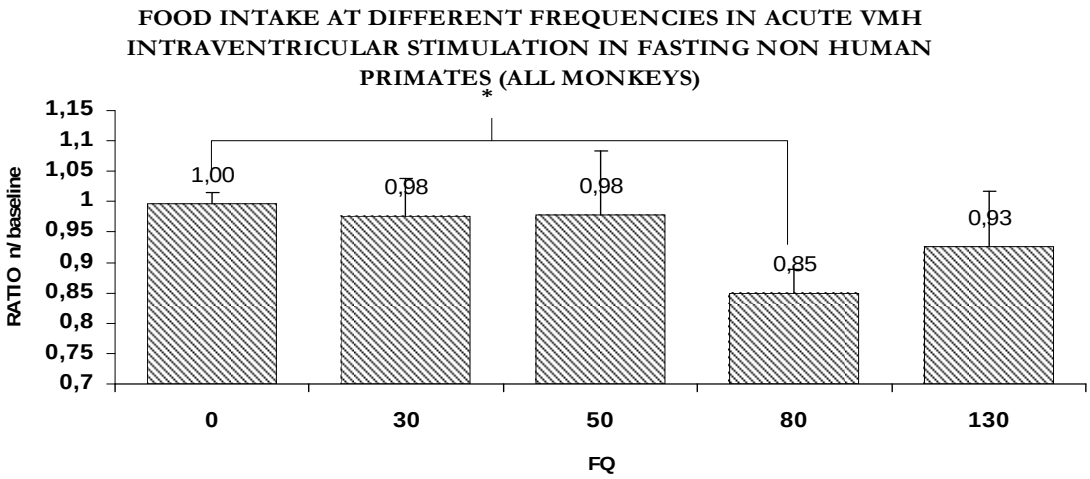


Figure 38: Mean change in meal size expressed in ratio (*n/baseline)* for VMH Intraventricular stimulated monkey at different frequencies, after 24 h fasting *p< *0.05* Friedman Test (Nonparametric Repeated Measures ANOVA) with Dunn post test comparisons) The P value is 0.0280, considered significant. Significative reduction in FI was seen at 80Hz stimulation when compared with baseline stimulation (0 means off stimulation)

Effects of VMH stimulation at different frequencies in velocity (cm/s) during postprandial period

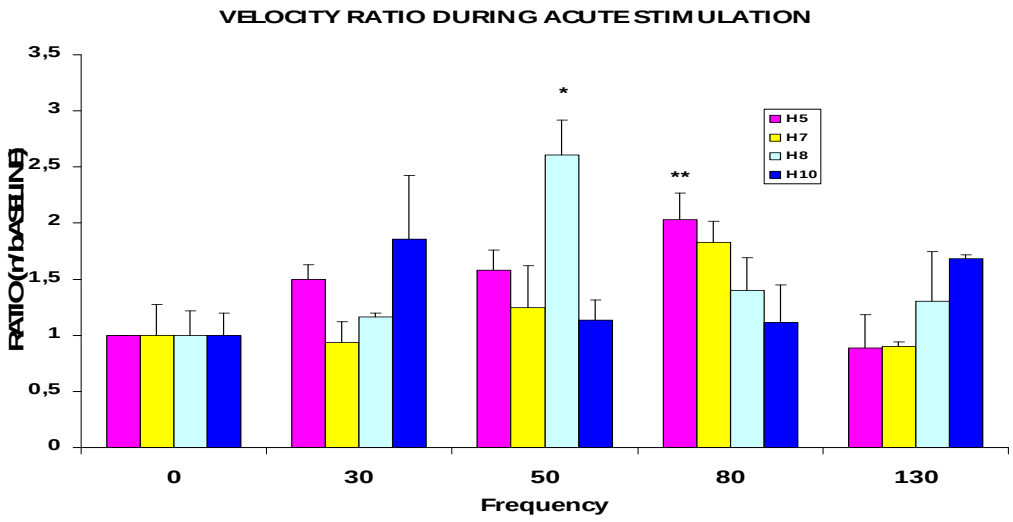


Figure 39: Velocity ratio (n/baseline) for each VMH Intraventricular monkey after 24 h fasting at different frequencies. A tendency to increase velocity during stimulation was seen in all subjects, and it was significant in H8 at 50 Hz and in H5 at 80 Hz. There was great variability between trials and subjects (* p< 0.05 ** p<0.01 Kruskal-Wallis non parametric test with Dunn post test comparisons)

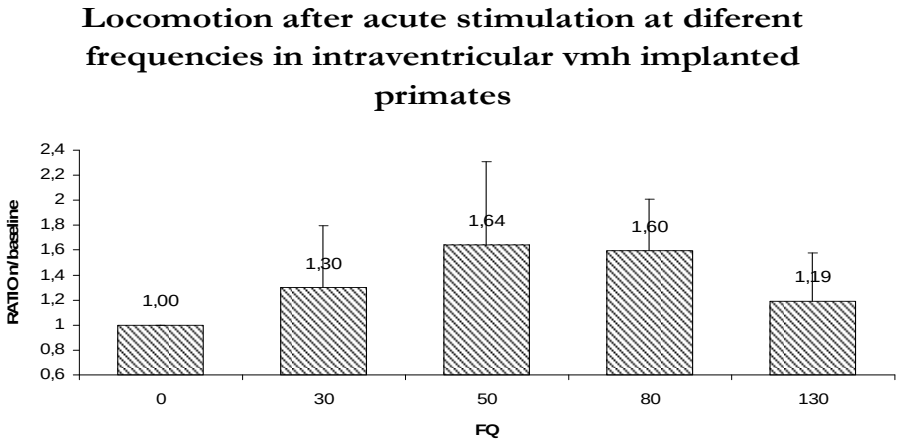


Figure 40: Mean change in velocity measured in cm/s ratio (*n/baseline)* for each VMH Intraventricular monkey after 24h fasting at different frequencies; no significative different was revealed between different frequencies(* p< *0.05* Friedman Test (Nonparametric Repeated Measures ANOVA) with Dunn post test comparisons)

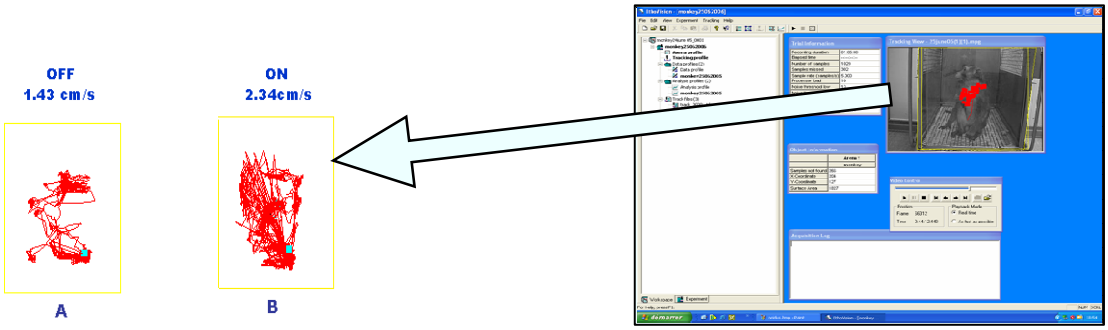


Figure 41 : Example of post trial analysis done after acute stimulation in hungry monkeys. Image A shows Monkey H8 movements during meal in off stimulation during one hour postprandial. Blue dot represents body centre and red line is the total trajectory of this centre during observation period. **Image B** shows H8 during on stimulation at 80 Hz. **In the right side**, there is an example of the behavior analysis done with Noldus observation software. It allows following the subject during trial and measuring several locomotion parameters. Behavior can be observed and readily record using keyboard commands. Total amount of biscuits left in cage also can be recorded.

In summary, the meal size was reduced at a stimulation frequency of 80 Hz in a ratio of 0.85 ± 0.04 from the baseline (or 15% reduction) when grouping all the data of all animals (*p< 0.05 Friedman Test with Dunn post test comparisons). Locomotor activity had a tendency of increasing when stimulating at all frequency, but no statistically significative difference were seen.

DISCUSSION

There is evidence to suggest that electrical stimulation of the Ventromedian hypothalamic (VMH) nucleus can produced reduction in food intake in fasting animals(Beltt and Keesey, 1975; King, 2006)The results of this study replicate earlier reports demonstrating acute decreases in food intake during electrical stimulation of VMH in food deprived animals(Beltt and Keesey, 1975; Takaki et al., 1992). However, this early reports were based on "*intraparenchymal*" electrode placements in VMH. Electrical stimulation was limited to the nucleus with special care of avoiding

surrounding structures. In contrast, we have introduced electrodes in the third ventricle adjacent to VMH and produced effects over the medial-ventral hypothalamic area.

The idea of stereotactically implant Intraventricular electrode adjacent to VMH was based on some important key points. Electrode introduction in ventral hypothalamic area is not a risk free procedure. Intraventricular electrode clearly avoids midline structures, thus inducing less risk associated to Transparenchymal passage. Using a single electrode to stimulate both sides theoretically reduce in a half the risk of complication associated to the introduction. Experience in Intraventricular electrode use has showed safety and marked effectiveness when stimulating nucleus proximal to the ventricular walls (Levy, 2003)Hosobushi et al and Richardson and Akril in 1977, reported effective pain relief after acute and chronic stimulation of the periacueductal and periventricular grey area at the level of posterior third ventricle in human patients (Hosobuchi et al., 1977; Richardson, 1982; Richardson and Akil, 1977a; Richardson and Akil, 1977b)Several studies have subsequently confirmed the phenomena: Intraventricular electrodes can modulate surrounding structures(Dieckmann and Witzmann, 1982; Hosobuchi et al., 1977)

The main finding in this study is that the observed effects over eating behavior were related to the stimulation frequencies. Even when using different types of contacts (1.5 mm vs. 3.5 mm long), the range of frequencies showed a marked decrease in total food ingestion at 80 Hz in all three subjects. These results differed from some previous reports showing marked feeding suppression at 50 Hz. (Takaki et al., 1992). Takaki and col. have found decrease in food intake in monkeys following trains of stimulation at 50 Hz, 100µA and 0.2 ms pulse width. His study, however was conducted using a different

type of electrode tip (30 µm diameter and 50 -80 µm long) implanted directly in the nucleus and using a bar-press feeding task instead of spontaneous feeding (Takaki et al., 1992)There is a fair amount of controversy regarding the results of VMH stimulation in monkeys. Although an electrical lesion placed in VMH in monkeys resulted in hyperphagia(Anand et al., 1955; Hamilton and Brobeck, 1964; Hamilton et al., 1976) an electrical stimulation in the same place did not constantly suppress the ad libitum feeding pattern even in partially satiated state (Robinson and Mishkin, 1962). Discrepancies between studies are thought to be related to the influence of the electrical field spreading to the surroundings structures with opposite effects, i.e. lateral hypothalamic area, arcuate nucleus(Takaki et al., 1992) .Our approach seems for the moment to advantageously stimulate bilateral medial structures like VMH, avoiding the lateral aspect of the hypothalamus and his anabolic effect. Surprisingly, high frequency stimulation (>130Hz) failed to show an increase in total food intake in the same fasting condition. In one monkey, food intake went to baseline, and in two other they were slight increase in food intake with a wide variability range. Monkey H5 even present a decrease in food intake at 185 Hz. Facilitatory effects on feeding behavior were not seen, may be due to experimental conditions.

Eating time was surprisingly independent of the different stimulation frequencies. In 1984, Brown and cols found that electrical stimulation in VMH can delay next meal in fasting dogs for a period ranging from 1 to 18 hrs (Brown et al., 1984). Our monkeys started eating at once, varying only the total amount of food meal. One explanation may be due to experimental design allowing monkeys to feed ad libitum with no bar press feeding task to be done. Hence, motivated behavior, an important part of the VMH

function, was not involved in feeding. In the same study, they have found that dogs, when stimulate twice a day for three day, reduced their daily food ingestion by 35% of the baseline (Brown et al., 1984). Electrode placement in the third ventricle could account for some difference as long as other nucleus in the area, notably arcuate nucleus might be modulated by the electrical field generated from the electrode.

Locomotion changed during meals under different stimulation conditions. Velocity (cm/s) increased at low voltages (<130 Hz) with a peak at 80 Hz frequency. At least 3 subjects presented a this increase at 80 Hz. Monkeys were having almost double the speed during and immediately after meals at 80Hz, when compared with baseline (H5: 16.37/8.06, H7:4.77/2.55, H8: 2.75/1.75 cm/s).Stimulation outside the region (H5 contact 1) failed to increase the velocity of the animal during meal (baseline 8.59, 80Hz on stimulation 6.71 cm/s), suggesting a clear involvement of ventromedian region of the hypothalamus.Only H10 was different, having maximal increase in locomotion at 30 Hz and 130 Hz, having only moderate increase at intermedian frequencies Studies suggested that stimulation bound activity was an important contribution factor to decrease weight gain and food intake during chronic VMH stimulation. .In rats, high activity groups (represented by running, jumping, climbing) during VMH stimulation showed significant reduction in food intake compare to control. VMH stimulation has been shown to both increase metabolic rate and facilitate locomotor's activity (Bielajew et al., 1994). Narita et al review of the literature on metabolic rate and locomotion suggested that VMH may be involved in the integration of motor activity and energy metabolism (Narita et al., 1993).In a study examining exercise metabolism, Vissing et al, looked at the relationships between VMH activity, running and substrate mobilization;

they speculate that exercise-induce increase in VMH activity may be regulated by the same CNS structures that also activate locomotion and substrate mobilization (Vissing et al., 1989). Pauwson have showed that when under sedatives/anesthetics, VMH stimulated rats reduced the otherwise remarkable increase in metabolic rate in 50%. It may be that muscle tone and activity were important contributing factors to the reduction in weight gain observed in chronic studies when rats were conscious and free to move about(Bielajew et al., 1994) . Large corps of evidence shows that VMH stimulation weight loss can not be solely attributed to a reduction in food intake, so VMH stimulation may augment energy expenditure. For example, studies carried on by Pauwson have shown that conscious rats receiving VMH and anterior hypothalamic stimulation increased more than 90% their metabolic rate (Bielajew et al., 1994).More recently, Challet has hypothesized that ventromedial hypothalamus (VMH) may also modulate locomotor activity. Using a test (wheel running) in rats with VMH lesion vs. sham operated, he has found that both groups have increased their activity during fasting but in VMH lesion group; this raise was significantly reduced and delayed. Motor activity plays a role in the stimulation induce reduction of food intake, even though correlation between food intake and velocity could not be established at the moment (Challet et al., 1995) .

The zone heterogeneity could account for the variability in the data presented (food intake, eating time and velocity). The stimulation in a region containing some connection more or less anabolic (like the NPY neurons connecting with arcuate nucleus) mixed with anorexigen neuronal highways (POMC) in a nucleus with a strong sympathetic connection can explain at least some failure to block the anorexigenic effects at high frequency stimulation (King, 2006). Low frequency stimulation did produce reduction in

food intake even when rewarding food was present with daily meal, in ad libitum feeding. Finally, stimulation in VMH induced increase in the motor activity represented by movements in cage during meals. The present study shows that Intraventricular electrode stimulation in VMH area could produced reduction in food intake in fasting monkeys in a frequency-dependent fashion and highlights the need of study their influence in weight gain in a chronic settings.

CHAPTER X PHASE 2: CHRONIC STIMULATION PROTOCOL

In the second phase of the study, the effects of chronic intraventricular stimulation in feeding behavior, global body weight, % body fat and Fat free mass were studied. Using parameter settings obtained in the first phase of the study, monkeys were stimulated following prefixed paradigms. The main objective of this experience was to evaluate the changes induced by three sets of frequencies in body composition. 130(HFS), 30(LFS) and the frequencies that produced maximum depression in food intake BFS (80) were tested. Complete fat related constants were obtained along with hypothalamic-pituitary axis function hormones in order to study changes in complex fat body and lean body composition. Food intake and stimulation induced behavior were all recorded and analyzed.

MATERIALS AND METHODS

BASELINE PERIOD

Monkeys H5, H7, H8, H10 and control (sham) H4 were kept in a regular chow biscuits diet and fruits, in order to establish a baseline food intake. During this period, monkeys are not stimulated and any “carry on” effect from the acute tests in the phase one should

decrease and eventually disappear. Baseline body constants are taken and several fat mass indicators established.

All data were collected after an overnight fast (12 hours) and with the animals sedated with ketamine (10-30 mg/kg IM). Blood samples were obtained following standard guidelines. Body weights were recorded in kilograms using a calibrated electronic scale (GSE, Chicago IL). Waist circumference was measured using a standard flexible tape with the animal lying on its back. The iliac crest was identified and the tape placed around the body just above the iliac crest and just above the umbilicus. Subscapular skinfolds and iliac skinfolds are measured with a special caliper. Fat mass and Fat free mass were obtained by bioimpedance (BIA) (see below section General principles of Bioimpedance Analysis)) using a Xitron multi-frequency bioimpedance analyzer (Xitron Technology Corp, San Diego, CA). The animal were placed in prone position (lying over their abdomen and sternum) on a plastic covered wood table and electrodes were attached to shaved areas on both wrists and ankles, and the proximal electrodes in the occipital eminence and base of the tail

PARADIGM 1:

Monkey H5 and H7 underwent Intraventricular stimulation using the optimal parameters (BFS) obtained in Phase 1 of the study (80Hz, 2volts, 60 µs) for a 8 weeks period .Monkey H8 and H10 received Intraventricular HFS (130 Hz, 2 volts, 60µs).Monkey H4 served as a sham (operated non stimulated control) subject: electrode was placed in third ventricle but not connected to the battery. As in baseline phase, Fat mass measured by BIA, weight, food intake, Subscapular and Iliac skinfolds thickness and abdominal circumference were obtained. Blood samples for glucose, cortisol, complete

electrolyte, total serum proteins were process for each subject at regular intervals. Plasma was separated and congealed to -80 degrees to further analysis (Leptin and hormones).

PARADIGM 2:

Monkey H8 and H10 underwent Intraventricular Stimulation using their optimal parameters obtained in Phase 1 of the study (80Hz, 2volts, 60 µs)for a period of 8 weeks .At the same interval of time Monkeys H5 and H7 received HFS (130 Hz, 2volts, and 60µs). Monkey H4 serve as a control (electrode placed in third ventricle but not connected to the battery). Measures were done as in the paradigm 1.

PARADIGM 3:

Monkeys H5, H7, H8 and H10, after a wash out period of 4 weeks, received Low frequency stimulation (30 Hz, 2 volts, 60 µs) during another 8 week period. Monkey H4 serve as a sham operated control (electrode placed in third ventricle but not connected to the battery).

Cross control in terms of architecture of the tip of the electrode will be achieved with these patterns of stimulation (H5 and H10 have electrode 4 contacts 3389; H8 and H7 have one contact electrode 3388). Between the paradigms, four weeks of non stimulation served to ensure the wash out of possible confounding effects.

General principles bioimpedance analysis

Bioimpedance analysis (BIA) measures tissue conductivity. Under stable conditions the conductivity of a body is directly proportional to the amount of electrolyte-rich fluid present. BIA can therefore be used to measure several fluid compartments, including Total body water (TBW),extracellular water(ECW) and Intracellular water(ICW). Fat is anhydrous and thus all body fluids, including the water present in adipose tissue, reside in the fat-free mass component.

The impedance of tissues is strongly dependent on frequency. At low frequencies the impedance of the cell membranes and tissue interfaces is too large for conduction of current within the cells to occur. As a result the current is conducted only through the Extracellular fluid. Thus the measured impedance is considered resistive with no reactive component (Lukaski, 1996). As frequency increases, reactance increases because the capacitant properties start to retard the current, and resistance decreases. At a critical frequency the reactance is maximal. At frequencies exceeding this critical frequency the current flow in the intracellular route will increase, as cell membranes and tissue interfaces start to lose their capacitive ability(Cox-Reijven and Soeters, 2000; Lukaski, 1996). This frequency dependence can be modeled empirically by a function. The most widely used model is the ***Cole-Cole equation***. A plot of reactance *vs.* the resistance at different frequencies results in a semicircular arc, the center of which is depressed below the real axis(Foster and Lukaski, 1996) Fitting the measured impedance data to this model the resistance at zero and at infinite frequency can be extrapolated which are the resistances of ECW and TBW respectively.

Several groups have found highly significant correlation between fat mass obtained by DXA and that obtained by bioimpedance (Cox-Reijven and Soeters, 2000) following the same procedure described here applied to non human primates (Comuzzie et al., 2003). Our only problem was that the presence of an electrode and the Soletra in the body of the monkey could produce some erroneous lectures. For avoiding that, we took advantage of the euthanasia of H3 and calibrate the bioimpedancemeter.

Calibration Xitron Bioimpedancemeter

Following the same protocol, Monkey H3 was measured with and without two Soletra and two extensions (104 grs total). Impedance in Extracellular water fluid (ECW) and Intracellular water fluids (ICW) where unchanged and total body free fat mass was calculated.

Calibration of Xitron bioimpedancemeter requires calculation of resistivity in ECW and ICW. To accomplish that, three trials of the afore mention procedure were done in each monkey and using Hydra acquisition utility software provided by Xitron Technology, we were able to calculate those constants. Then using published data about body composition in *Macaca fascicularis*, Fat Free mass and total fat content were calculated.

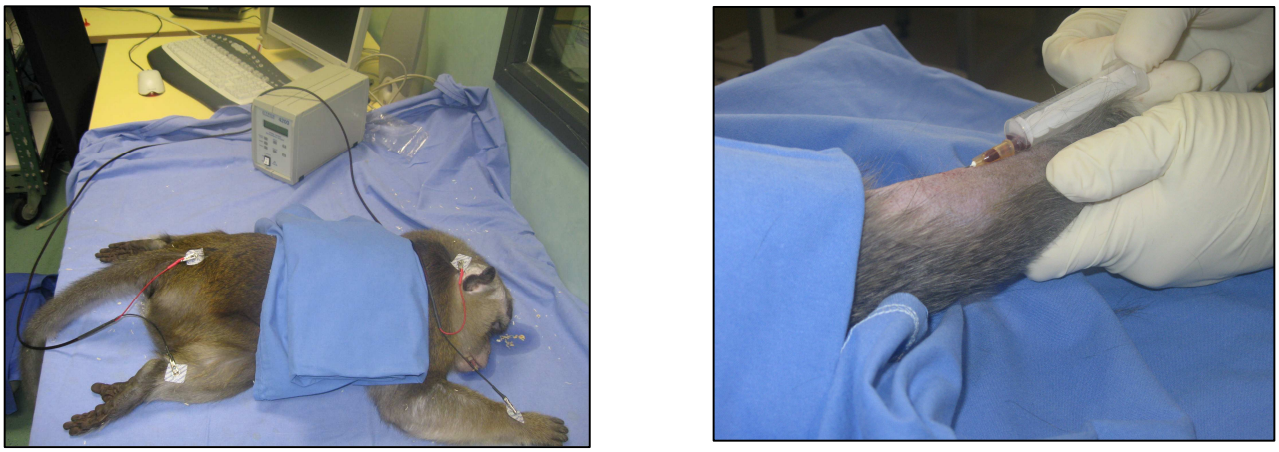
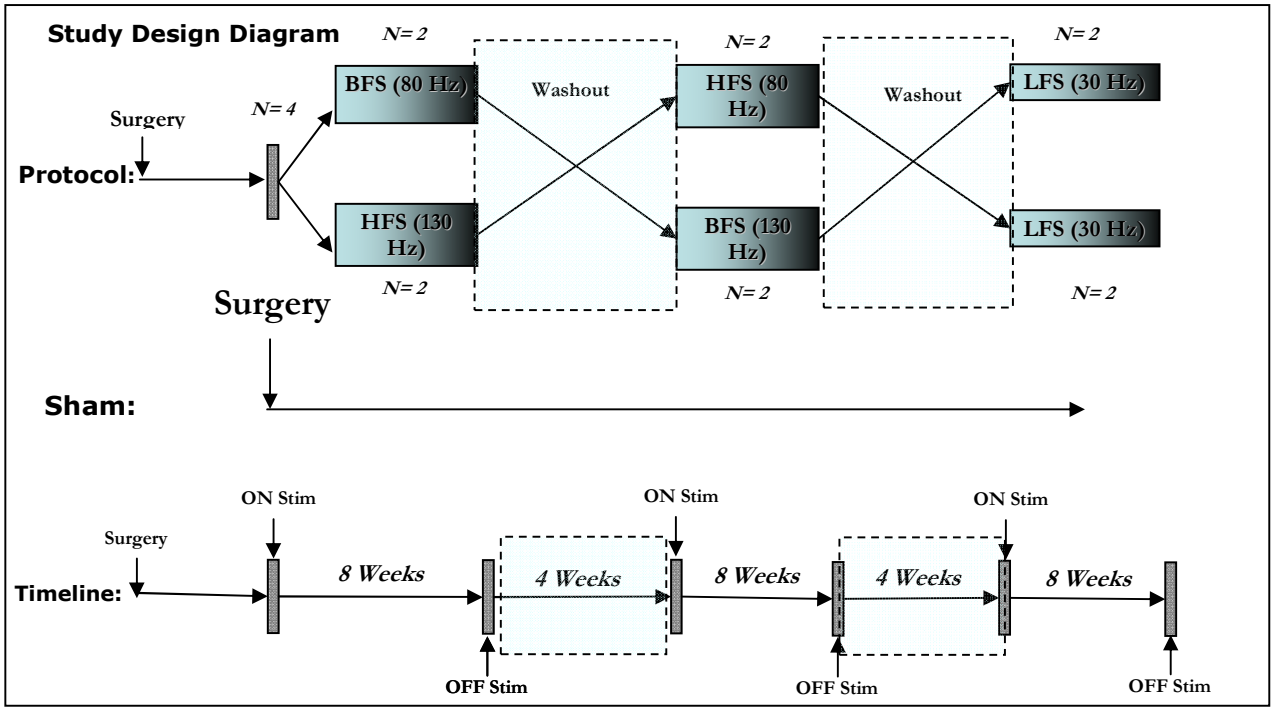


Figure 42: Images from the phase 2 or chronic protocol trials. Left: monkey in sternal recumbency during BIA testing. A multi impedance analysis is performed in order to calculate total body, extracellular and intracellular water. **Right:** in the same session, Blood samples were taken (all under general anesthesia)

RESULTS

CHRONIC STIMULATION PROTOCOL



WEIGHT, FAT BODY COMPOSITION AND INDIRECT FAT MEASURES

Animals (H4, H5, H7, H8, and H10) were weighted at regular intervals. At each time, intravenous anesthetics were used for sedation (Imalgene, lab Merial Lyon France) at 10-30 mg/kg loading dose and maintenance doses were employed if necessary. A good sedation is required for the adequate use of the BIA. The individual has to rest motionless and in a fixed position at least 15 min before and during the test to assure reproducibility. Wrist and ankles along with occipital and sacral areas were shaved and served as Xitron electrodes placement(Figure 42)

Using Hydra utilities, total body single measure multiimpedance analysis were carried out and results were stocked for later analysis. At the same time, abdominal

circumference, iliac and subcutaneous skinfolds measure and blood samples were obtained. The baseline values at the start of the study are depicted in Table 8:

Table 8 : *BASELINE CHARACTERISTICS OF THE ANIMALS*

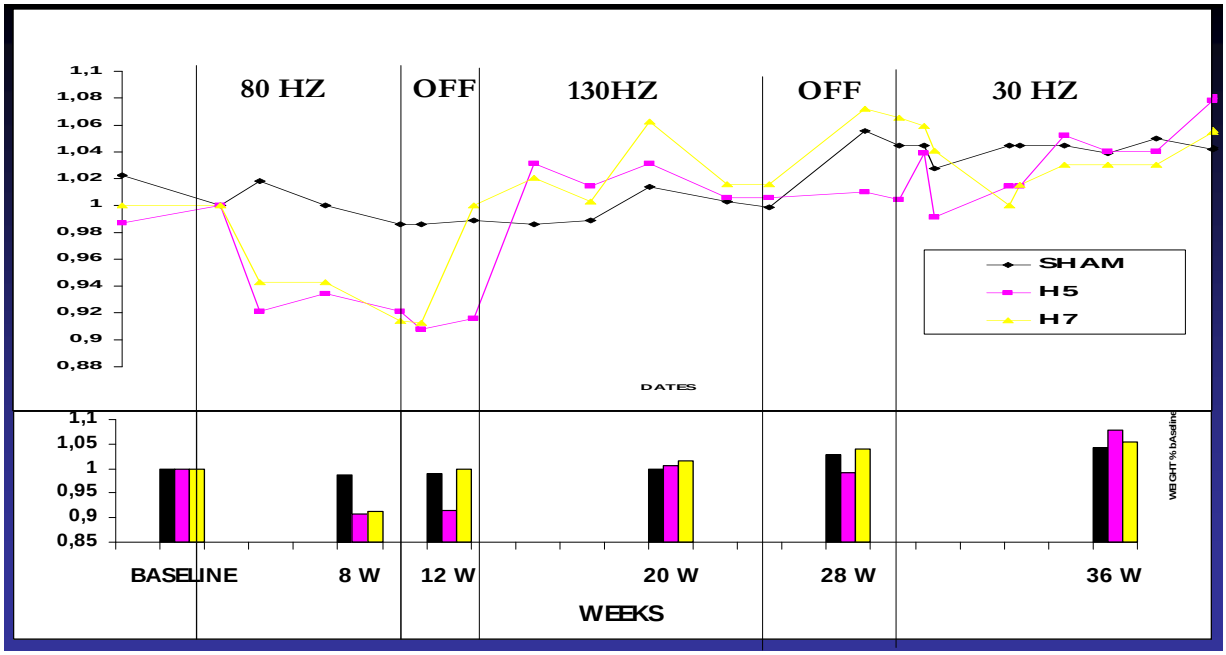
Monkey	Sex	Crown-rump Size (cm)	Weight(Kgs)	BMI	Abdominal circumference	Subcutaneous skinfolds	Iliac skinfolds	Food Intake
H4	M	37,00	7,20	52,59	45,00	6,92	16,55	340.82
H5	M	41,00	7,60	45,21	31,50	4,00	9,47	407,23
H7	M	39,00	7,00	46,02	36,50	5,36	9,87	410.95
H8	M	40.50	7,28	44,38	35,00	5,52	6,44	466,22
H10	M	41,00	6,10	36,29	32,00	4,60	3,55	376,36

Table 8: Baseline values characteristic in all 5 monkeys. The animal size was measured between crown (head) and rump (tail base) and used to calculate Body weight index. Food intake was an estimated averaged of daily intake of *ad libitum* food ingestion.

Changes in Weight and BMI During Chronic Stimulation

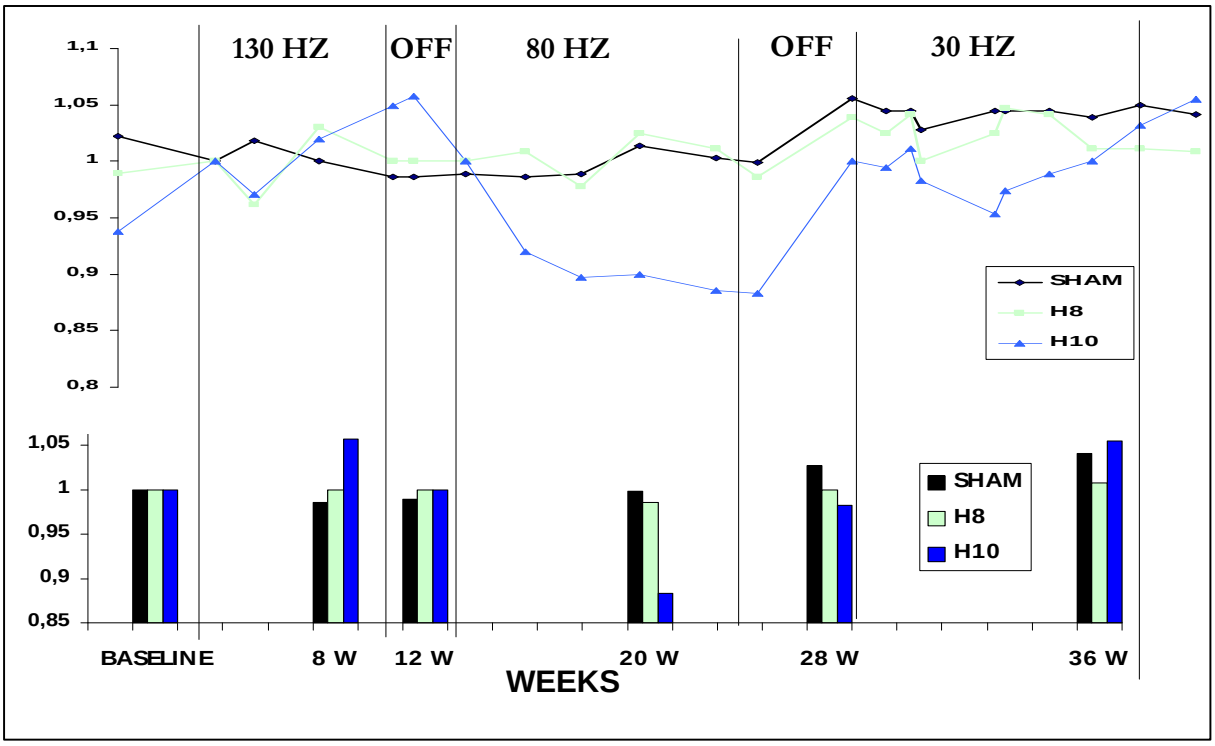
Changes in weight and BMI are divided in two groups: animals who received the BFS (80 Hz) HFS (130 Hz) and LFS (30Hz) (Graphic 5) and animals who received first HFS, then BFS and LFS (Graphics 6). Results are shown in ratio: weight divided by initial weight at the beginning of each paradigm (<1 meaning a decrease from initial weight/BMI and >1 meaning increase in weight/BMI from the baseline). A washout period of 4 weeks was completed between the stimulation paradigms.

Graphic 5: Evolution of the ratio weight/baseline at different frequencies during Intraventricular stimulation chronic protocol monkeys H5 H7 and sham



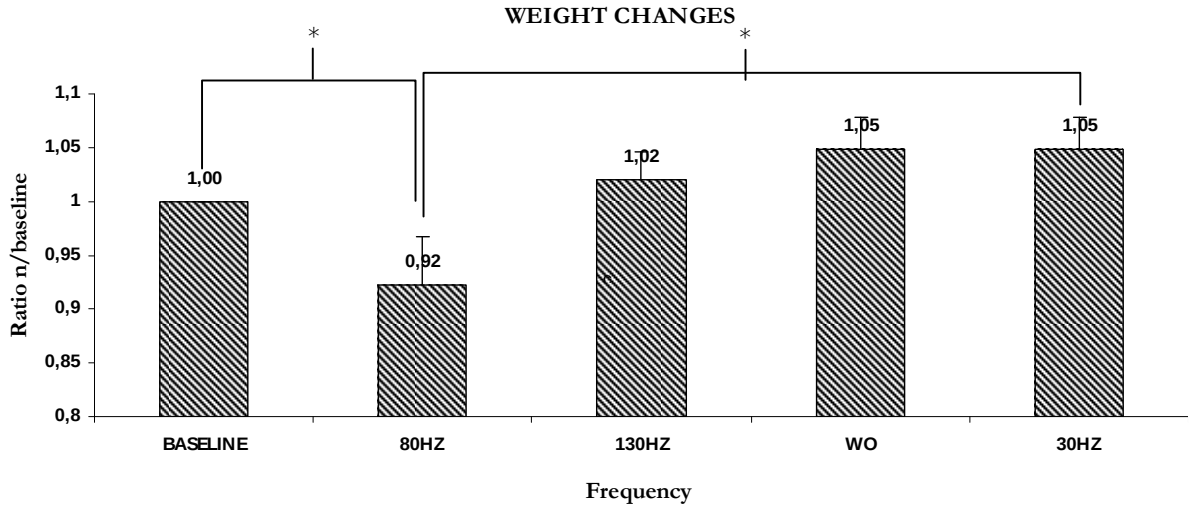
From baseline weight of 7.60 and 7.0 Kgs (BMI 45.21 and 46.02 respectively), a significant reduction of 9% (0.91 for both H5 and H7) in body weight occurred in BFS 80Hz in Monkeys H5 and H7 at the end of 8 weeks (w) (*$p<0.05$). During the washout period of 4 w, H5 monkey maintained the weight (0.92) but monkey H7 returned to baseline. Stimulation at 130 Hz (HFS) produced an increase of 1-2% in both animals. Finally LFS (at 30Hz) produced 8-5% (1.05-1.08) in weight gain.

Graphic 6: Evolution of the ratio weight/baseline at different frequencies during Intraventricular stimulation chronic protocol monkeys H8 H10 and sham

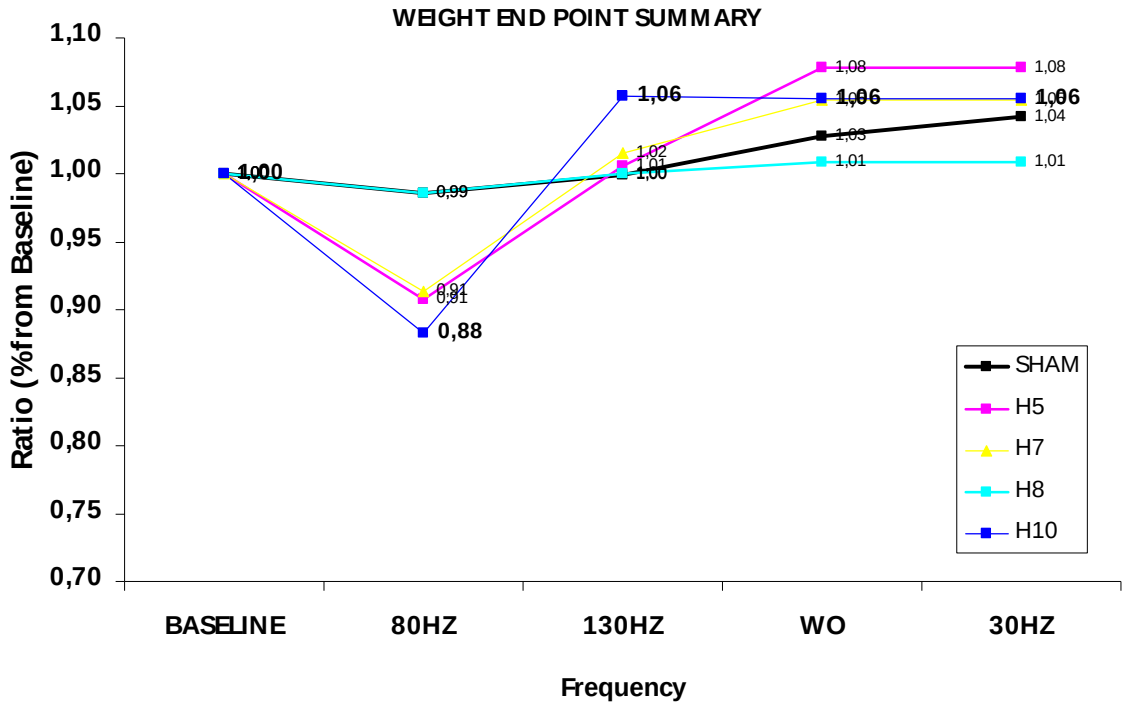


In the second group of animals (graphic 6), H10 increased body weight at HFS in 6% (1.06), returned to the baseline weight during the washout and lost 12% (0.88) body weight at BFS. At LFS, H10 increased again body weight in 6%. Animal H8 did not change during the whole study (0.99, 1.0 and 1.01 at 130, 80 and 30 Hz respectively). In non stimulated sham monkey, weight and BMI increased slightly (1.04) at the end of the study. Overall average reduction in BMI and weight was 8% (ratio 0.92±0.04) at 80Hz (*$p<0.05$) (Graphic 7). There is a non-responder animal (H8), weight/BMI did not change. When only the responder animals were taken into account, the average ratio weight/BMI loss during BFS (80 Hz) was 10% (ratio 0.91 ± 0.017).

Graphic 7 Changes in weight at different frequencies during Intraventricular stimulation chronic protocol non human primates



Average weight ratio (*n/baseline)* during chronic stimulation protocol at different frequencies (* *p< 0.05* ** *p<0.01* Friedman's non parametric paired test with Dunn post test comparisons). Baseline means off stimulation before the start of the study, and WO wash out period after paradigm II. Each period of stimulation lasted between 8 to 10 weeks. (continuous monopolar stimulation at 2 volts and 0.6 ms pulse width)



Changes in Indirect Fat Measures (Skinfolds and Abdominal Circumferences during Chronic Stimulation)

Indirect fat measures were obtained in fasting monkeys in sternal recumbency position, and shaving the areas used for measure (Figure 43).Subcutaneous subscapular skinfolds (SS) and abdominal circumference (CC) give an indirect indication of fat content and fat distribution in mammals and it has been associated in humans to hypertension and diabetes (Juhaeri et al., 2003; Larsson et al., 1992).Results have been summarized in Graphic 8. Skinfolds obtained in the iliac region (IS) followed a pattern of similar to the weight loss seen during 80 Hz Intraventricular stimulation. Average IS at 80 Hz was 0.69 ± 0.08 (**p<0.01), while at HFS (130Hz) IS was 1.26 ± 0.15 and at 30Hz was 0.88 ± 0.16. Results in abdominal circumference followed the same pattern with

more discreet effects: average AC ratio at 80 HZ was 0.95 ± 0.09, 130Hz and 30Hz were 1.06 ± 0.05 and 0.96 ±0.06 respectively. SS showed no changes during the 3 series of 8 weeks of stimulation (80, 130 and 30 Hz produced averaged ratio of 0.94±0.23, 0.96±0.11, 1.10±0.35 respectively).

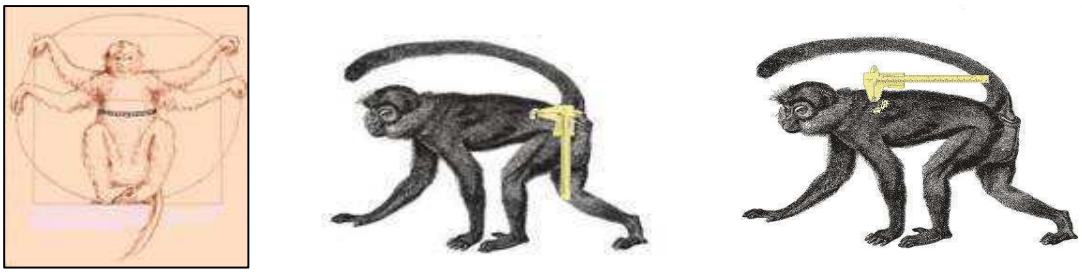
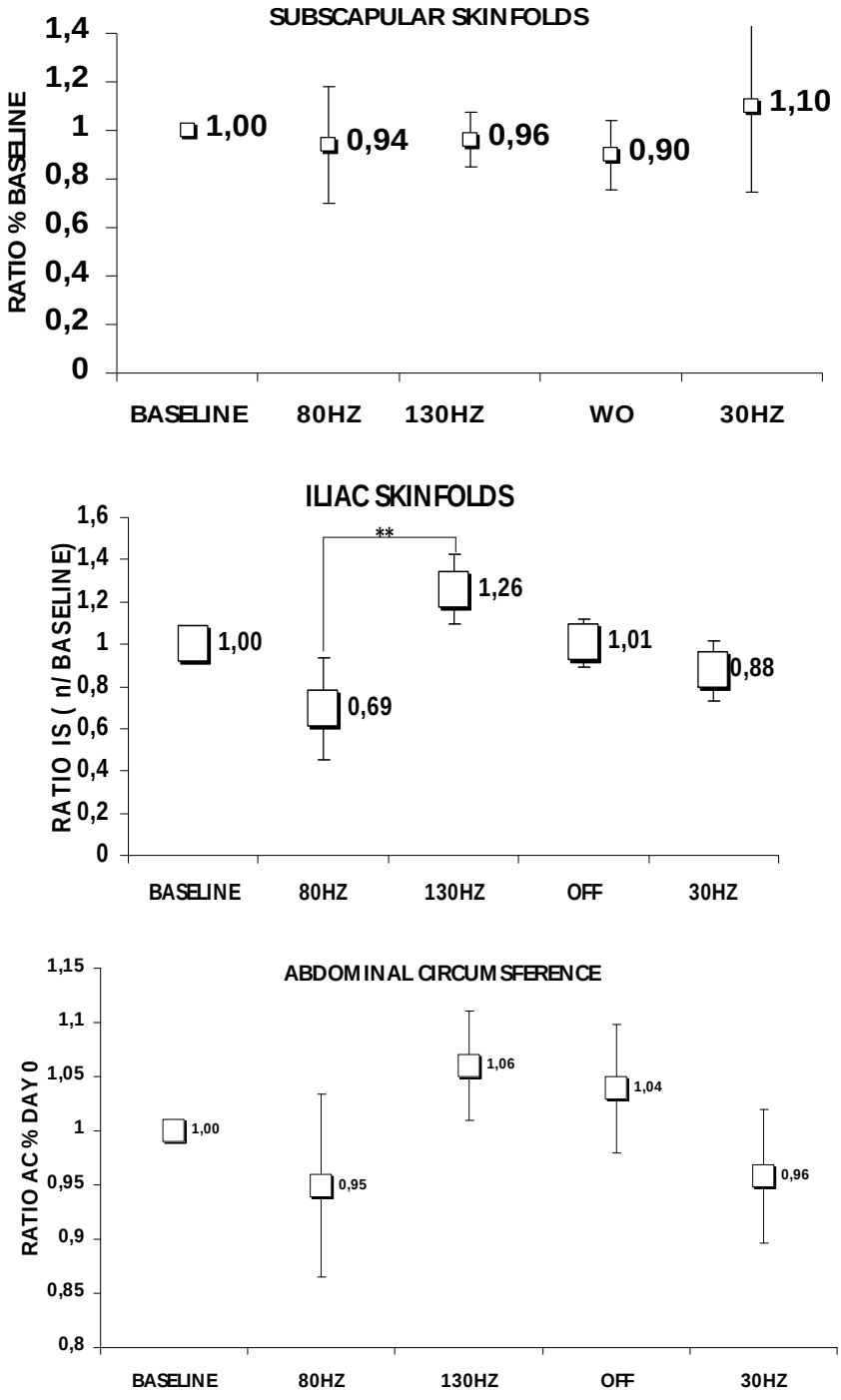


Figure 43: ABDOMINAL CIRCUNSFERENCE, ILIAC AND SUBSCAPULAR SKINFOLDS: With the primate in recumbent position over an isolating mattress, abdominal circumference was obtained using a tape meter, taking great care of passing the band over the supraumbilical area

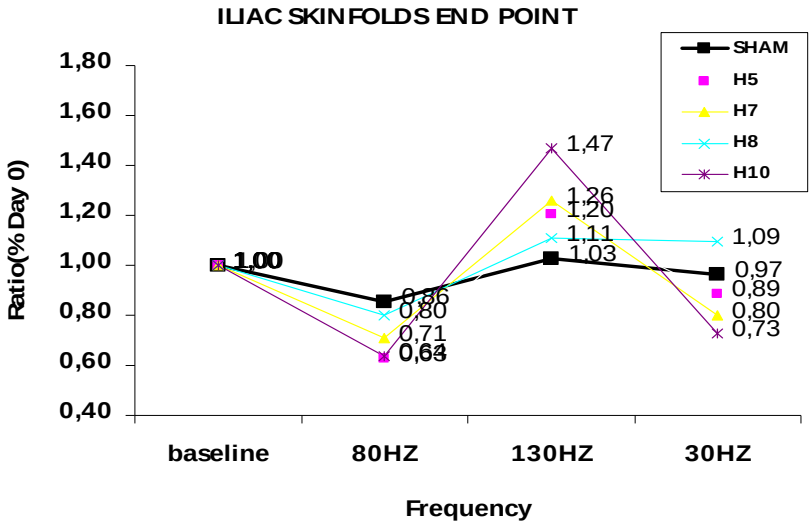
Graphic 8 Abdominal circumference and Iliac and Subscapular subcutaneous skinfolds during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



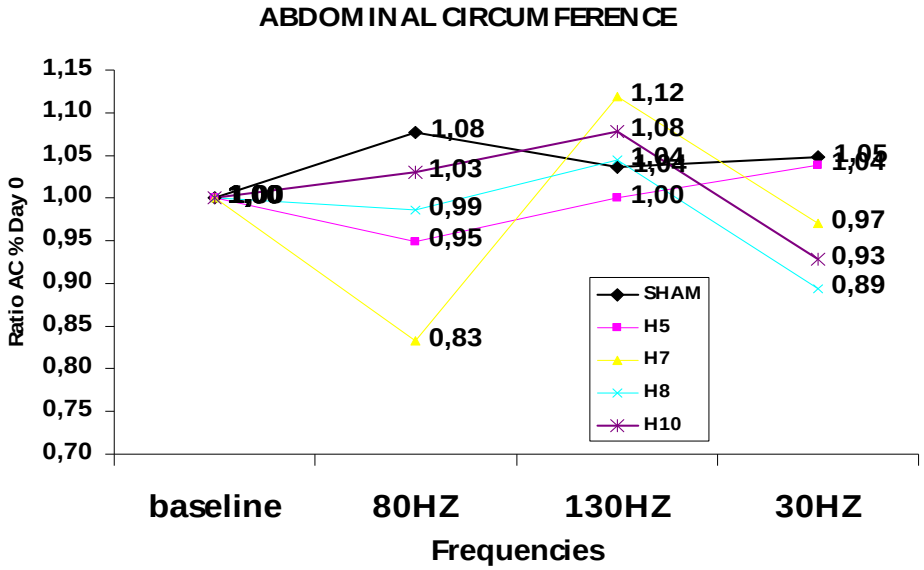
Considering the animals individually, results are shown in ratio: centimeters divided by the initial measure in day 0 at the beginning of each paradigm (<1 meaning a decrease from initial IS, SS or AC and >1 meaning increase from the baseline). A washout period of 4 weeks was completed between the stimulation paradigms.

Iliac skinfolds followed the trend seen in weight (Graphic 9). Monkey H5 reduced IS at 80 Hz to 0.63 (37% reduction), gained IS at 130 Hz (ratio 1.2) and reduced again at 30 Hz (0.89). H7 also reduced weight during 80 Hz (0.71) gained at 130 Hz (1.26) and reduced at 30 Hz (0.8). Less important H8 was only reduced to 0.8 at 80 Hz, gained 11% (1.11) at 130 Hz and also gained in IS at 30 Hz (1.09). H10 showed a sharp reduction in IS Ratio at 80 Hz (0.64), a rapid gain at 130 Hz (1.4) and finally a reduction at 30 Hz to 0.73 (IS is a ratio from the baseline-measure at day 0). Sham macaca had 0.86, 1.03, and 1.09 during the 3 consecutives periods of the duration of the trials.

Graphic 9 Iliac skinfolds changes during chronic stimulation for each monkey in V3 Stim *Macaca fascicularis*

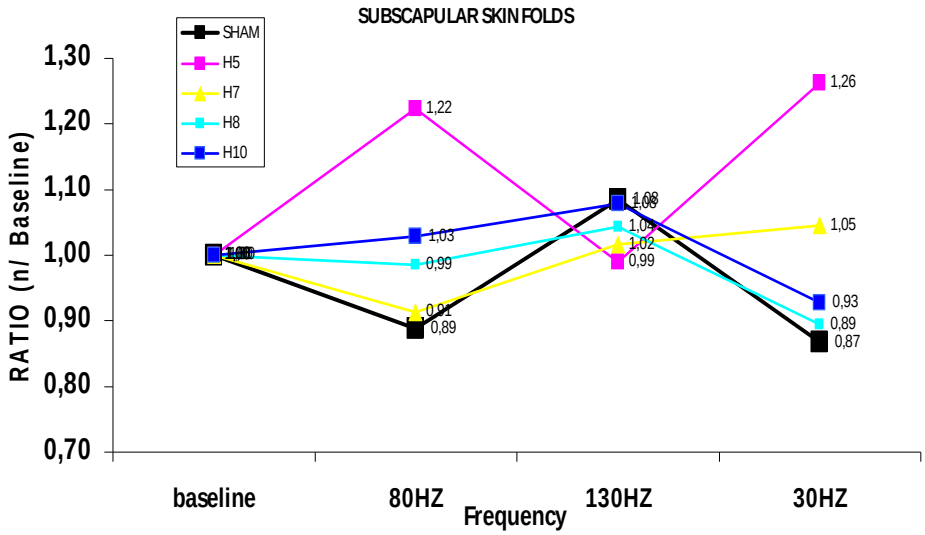


Abdominal circumference changes during chronic stimulation for each monkey in V3 Stim *Macaca fascicularis*



Abdominal Circumference (AC) when consider individual monkeys, had a large variability with a trend towards weight reduction at 80Hz. H5, H7 had a ratio of 0.95 and 0.83 respectively at 80 Hz, whereas H8 did not change(0.99) and H10 increased to ratio 1.03. At HFS (130) values were in baseline or superior for all monkeys H5, H7, H8 and H10 (1.0, 1.12, 1.04 and 1.08 respectively). At LFS (30 Hz) the results were (1.04, 0.97, 0.89 and 0.93 respectively). Sham monkey increased AC during the experiment from baseline, to 1.08, 1.04 and 1.05.

Subscapular Skinfolds changes during chronic stimulation for each monkey in V3 Stim *Macaca fascicularis*



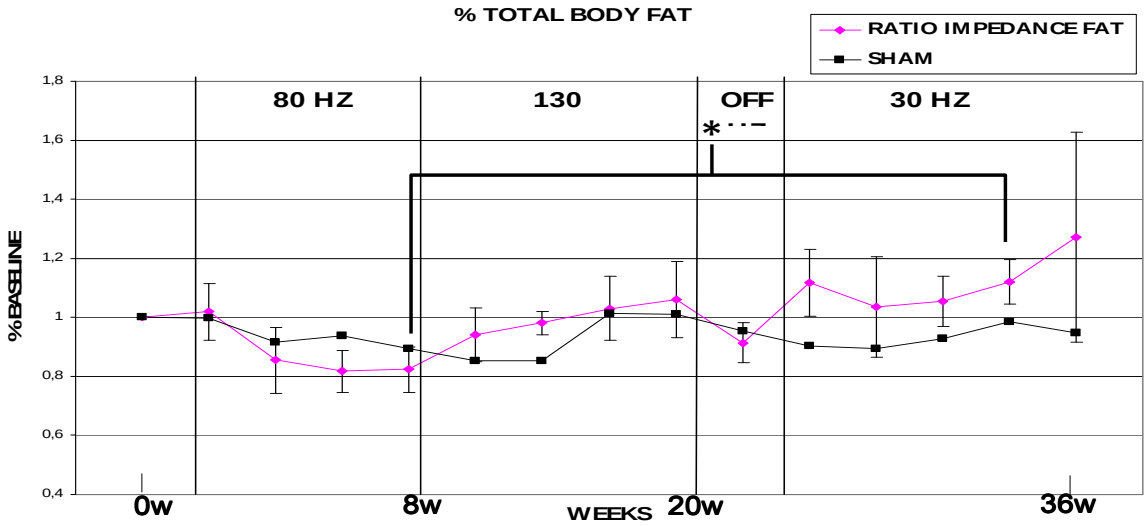
Subscapular skinfolds (SS) when consider individual monkeys, had a large variability. At BFS (80Hz) H5 had a ratio of 1.22(22% increase from baseline skinfolds), H7, H8 and H10 showed ratio of 0.91, 0.99, 1.03.At HFS (130) values were 0.99, 1.02, 1.04 and 1.08 respectively. At LFS (30 Hz) the results were scattered with an increase in SS in H5 at 1.26 and H7 at 1.05; and decrease in H10 0.93; H8 0.89 and. Sham monkey (H4) changed during the observation period with no specific pattern.

Changes in Fat Content Measured Using Bioimpedance Analysis (BIA) During Chronic V3 Stimulation

As explained above, animals were measured using BIA at regular interval during each 8 weeks stimulation period plus wash out. Bioimpedance analysis (BIA) measures tissue conductivity. Under stable conditions the conductivity of a body is directly proportional to the amount of electrolyte-rich fluid present. BIA can therefore be used to measure several fluid compartments, including total body water (TBW), Extracellular and intracellular water (ECW and ICW). Fat is anhydrous and thus all body fluids, including the water present in adipose tissue, reside in the fat-free mass component. This characteristic allows researchers to calculate the fat component in an organism.

The primates were anesthetized and put in recumbent position over an isolating mattress. Xitron® special skin electrodes were used in wrist and rump area. A period of absolute rest under general anesthesia of 15 minutes was allowed before the start of the test. The results are depicted in Graphic 10.

Graphic 10 % Fat content measured using BIA during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



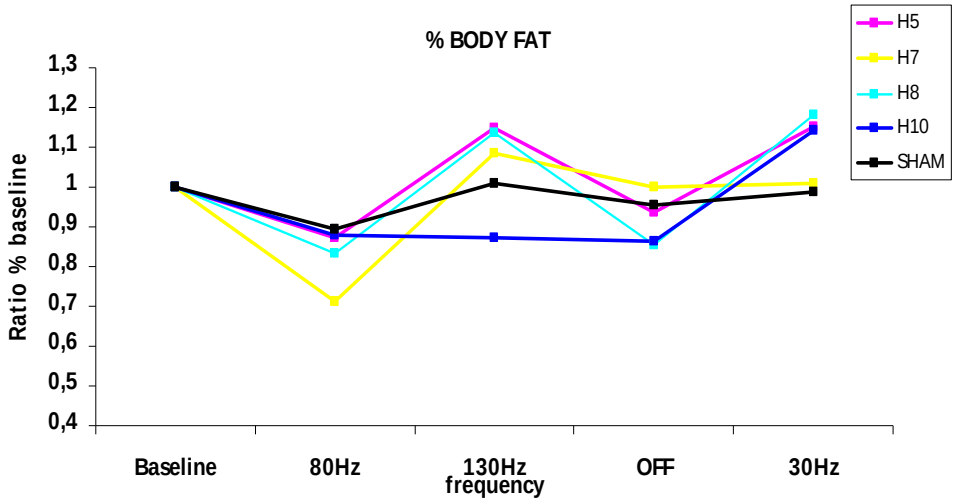
Average Ratio (*n/baseline)* Fat content during chronic stimulation protocol at different frequencies (* *p< 0.05* ** *p<0.01* Friedman's non parametric paired test with Dunn post test comparisons).

Fat content was calculated from the fat free mass obtained directly from Xitron bioimpedancemeter. Three trials per session were attempted and averaged. A reduction in the fat content was observed at the end of the 80 Hz period. A ratio of 0.82 ± 0.08 was seen at the end of 80 Hz period, so a statistically significative reduction of 18% in fat mass in animals, (p< *0.05,* using Friedman non parametric paired test with Dunn post test comparison). During HFS, we observed a tendency to return to baseline and during LFS (30Hz) an increase in fat mass, becoming significant at 8 weeks stimulation (1.12 ± 0.08*, P< 0.05*). Observation in non stimulated sham monkey showed changes unrelated to periods of stimulation.

Considering each subject individually, at 80 Hz a considerable reduction in body fat content was seen in all subjects(0,87; 0.71; 0.83; 0.88 for H5, H7, H8, H10); while

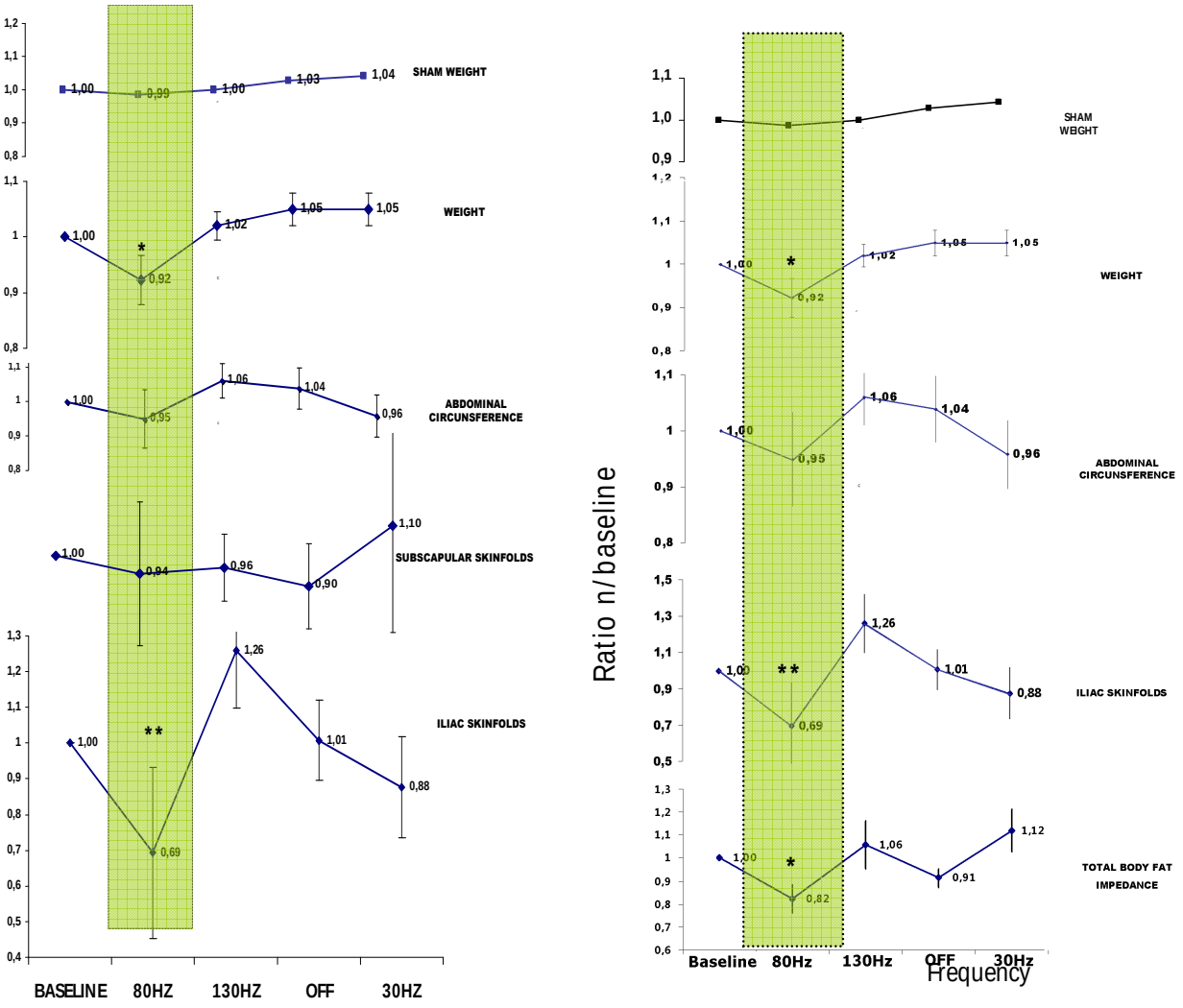
during low frequency(30Hz) there was mostly an elevation in fat content in relationship with baseline(1.15; 1.01; 1.18; 1,14 for H5, H7, H8, H10). During 130 HZ there was mixed results (increase in H5, H7 and H8 to 1.15; 1.08; 1.14 respectively; and decrease in fat content to 0.87 in monkey H10). Sham animal changes in fat content during chronic protocol showed little variability spreading between 0.89-1.01 (1.00 0.89 1.01 0.95 0.99 at the end of each period) (see Graphic 11)

Graphic 11 % Fat content for each animal measured using BIA during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Average Ratio (*n/baseline)* Fat content during chronic stimulation protocol at different frequencies. In the graphic, final point at the end of period is represented. 'Off' is the wash out period before Low frequency stimulation(30Hz). Bioimpedance testing was performed with animal in fasting and at the same hour under general anaesthesia

Graphic 12 Summary: Weigh and Indirect fat measures during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Average (*n/baseline)* weight, Abdominal circumference and iliac and subscapular skinfolds during chronic stimulation protocol at different frequencies (* *p< 0.05* ** *p<0.01* Friedman's non parametric paired test with Dunn post test comparisons). Baseline means off stimulation before the start of the study, and WO wash out period after paradigm II. Each period of stimulation lasted between 8 to 10 weeks. (continuous monopolar stimulation at 2 volts and 0.6 ms pulse width) First plot is the evolution in weight of sham monkey during the same 8 weeks periods

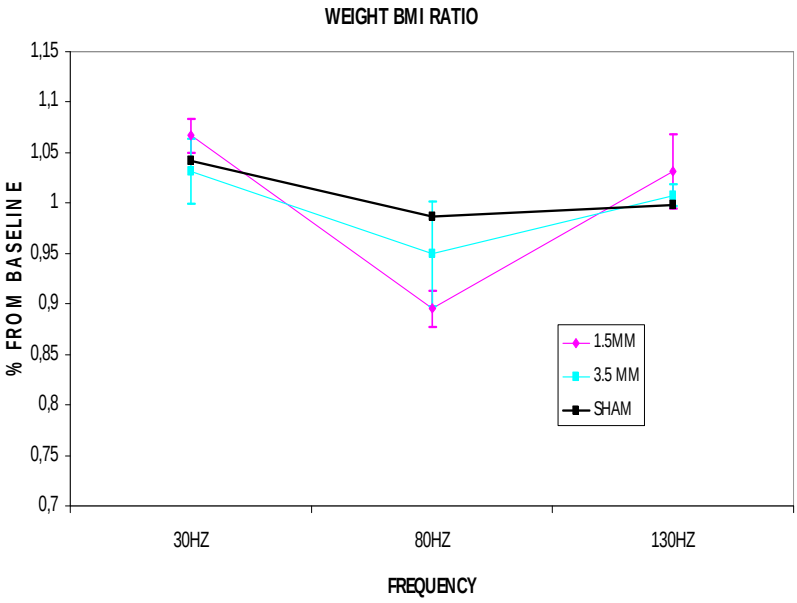
Comparison in weight and fat content using different electrode geometry

Animals were implanted with two different kind electrodes, allowing extensive coverage of the surrounding tissue. Two animals underwent stimulation using contact 0 (deepest possible) in the electrode 3389 Medtronic, which has a 1.5 mm in height, and the other two received stimulation thought a contact of 3.5 mm in height, using the electrode 3387 Medtronic. The current intensity was fixed to 2 volts and the rest of parameters were the same used during DBS for PD of monopolar stimulation (case+, contact -) and 0.60 msec in pulse width. The area of modulation of the electrical field was increased in height producing modulation to more dorsal structures in 3.5 mm than in 1.5 mm plot. Considering this difference, we analyzed the influences over Weight and Indirect Fat measures.

Weight and BMI is taken at the end of the 8 weeks period stimulation at different frequencies. Animals were assembled in two groups: each one of them having one monkey implanted with 1.5 mm and the other with the 3.5mm contact electrode. The cross over protocol was already explained. For analysis, the variables at the end of each period were divided by the baseline value at the beginning of each period. Weight/BMI results are showed in the following table and graphic

Table 9 Changes in weight considering contact size at different frequencies during Intraventricular stimulation chronic protocol monkeys

WEIGHT/BMI			
PLOT	**30HZ**	**80HZ**	**130HZ**
1.5mm	1.07	0.89	1.03
SD	0.016	0.018	0.036
3.5mm	1.03	0.95	1.01
SD	0.033	0.05	0.011
SHAM	0.998	0.97	1.04



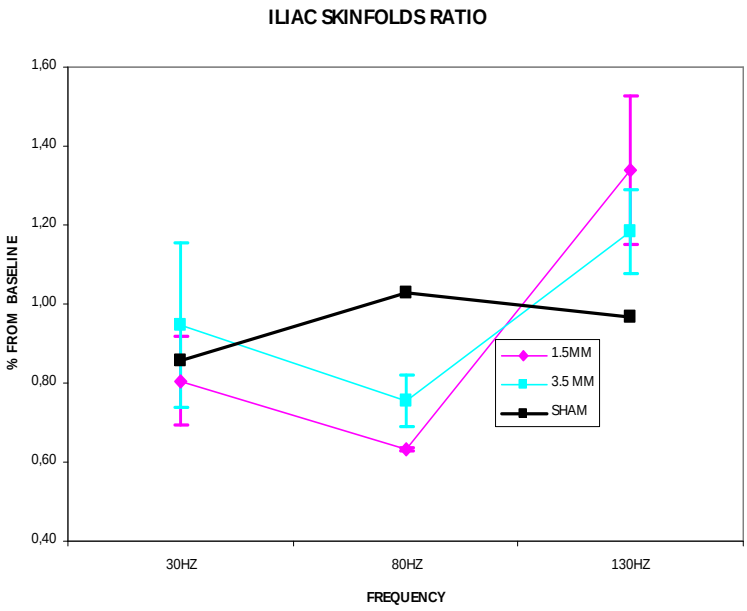
During effective weight reduction stimulation at 80 Hz, the contact 1.5mm produced 0.89± 0.018 (11%) reductions in weight/BMI in monkeys after 8w continuous stimulation. At the same parameters using electrode 3.5mm, reduction in weight was 0.95 ± 0.05 (5%), meaning an additional 6% weight loss when using the 1.5mm. At 30 Hz, increased in weight was seen in both plot 1.5mm (1.07±0.016) and plot 3.5 mm (1.03 ±0.033). Also at 130 Hz a modest increase in weight was seen for 1.5 mm (1.03±0.036) and for 3.5 mm (1.01±0.036). Sham monkey changed weight during the protocol, increasing 4% at the end of the 36 w of

observation. The trend seen in BMI and weight is found also when analyzing iliac skinfolds measures. Monkeys implanted with 3389 Contact of 1.5mm had an important reduction in IS during 80Hz to 0.63±0.01 (37% reduction). Monkeys implanted with the larger contact 3.5 mm had also a reduction (0.75±0.06) in IS but less marked (6% difference). There is a larger difference at HFS (between 1.34 ±0.19 and 1.18±0.11 for contact 1.5mm and 3.5mm respectively) and the difference at 30 Hz is also important (14%). (Table 10)

Table 10 Changes in IS in relation with contact size at different frequencies during Intraventricular stimulation chronic protocol monkeys

ILIAC SKINFOLDS			
PLOT	**30HZ**	**80HZ**	**130HZ**
1.5mm	0.81	0.63	1.34
SD	0.11	0.01	0.19
3.5mm	0.95	0.75	1.18
SD	0.21	0.06	0.11
SHAM	0.86	1.03	0.97

SS skinfolds and abdominal circumference did not show appreciable difference between contact 1.5 mm and 3.5 mm. In summary, analyzing the data regarding the different benefits in relation with the contact size, it seems that the 1.5 mm contact produced 6% more reduction in weight and IS than the 3.5 mm plot.



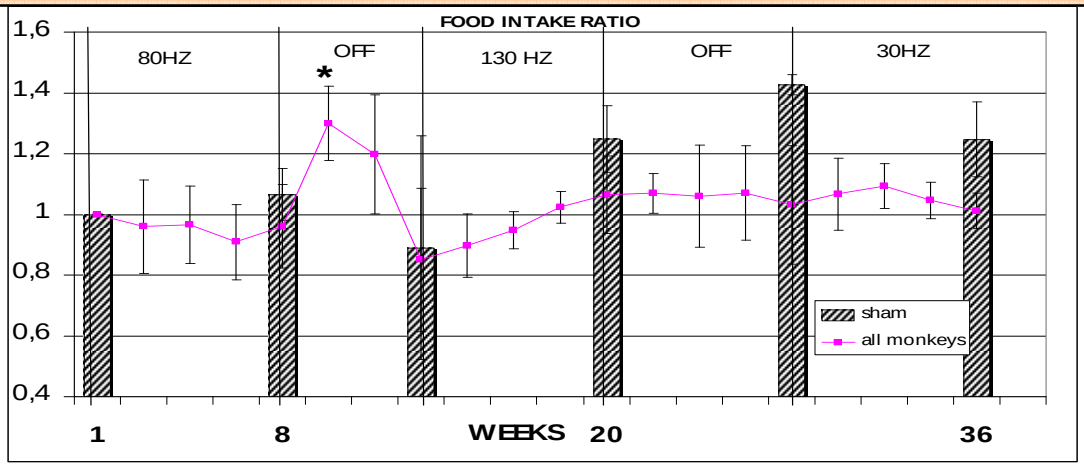
Number of observation is very limited (n=2) to find something significant between the two groups. Also the fat content did not follow the trend towards a more effective small electrode, because at 80Hz the 3.5 mm produced 0.77±0.09 or 23% reduction in fat measured by bioimpedance, instead the 1.5mm produced 0.87±0.004 or 13% reduction in fat. A more important number of subjects are necessary to answer this question about which active surface is more appropriated to produce the catabolic effect.

Changes in food Intake during Chronic V3 Stimulation

Animals were fed with more than the required daily allowance: fruits (300grs) and primates chow biscuits (200-250grs), in order to attain a food intake as *ad libitum* as possible. A daily account of food left allows us to calculate total food intake. Water was not measured due to technical problems with the water dispenser (which is easily reachable by the monkey and present leakages that makes measure difficult). Baseline food ingestion was reached before starting chronic stimulation protocol. During almost two month careful observation in behavior allowed us to determine for each individual

meal preferences and quantities. Food ingestion depends in several factors and can be very variable inter-individually. The way chosen to compare different states was to measure in *off* stimulation the food intake during two month and then calculates ratios in relation with this off period. Results will be express as an increase in FI when >1 or a decrease when <1. Results are shown in Graphic 13

Graphic 13 Food intake ratio during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*

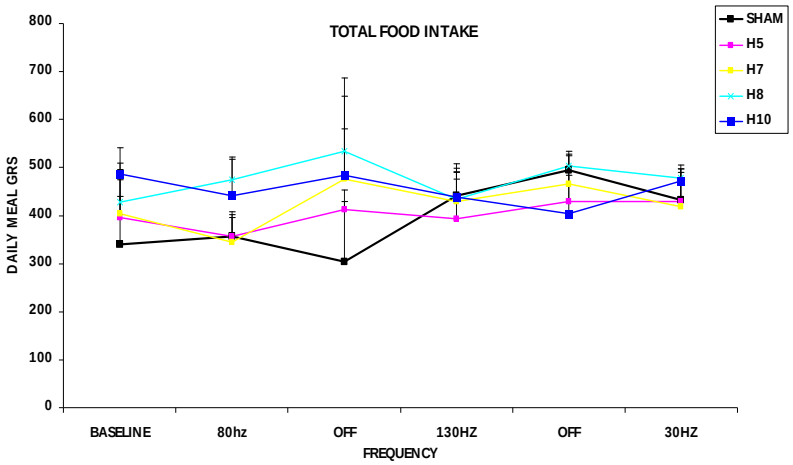


Average food intake during trials and washout periods in stimulated and sham control subjects Ratios were obtained using two month off-stimulation prior to the beginning of the trials as a baseline FI. There is a significative increase in FI during WO period after effective weight reducing stimulation of 80 Hz (* $p < 0.05$ ** $p<0.01$ Friedman's non parametric paired test with Dunn post test comparisons).

A significative increase in FI was observed in subjects during wash out period after Efficient V3 stimulation at 80 Hz frequency *(1.29* ± 0.12 p*<0.05*). This hyperphagia lasted the time of the wash out period, descending sharply at the beginning of HFS 130 Hz (0.90 ± 0.10 at week 14) and then keeping around baseline during the rest of the trials. Sham monkey had a tendency of increase food intake through the study from the baseline. Considering each monkey individually, we have represented de average food intake in monkeys during each period of stimulation at difference frequencies. At the first glance, it is possible to realize that total daily ingestion lies between 300 to 600 grs in all

monkeys. In the off period after effective weight reduction, an increase in food ingestion is seen in all animals except sham. In the rest of the periods, average food intake has maintained stability and less variability. (See Graphic 14)

Graphic 14 Average FI in grs during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Average food intake during trials and washout periods in stimulated and sham control subjects Total Food intake is displayed in average FI during each of the periods. It is possible to see some increase in FI after effective weight reduction (after 80Hz stimulation) except for sham monkey

LABORATORY ANALYSIS, BLOOD CHEMISTRY

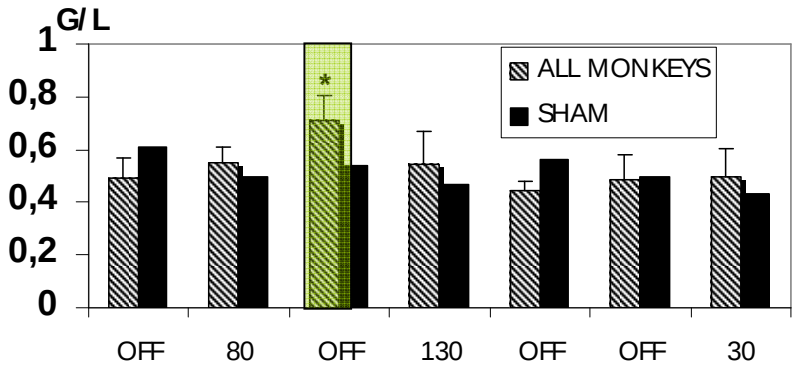
Plasma samples were taken for blood chemistry analysis during trials and processed using Hospital protocol and equipment. Animals were sedated with ketamine and disposed in a mattress as described for impedance analysis and skinfolds measures. Intravenous sampling was done using femoral veins at inguinal level or small saphenous vein in the posterior leg. At each trial, 1 ml of blood was withdraw and centrifuged (3500 rpm during 10 min at 4℃) and then separated in two 500 ml samples, one send to the Biological Integrated Laboratory in the Grenoble Universitary Hospital and the other stored at -80 ℃ for further analysis. Glucose, pro teins and electrolytes were directly

processed and Hormones and Leptin waited until the end of the study at -80° C to be processed. The Laboratory of « *Hormonologie Pédiatrique et Maladies Métaboliques Hôpital Saint Vincent de Paul* » collaborated with the project measuring serum Leptin and Testosterone.

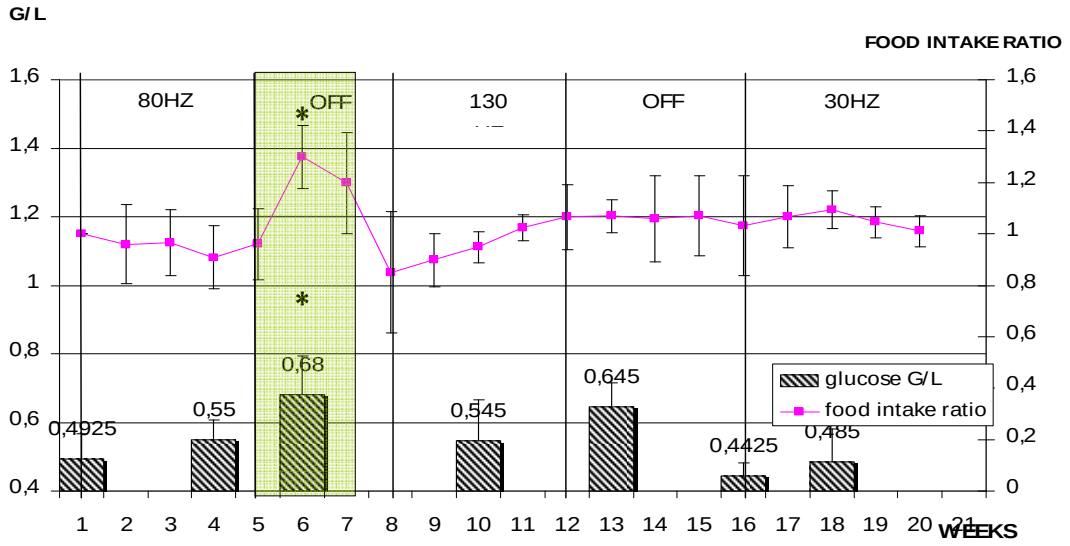
Changes in Blood Glucose and Electrolytes

Changes in blood glucose and electrolytes are summarized in Graphic 15 and 16. Glucose is represented in G/l. The first wash out period, after the statistically effective weight loss trial (80Hz), presented an increase in plasma glucose to 0.68 G/l ($p<0.05$ Friedman's non parametric paired test with Dunn post test comparisons). This rise in plasmatic glucose corresponds to a hyperphagia period when animals regained weight after effective 80 Hz stimulation (Graphic 16). Glucose results maintained low normal values during the rest of the study (reference values for macaca 0.66 ± 0.15).

Graphic 15 Serum glucose during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Graphic 16 Serum glucose and Food Intake during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



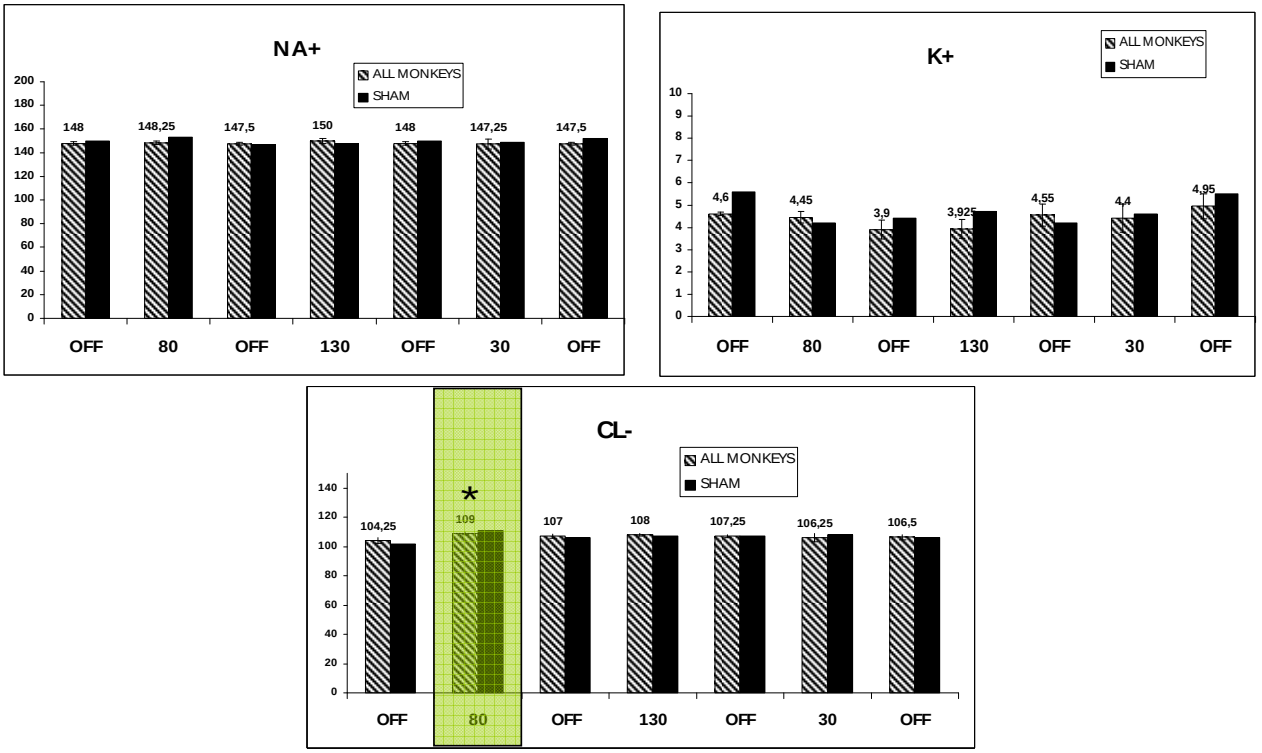
There was no statistically significative difference in the electrolytes during the study, with the exception of Cl^- during 80Hz trial (p<0.05) (Graphic 17). Reference values were in the normal range for Na, K and Cl (Table 11).

Table 11: ***Reference Values in Macaca of Serum Electrolytes***

Na	145,5	156,8	mmol/L
K	3,18	4,8	mmol/L
Cl^-	104	110	mmol/L

Hypothalamic stimulation and Lateral hypothalamic lesion can produce severe adipsia, characterized by the absence of thirst and presenting typically as hypernatremic dehydration. In our study as said before, measuring water intake was technically difficult, so electrolytes in serum can reflect the state of hydration of the subjects.

Graphic 17 Changes in Na, K and CL during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Average serum levels of Electrolytes during trials and washout periods in stimulated and sham control subjects. Measures were done using automated modular instruments (Coulter) used in human subjects in clinical facility (Biology Integrated Laboratory Grenoble University Hospital)

Changes in Hormones during stimulation

Changes in hypothalamic relevant hormones were investigated during the study. Precise dosages of hypothalamic releasing factors specific for Macaca genre were not available. We use pituitary hormones and some final gland hormones (like adrenal cortisol) in order to asses if changes seen in fat content and weight could reflect a hormonal imbalance or a clinical endocrinological syndrome. The results could be grouped in several axes: Corticotropic axis (including GH, cortisol); thyrotrophic axis (TSH, T3L and

T3L) and gonadotropic axis (prolactin, FSH, LH and Testosterone). Results are summarized in table n 12

Table 12 ***Hormones during chronic stimulation at different frequencies in V3***

HORMONES	BASELINE		80 HZ		130 HZ		30HZ		REFERENCE VALUES		
CORTISOL	600.75	±148.9	700.13	±146	510	±104.3	840.2	±326.	275	689	nmol/l
GH	6.52	±1.79	6.59	±3.74	6.72	±6.97	7.4	±7.9	3	60	uUI/ml
T3L	4.88	±0.74	5.71	±0.72	5.54	±0.61	6.29*	±0.43	3	6	pmol/l
T4L	10.23	±1.27	9.66	±0.97	10.4	±1.03	12.84	±3.10	12	22	pmol/l
FSH	2.43	±1.13	2.25	±1.14	2.54	±1.24	2.59	±1.96	1.3	11.5	mUI/ml
PROLACTIN	97.75	±70.8	223.88	±112	123.13	±62.71	263	±341.	30.3	212.1	uUI/ml

Table 12 ***Hormones during chronic stimulation at different frequencies in V***
Hormones values during V3 Intraventricular Stimulation in *Macaca fascicularis*
Marked in yellow are the hormones over the range of the reference values published in tl literature.*p=0.056 (Friedman's non parametric paired test with Dunn post test comparison All values were obtained using human reactive and standard clinical laboratory process.

Corticotropic axis: ***Cortisol*** was found to have large variation between tests with no significative difference between frequencies and baseline values. Mean cortisol levels were higher than reference values at 30 Hz and 80 Hz. ***Growth Hormone (GH)*** were done using human recombinant hormone (hGH-RIACT from CISBIO)(table 10). Differences found were no statistically significative.

Thyrotrophic axis: Thriiodothyroxine (***Free T3***) was very tight regulated, with a tendency to increase at 30 Hz LFS (6.29 p= 0.056) value also slightly over the reference normal range. ***Free T4*** presented almost no variation between different frequency stimulation. Thyroid Stimulant Hormone ***(TSH)*** could not be analyzed because poor cross reaction

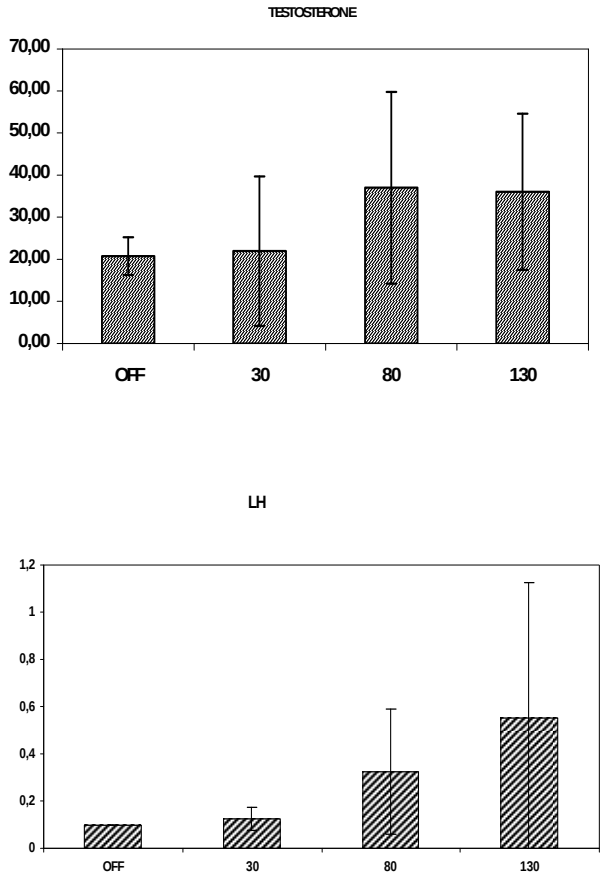
between human TSH tests and Macaca genu, which is not the case with “superior” non human primates like chimpanzees or gorillas (genu *Pan, Pongo* and *Gorilla* from *Hominidae* Family)

Gonadotropic axis: Variations in Hormone ***FSH*** were not statistically significative. High variability between each trial was seen in the levels of ***prolactin***, with mean values slightly superior to reference at 80 and 30 Hz frequency stimulation, no statistically significative. Levels of ***LH*** were non detectable in two monkeys and there was a tendency to increase the levels of ***Testosterone*** during on stimulation. The two hormones seem to be correlated and with the increase of frequency. However, observed differences in testosterone and LH are not significative. (Graphic n 18)

Table 13 ***TESTS USED FOR MEASURE AND HORMONAL SERUM SENSIBILITY***

HORMONES	Test used for dosage	Sensibility
CORTISOL	Roche diagnostics Modular E170	<8nmol/L
GH	H GH RIACT CISBIO	0.03 uUI/ml
T3L	BECKMAN COULTER	0.5 pmol/l
T4L	BECKMAN COULTER	0.5 pmol/l
FSH	IMMUNOTECH (IRMA)	0.2 mUI/ml
PROLACTIN	IMMUNOTECH (IRMA)	0.2 mUI/ml
LH	IMMUNOTECH (IRMA)	0.2 mUI/ml
TESTOSTERONE		
GLUCOSE	MÉTHODE COLORIMÉTRIQUE À L'HEXOKINASE(Modular) ROCHE Diagnostics)	
Na, K, CL	POTENTIOMETRIE INDIRECTE(Modular) ROCHE Diagnostics)	

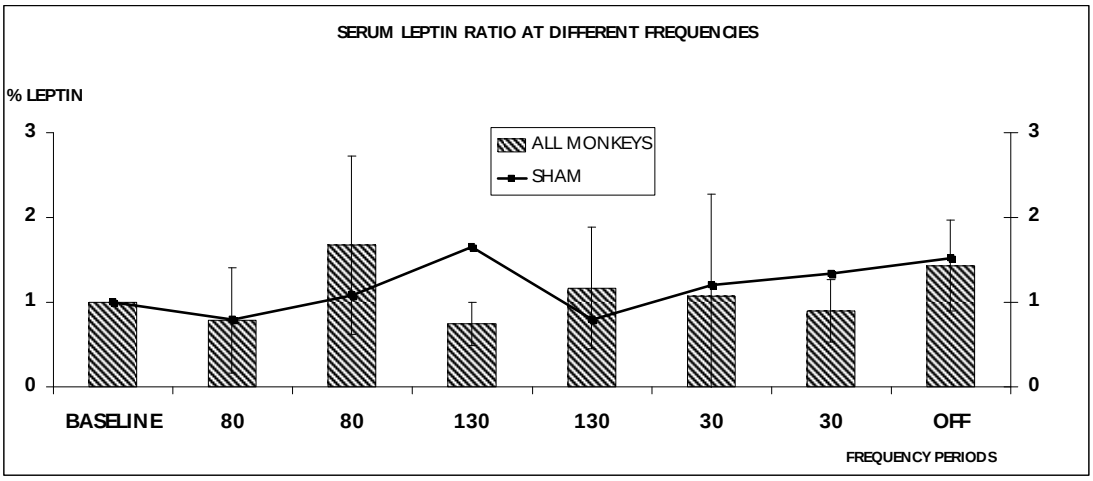
Graphic 18 Variation in Testosterone and LH levels during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Changes in Leptin during stimulation

Leptin (Greek *leptos* meaning thin) is a 16 KDa protein hormone, derived from adipose tissue. It plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin act as adiposity signal, and is level is proportional to adipose tissue. Leptin was measured from plasma samples stored at -80 C and send to Laboratory of « *Hormonologie Pédiatrique et Maladies Métaboliques Hôpital Saint Vincent de Paul* » in Paris at the end of the study. The results were analyzed and organized in function of period of stimulation, averaging all subjects during 80 Hz, 130Hz, and 30 Hz. The results can be seen in Graphic 19

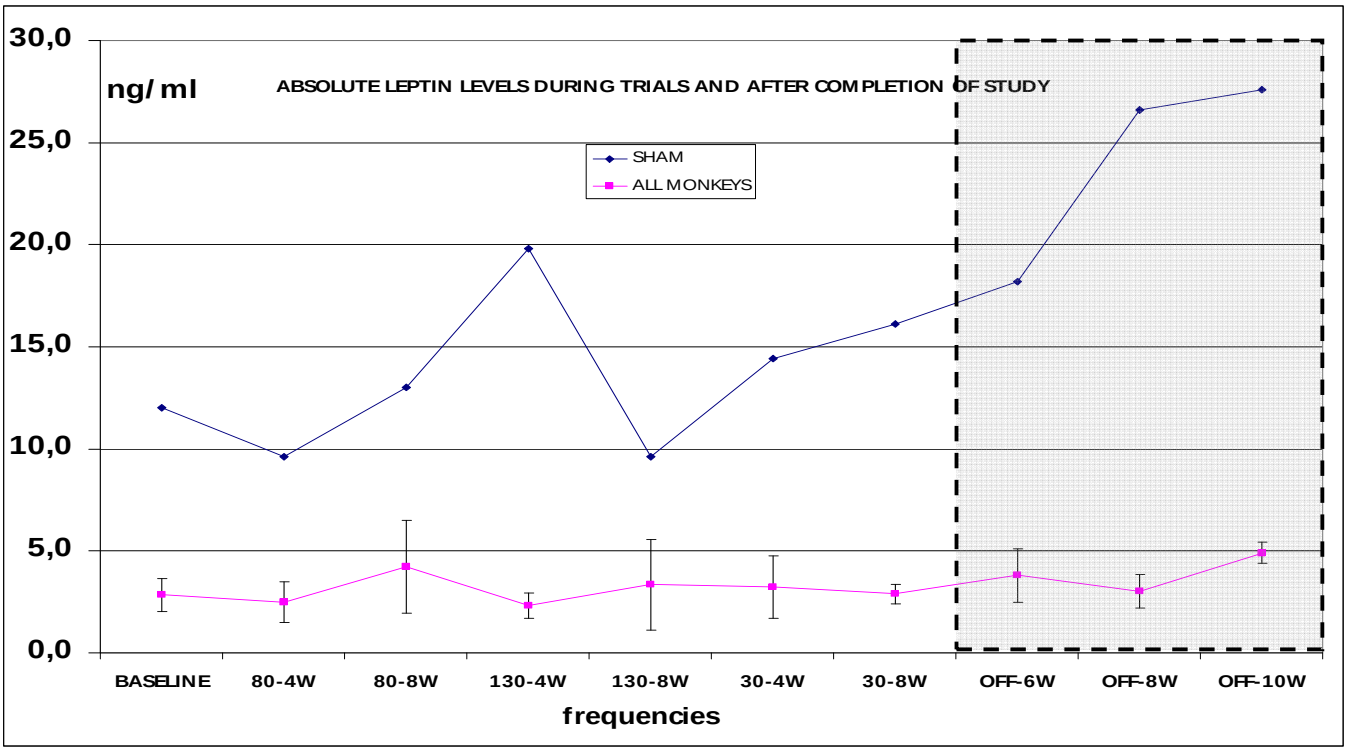
Graphic 19 Changes in Serum Leptin levels expressed in ratios (n/baseline) during chronic stimulation at different frequencies in V3 Stim *Macaca fascicularis*



Ratio of serum Leptin in macaca during chronic Intraventricular stimulation trial: Serum leptin was divided by baseline serum leptin before the beginning of the trials. Leptin was analyzed as we have done with fat measures which have an accumulative effect (Leptin is an adipose signal).

Great variability in levels of leptin was observed during the experiment, even in non stimulated sham subject. Observed Differences are no statistically significative (using

non parametric paired ANOVA: Friedman Test). In general, sham monkey has higher levels of leptin, as expected given its superior BMI, than other subjects and seems to increase during the experience (Graphic 20). After the end of the experience, all subjects experienced an increase in absolute leptin levels.



Absolute levels *(ng/ml)* of serum leptin during the chronic stimulation protocol plus ten weeks after the end of the experiment. Absolute values are larger in control monkey than stimulated animals and there is a tendency to increase levels of leptin toward the end of the study. Stimulated animals maintained the leptin levels and only at ten week post stimulation seem to augment the leptin values. Increasing leptin values may be related to adiposity accumulation seen in animal with long periods of captivity.

Laboratory analysis summary

Several interesting finding were observed during Hypothalamic V3 chronic stimulation in non human primates. In the first place, electrolytes, an indirect indication of adipsia and

dehydration, remained in the normal reference values for *Macaca sp*. Chloride was increased during 80 Hz ($p<0.05$) but its absolute values remained in normal range.

Other positive finding was concerning glycemia. During the wash out period after 80Hz effective weight reduction stimulation, serum glucose augmented significatively ($p<0.05$) coinciding with an increase in food intake (rebound hyperphagia).

GH, Cortisol, Prolactin, and FSH presented high variability with no statistically significative difference between frequencies. Cortisol and Prolactin values at 80Hz and 30Hz are slightly elevated.

There is a tendency of increasing values of Free T3 at LFS (30Hz) ($p=0.056$). No significative difference was seen in T4L.

LH was undetectable in two monkeys; Testosterone was within normal values but with a tendency to increase with the frequency. Leptin presented high variability with no changes during the study but with a tendency of increasing its levels in all subjects after the end of the experience.

ELECTRODE PLACEMENT: MRI IMAGES AND STEROTACTIC VENTRICULOGRAPHY.

As discussed in methods, we used two imaging methods for localization of the electrode and the active contact. First, the Ventriculography and the X-ray in stereotactical condition; which allows transforming the contact position into spatial coordinates, permitting the comparison between different subjects and the atlas (after normalization using internal landmarks like ac pc line or height of the thalamus). (Percheron et al., 1986b) . And in second place, MRI 3Tesla adapted to the small animals; which can be used to localize the electrode in the post mortem piece (Pfefferbaum et al., 2004). Using

both methods is possible to have a clear idea of where the contacts were. In the following table, we have summarized all the coordinates for each active contact. The contacts coordinates are expressed as raw data, which means the distance measured directly in the three planes without any further transformation (besides the coefficient of magnification for all X-rays). The normalized data was created following the same rules applied to human subjects: division in 1/12th the ac-pc line and in 1/8th the thalamus height and expressing all the distances in twelve's of the ac-pc line(Y coordinate) and in eighths of the thalamus height (the coordinate Z). For review into this, please see(Benabid et al., 2002a)(Table 14)

Table 14: Active contact coordinates for each monkey, measured using Stereotactical X-rays and Ventriculography.

SUBJECTS	TYPE ELECTRODE	AC-PC	TH	ELECTRODE MM RAW DATA						NORMALIZED DATA					
					Y		Z		X		Y	Z			X
				TIP INF	TIP SUP	TIP INF	TIP SUP	TIP INF	TIP SUP	TIP INF	TIP SUP	TIP INF	TIP SUP	TIP INF	TIP SUP
H4 monkey	3389	8,432	6,664	0,45	0,45	4,34	2,84	2,60	2,60	0,65	0,65	-5,21	-3,41	2,60	2,60
H5 monkey	3389	9,258	5,386	0,94	0,94	5,10	3,60	0,25	0,25	1,22	1,22	-7,49	-5,29	0,25	0,25
H7 monkey	3388	10,47	6,112	1,53	1,53	5,20	1,70	0,00	0,00	1,76	1,76	-7,03	-2,30	0,00	0,00
H8 monkey	3388	10,03	6,62	0,96	0,96	5,10	1,60	0,12	0,12	1,14	1,14	-7,25	-2,27	0,12	0,12
H10 monkey	3389	10,45	7,33	0,00	0,00	6,03	4,53	0,55	0,55	0,00	0,00	-6,57	-4,94	0,55	0,55

Table 14 Showing contact coordinates in X, Y and Z planes: X is the laterality from midline, Y is the antero-posterior distance from the AC, and Z is the depth from AC-PC plane. Normalization was made taking into account the ac pc distances for each monkey. TH is the thalamus height. 'Tip inf' and 'Tip sup' means inferior and superior contact tip.

All the contacts lied in the 3 Ventricle. The X coordinates (laterality) were between 0 to 0.55 mm from the midline, with the exception of monkey H4 or sham control, who was slightly off-center (2.6mm from the midline). The depth from the ac pc plane was

between 4.34-6.03mm (5.21 to 6.21 normalized). And they were just posterior the anterior commisure between 0-1.76 normalized data. Using the atlas of Paxinos and normalizing the distances, we were able of represent the VMH in this stereotactical space. Results are shown in Figure 44

Figure 44: VMH in Sagittal and Coronal views showing active contacts projections. The contacts lied on the dorsal part of the nucleus.

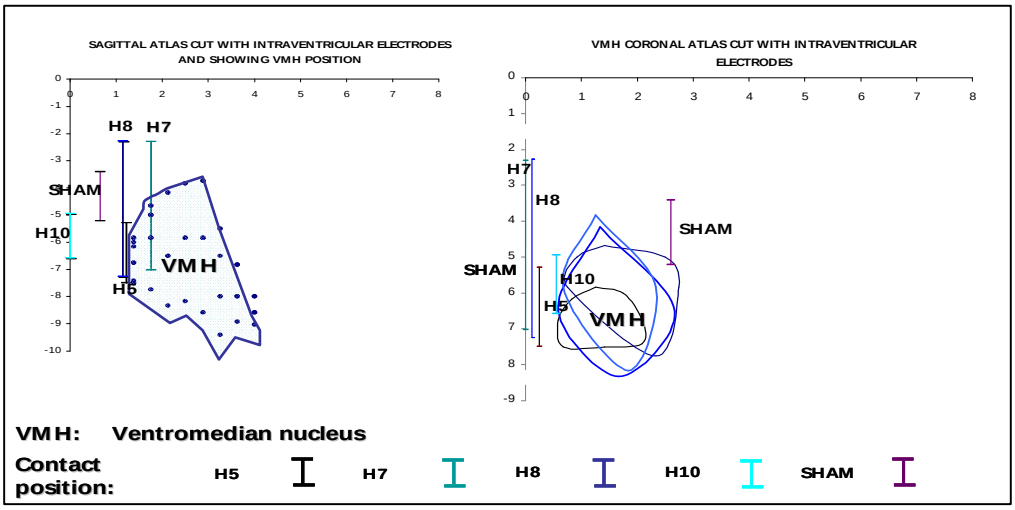
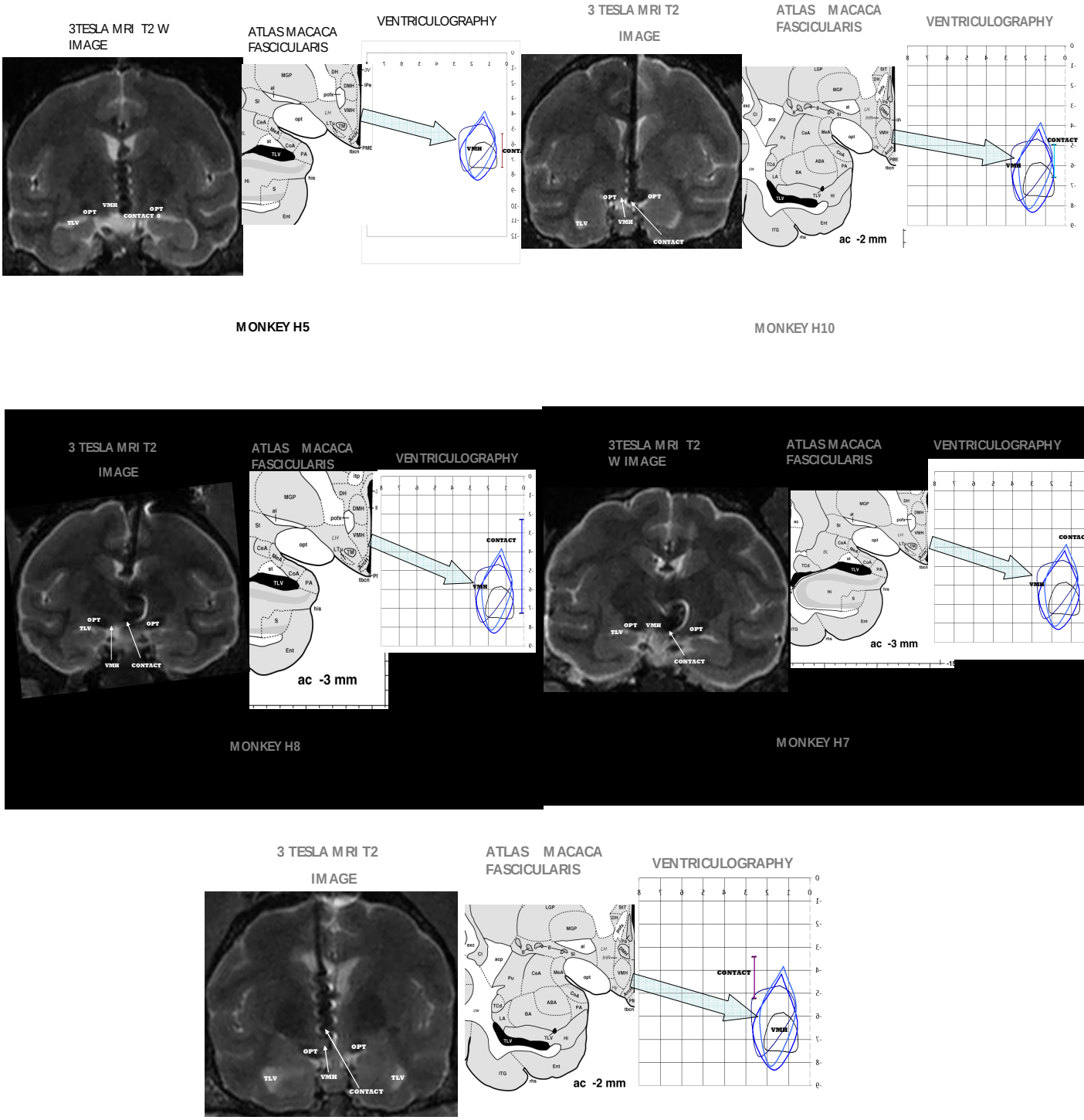


Figure 44: Active contact coordinates represented in the stereotactical space in coronal and sagittal view. VMH is shown in the coordinates obtained from Paxinos and Huang Atlas Macaca Mulatta. (Paxinos et al., 1999)

The results from the MRI images were obtained and the centre of the hypo-intensity artifact was used as a contact center. The electrodes 3389 had a smaller artifact than electrode 3388, and it was further decreased when we orientated the lead parallel to the magnetic field. In the figure 45, the position of the electrode relative to other brain landmarks and to VMH is displayed.

Figure 45: Coronal MRI images showing the electrode position: Comparison with Atlas cuts and X-rays AP projections.



The monkeys H5 and H10 were implanted with a quadripolar electrode and stimulated with the lower contact (contact 0). In the control MRI, the contact 0 was in contact with the floor of the third ventricle in both monkeys. The main difference was that in the monkey H5 the contact seems posterior; corresponding with the atlas cut ac –3mm and in the monkey H10 the center was more anterior corresponding to a cut ac -2mm. In either case, the contacts were in close relationship with VMH nucleus. Both electrodes were lying more to the left wall of the third ventricle. The monkeys H7 and H8, implanted with a monopolar, monocontact of 3.5 mm in height generated an artifact in "arrow's point". This characteristic made difficult to identify properly the center of the electrode contact. If we consider the tip of the artifact as the tip of the electrode, both electrodes lied in the floor of the 3rd ventricle in the corresponding atlas coronal cut ac–3mm (3mm posterior to ac). There were discrepancies in the measures MRI and Ventriculography, but both methods confirmed the Intraventricular placement of the electrode and the close relationship with VMH. Sham subjects had as expected, a contact that is dorsal to VMH and off center to the right, but inside 3V. (In the X ray correspond to 2.6 mm to the midline).

In conclusion, the position of the active contacts in general, was at the level of the VMH nucleus, Intraventricular but lying slightly off center to the Left, and with the tip touching the floor of the third ventricle.

DISCUSSION

This is the first report to our knowledge, of effective decrease in total weight and fat mass in non human primates following DBS in the hypothalamic region. This is also the first time that the Intraventricular approach has been used to modulate hypothalamic

structures in monkeys. Essential in our study is the use of this new, less traumatic approach to central median brain structures: the Intraventricular way, which has been proved easy to perform and safe. And because of the preclinical characteristic of this work, Blood chemistry and hormones in serum were measured revealing no clinical condition (endocrinological or related to electrolytes) that could hamper is application in human settings. Details of the different findings are discussed.

Weight and fat content modulation

The most important finding of this study is that weight and fat mass can be modulated in non human primate using the adequate electrical stimulation parameters in the hypothalamic medial region. Animals reduced in 8-10 % in body weight and 18 % reduction of fat content at the end of 8 week after stimulation at 80 Hz. Other frequencies produced no changes in weight. The reduction in weight was accompanied with reduction of fat mass and a reduction in iliac skinfolds and a tendency in reduction in abdominal circumference. The reduction in weight was done primarily in fat content and was achieved in the local areas where fat accumulates, like abdomen or iliac region. On the contrary, Subscapular fat was not changed during the experience. Localized abdominal fat reduction was related in humans with reduction in the risk of obesity associated diseases like hypertension or diabetes (Larsson et al., 1989; Pender and Pories, 2005). Several authors have showed modulation in weight in rats following lesion of lateral structures in the hypothalamus or electrical stimulation in ventromedian hypothalamus (Anand and Brobeck, 1951b; Bielajew et al., 1994; Sani et al., 2007; Stenger et al., 1991)But the results in monkeys are limited and far from definite(Lacan et al., 2008), and several conflict reports are published(Robinson and Mishkin, 1968)

The contradictory reports in monkeys have a possible methodological reason. For the most part, they use stimulation parameters (frequency, voltage etc.) coming from Basal ganglia stimulation in Parkinson's patients (Lacan et al., 2008; Takaki et al., 1992). In this area, it is maybe critical to study stimulation parameters setting due to the complexity of the region. In this study, careful acute stimulation protocol allowed to set parameters according to acute reduction in food intake. This long period of testing might be essential to produced effective weight reduction.

Other element in published studies that contrast with the present work was the observation period. Very short protocols (Lacan et al., 2008) produced possibly modulation in food intake, but fail to show weight loss. Long term assessment is maybe needed. The present work presented a long period of acute stimulation and a cross over protocol with periods of 8 to 10 weeks with washout of 4 weeks. Longer periods of stimulation are sometime required in several disorders to achieve maximum benefit (like dystonia and Obsessive compulsive disorders) (Krauss et al., 2003; Vercueil et al., 2001)

A final element that distinguishes this study from others and could explain divergences is the targeting and final active contact position. Targeting the VMH in the literature is made by putting contact directly in the center of the nucleus. It is possible that spreading to other areas and to the fiber of *passage* make difficult to evaluate the electrical stimulation of VMH in those conditions. The difficulty with the intraparenchymatous approach is that it is easy to modulated sub areas in the hypothalamus that are involved in opposite function to VMH. Also the introduction of the lead can produce mechanical lesion in VMH and therefore, the reverse effect. Intraventricular stimulation, which at the first glance is less specific, present the advantage of complete covering medial

hypothalamic areas (areas susceptible of anorexia and weight loss), and saving lateral hypothalamus, a mostly fiber area(Beltt and Keesey, 1975)of modulation and antagonizing the weight loss effect.

Modification in food intake

FI was stable during the stimulation periods. But after effective lost of weight and reduction in fat mass (8 % in weight loss and 18% fat mass reduction at the end of 8 week 80Hz stimulation period) during washout there was an increase of 25% in FI ($p<0.05$). Hyperphagia was accompanied by an increase in glucose and rebound weight gain. Literature in Obesity usually finds periods of hyperphagia and rebound weight gain after effective weight loss(Hensrud et al., 1994; Masuo et al., 2005)In the first part of the present work, acute test showed decrease in food intake in hungry monkeys at particular frequencies. During short periods of time and in specific condition of fasting, animals reduce FI measured as total meal size and increased locomotion at effective frequency stimulation parameters. Those parameters that induced reduction in FI during acute trials produced afterwards weight reduction during the 8 to 10 week chronic stimulation crossover protocol. These finding suggest that a behavioral effect over FI is obtained when the stimulation is put on. The acute effect over FI fades away in time and a catabolic metabolism is established producing finally the necessary energy consumption to provoke weight loss and fat mass reduction.

Finally, the increase in FI during off stimulation after weight loss and rebound weight gain rise the question of how long the stimulation should continue to produce a stable BMI and possibly a “new set point” for fat content in the subjects.

Endocrinological and Blood Chemistry Changes

Electrical hypothalamic stimulation could induce several endocrinological conditions secondary to the secretion of releasing factors into the hypothalamus pituitary portal system or secondary to direct production of hormones(Fink, 1976; Martin and Reichlin, 1970; Martin and Reichlin, 1972)Some of those conditions could have explained the weight loss and the reduction in fat mass during effective V3 medial hypothalamic stimulation (hyperthyroidism)(Martin and Reichlin, 1970).

More importantly, some of those endocrinological conditions might have prevented or at least delayed the possible clinical application of our study. Also, besides hormones, the electrolytes and glucose might produce some undesirable side effects, like dehydration (Szczepanska-Sadowska et al., 1979)or diabetes and put into risk the animal well being and potentially human patients. So, for evaluating the safety of the procedure, is necessary to take into account not only surgical tolerance or acute behavioral symptoms, but also hormonal and ancillary laboratory tests.

In the other hand, Effective electrical stimulation of V3 seems to produce a catabolic state associated to hyper-metabolism, which increases basal energy expenditure(Bielajew et al., 1994) So in general, laboratory measures look to unveil some of the causes of weight loss during V3 Stimulation and dissipate safety concern that could eventually hamper further clinical experiences.

In the study, some interesting finding might help explain some of the physiological characteristic of the fat and weight loss in the subjects during stimulation. And more importantly, these data help clarify safety concerns associated to hypothalamic stimulation.

In the first place, ***electrolytes*** remained in the normal reference values, and only CL was significative increased (but in the normal range) during effective weight loss stimulation. In the literature, Lateral hypothalamic lesion and VMH electrical stimulation (Anand and Brobeck, 1951b; Bernardis and Bellinger, 1996) (Stenger et al., 1991; Teitelbaum and Epstein, 1962) in rodents were often accompanied with adipsia and hypernatremia during weight loss. During the trial, it was difficult to measure water intake for several technical reasons. So, measuring electrolytes provided at least an idea of the degree of dehydration produced. The syndrome adipsia-hypernatremia was not present in our study, meaning probably that thirst center were not compromised by the stimulation or at least they maintained the compensatory mechanisms.

Glucose remained in the normal reference values throughout the experiment. Nevertheless, an increase in the glycemia was seen immediately after reduction in body weight and fat content following V3 80Hz effective stimulation. During this wash out *off*-period, a surge in food intake and a partial rebound in weight were also noticed. Even thought the levels of glucose increased, they maintained in the published normal range for macaca.

Obesity treatment usually reports rebound weight gain after successful weight loss, accompanied with hyperphagia and a peak in serum glucose. The fact that levels of glucose remained in the normal range even during the increase indicate that pancreatic regulatory mechanism compensate adequately this anabolic period.

GH, cortisol, prolactin, FSH, presented large variability with no significant variation. ***LH and Testosterone*** seems correlated with a tendency of increasing with the stimulation (at all frequencies) but no significative difference was found. No changes were found in Thyrotropic axis, with the exception of free ***T3***, which has a tendency of increase when

the stimulation was at LFH 30 Hz (p=0.056). VMH and possibly DMH electrical stimulation does not produce direct secretion of hypothalamic releasing factors (like Somatostatin or ACTH). However, it is well known that VMH stimulation is associated to an elevated sympathetic tonus, with an increase in noradrenalin exchange rate in rodent's brown fat tissue(Minokoshi et al., 1986) Increase sympathetic tonus associated to increased metabolic basal energy expenditure can lead to changes in some stress hormones (Vissing et al., 1989). Compensatory mechanism might be at work in our study and help explain why hormones remained in reference levels. Also, additional measurements of the metabolic rate should be done in metabolic chamber capable of measure O2 consumption, heat, cardiac frequency, respiratory rate and other variables reflecting the catabolic state, helping unveil mechanism of modulation in weight following hypothalamic stimulation. Broad serum investigation in the search of markers should be undergone for explaining the physiology of the electrical stimulation hypothalamic weight loss.

Also, the Intraventricular V3 approach to the stimulation rather than intraparenchymatous, might have been important for avoiding spreading to neighboring areas. Some areas near ventromedial hypothalamus can effectively secrete hypothalamic releasing factors putting into a risk the general health state of the implanted subject. Specific medial hypothalamic stimulation, avoiding other nuclei and *fibers of passage* seems to be reached with Intraventricular approach.

Finally ***leptin*** serum levels were very variable and did not follow a discernable pattern; no statistical difference was found. Leptin is an adiposity signal that is proportional to the amount of fat content. Thus, leptin levels should decrease after effective weight loss treatment. However, authors have found that during weight loss, leptin do not follow

exactly the fat content in treated subjects .Other factor involved was that leptin samples were processed after a long period of storage and were sent to an external laboratory. In consequence, storage and transport maybe in part responsible for high leptin variability. And the decrease in fat content was not as important or long in time to produce variation in those levels.

Safety Issues

The recent demonstration of the effectiveness of DBS in posterior hypothalamus has opened several research pathways using the hypothalamic areas (Leone et al., 2005)Unfortunately, some safety concerns in targeting this area remain (Pinsker et al., 2008; Schoenen et al., 2005)These concerns have made us explore new approaches to reach in a less invasive fashion these medial areas of the brain. Intraventricular DBS electrodes theoretically should have a risk comparable to the insertion of ventricular catheter use in hydrocephalus.

The first safety issue was the surgical procedure. Surgery was well tolerated in all animals. One of them has an infection in the IPG that forced us to withdraw the battery. After total recovery, this animal was used as a sham operated subject. In general, the procedure generates weight loss during the first weeks before the starting of the trials. During acute trials when the monkeys were recorded and analyzed, no behavioral changes were seen. In the chronic protocol, no evident side effects were seen. Overall, monkeys were healthy without signs of pain or reduction of movement. Some stereotypical movements were observed in two monkeys, but were related to the long captivity periods in small cages. When the animals were put in bigger cages and in-pairs, the repetitive movements stopped.

The most important limiting factor for using this stimulation in a clinical setting could be theoretically the disruption of hormonal balance caused by directly stimulation and secretion of hypothalamic releasing hormones producing potentially dangerous condition (like acromegaly or hyperthyroidism). Careful analysis of serum samples allowed us to conclude that stimulation were not inducing modulation in vital plasma elements that could become a threat to the animals. The subjects were observed during a long period of time, almost three years from surgery to euthanasia. During all those years, monkeys tolerated well the hardware (IPG and Electrodes) .The position of the tip of the electrode did no migrate to other areas, keeping the hypothalamus stimulated, without lesioning V3 walls or other areas. Hypothalamus was modulated without risking producing associated lesion, which could have had an antagonistic effect over weight control (in contrast to STN stimulation in which lesion or HFS has the same effect).

In conclusion, this procedure could be adapted to human subjects in clinical settings without evident risk to patients.

CONCLUSION

New indication for DBS are flourishing as our knowledge of the anatomo-physiology of diverse region in the Central Nervous system develops. And the characteristics of reversibility and minimum lesion to tissue of the DBS allow researchers to explore new areas in Brain. Encouraging results in the modulation of posterior hypothalamus in cluster headache and recent experiences in ES of Ventromedian hypothalamus in obesity models in rodents has prompted investigation in this area. This is the first report of DBS clear reducing weight and fat content in non human primates. Reduction in weight and fat was not followed by important hormonal or electrolytes imbalance, or

conditions that could have prevented the future application in clinical environment. Also key in our study, the new approach or the revival and adaptation of the old Intraventricular stimulation to reach medial structures has prove to be safe, stable in time and possibly more effective in modulation of circumventricular structures.

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