The neural bases of consciousness in the healthy and in the pathological brain

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Abstract

The study of consciousness is a fascinating topic of investigation with a wide field of applications and implications. Consciousness processes can be divided into two orthogonal though intimately linked components: the conscious state, that is the state of vigilance or arousal, and the conscious content which refers to the external inputs perceived and manipulated in a conscious space. Although consciousness represents the most important human dimension where people's personal events are continuously experienced and remembered, it is somewhat surprising that its underlying neural processes still sparks lot of debates.

In the first part of this PhD thesis, I took advantage from a well-known sensorimotor conflict paradigm, the Nielsen task (?), to investigate the neural correlates of the emergence of consciousness. Starting from the principle that much of motor processing occurs outside of awareness, I adapted the Nielsen paradigm to neurally investigate how the perception of a motor conflict in healthy subjects smoothly shifted along the unaware/aware state (i.e. point of subjective equality). Using EEG recordings, I then identify the brain sources which I consider the neural fingerprint of awareness. I found that the precuneus was critical for bringing the sensorimotor conflict into awareness. I also investigated this issue from a developmental perspective by examining the performance of healthy children. Although the timing of movement correction and the quality of movement trajectory in children was similar to adult subjects, motor awareness was shifted towards higher perception thresholds while parietal cortex activity was not found. Rather, children's response to conflict awareness was linked to SMA. After having addressed the topic of awareness in this first part, I will focus more on the second component, wakefulness. Usually these two components evolve together, however there are some pathological states in which they can be dissociated. It is the case for vegetative state patients who experience a state of wakefulness without awareness.

In the second part of the thesis, I investigated the challenging hypothesis of a potential return to a conscious state, in a patient lying in a vegetative state for 15 years, after vagus nerve stimulation (VNS). We report beneficial effects of VNS including improved behavioural responsiveness and reinforced brain connectivity patterns as key signs of increased consciousness. The results showed an increase of information sharing a measure of functional connectivity particularly prominent across centro-posterior regions. Converging findings, coming from different methods, showed that VNS promoted the spread of cortical signals and metabolism which we found correlated with behavioural improvement as measured with the CRS-R scale. The VNS-induced changes are promising since they seem to follow an already known connectivity pattern characterizing state of consciousness improvements.

Taken together, these findings indicate that the parietal lobe constitutes the neural correlate of both state and content-specific consciousness and suggest that this region is a "hot zone" for its emergence. Moreover, our first findings in a vegetative state patient also suggest that consciousness can be potentially repaired, thus opening the way to a new avenue of research in a domain where brain plasticity was underestimated.

Key Words: consciousness, motor awareness, disorders of consciousness, vagus nerve stimulation, parietal cortex, precuneus/posterior cingulate cortex.

Résumé

L'étude de la conscience est un sujet d'investigation fascinant avec un large champ d'applications et d'implications. Les processus de la conscience peuvent être divisés en deux composantes indépendantes quoiqu'intimement liées : l'état conscient et le contenu conscient. L'état conscient correspond aux processus de variation de la vigilance, tandis que le contenu conscient fait référence aux expériences sensorielles perçues et manipulées dans un espace conscient. Bien que la conscience soit un élément essentiel de la cognition humaine, qui conditionne ce que les gens vivent et peuvent se remémorer, la légitimité et le bien-fondé de l'analyse scientifique et rigoureuse des corrélats neuronaux de la conscience soulèvent encore des débats houleux.

Dans la première partie de cette thèse, j'utilise un célèbre paradigme de conflit sensorimoteur pour identifier des corrélats neuronaux de l'émergence de la conscience. Les travaux initiés par Torstein Nielsen (Nielsen 1963) ont démontré que la majorité des traitements sensorimoteurs s'effectuent sans nécessiter une analyse consciente. L'émergence de phénomènes conscients apparaissant à partir d'un seuil subjectif de conflit sensori-moteur appelé point d'égalité subjective. A partir d'enregistrements électroencéphalographiques, effectués chez une population de sujets adultes, il est possible d'identifier des sources d'activités corticales indépendantes des intensités des stimulations sensorielles expérimentées et spécifiques de l'émergence d'une sensation perçue consciemment. Ainsi, j'ai pu démontrer que le précuneus était une structure centrale dans les processus qui transforment un conflit sensorimoteur en une expérience consciente. J'ai également étudié ce phénomène d'un point de vue développemental en examinant les performances comportementales et des enregistrements EEG recueillies chez l'enfant. Bien que le moment de la correction du mouvement et la qualité du tracé de la trajectoire étaient similaires aux données mesurées chez les sujets adultes, le seuil de conscience motrice s'est montré plus élevé et l'activité du cortex pariétal n'a pas été retrouvée. En revanche, l'aire motrice supplémentaire a été identifiée comme un corrélat important de l'émergence d'une sensation consciente d'un conflit sensorimoteur chez l'enfant.

Dans une seconde partie, mes travaux ont été consacrés à l'hypothèse audacieuse qu'une stimulation électrique du nerf vague pourrait modifier l'état de conscience d'un patient se trouvant dans un état végétatif depuis 15 ans. Nous rapportons les effets bénéfiques observés après cette thérapeutique expérimentale au niveau comportemental, clinique et neurophysiologiques. Les enregistrements EEG et les méthodes de mesure de connectivité fonctionnelle m'ont permis d'observer chez ce patient une augmentation du partage d'informations corticales particulièrement importante dans les régions pariétales. L'effet de la stimulation a été également confirmé par d'autres méthodes. L'imagerie métabolique a montré une augmentation généralisée de l'activité corticale et sous-corticale et les évaluations cliniques par la CRS-R ont montré une amélioration de l'état de conscience corrélée aux observations électroencéphalographiques. Ces changements induits par la stimulation du nerf vague sont prometteurs car les modifications cérébrales observées sont caractéristiques de l'amélioration des états de conscience chez les patients gravement cérébrolésés.

L'ensemble de ces résultats suggèrent que le lobe pariétal constitue à la fois un corrélat important de l'état de conscience et du contenu conscient, faisant de cette région une composante essentielle de l'émergence de la conscience. De plus, nos résultats préliminaires suggèrent que la conscience peut être, au moins partiellement, restaurée. Cette découverte ouvre de toutes nouvelles perspectives pour le futur des recherches en neurosciences où, l'hypothèse d'une modulation de la plasticité cérébrale avait été oubliée.

Mots clés: conscience, conscience motrice, troubles de la conscience, la stimulation du nerf vague, cortex pariétal, precuneus / postérieur cortex cingulaire.

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Contents

Chapter 1

Introduction

Around the definition and the interpretation of the concept of consciousness there is a long-lasting multidisciplinary debate involving scientists from different domains such as neuroscience, psychology, philosophy, brain science, medicine and physic. This topic seems to have fascinated the human being since the beginning. Seth et al. in their editorial dare to say that "People have been wondering about consciousness since they have wondered about anything". The ancient Greeks already wondered about conscious perception, when long time ago Hippocrates identified the brain as the primary organ of experience (**?**). Much later, St. Augustine formulated the notion of two kinds of "presence", a first one referring to something that exists at the term of a cognitional act and the second one which refers to an experience of self-presence. However, he did not explicitly speak about *consciousness* and *self-consciousness*. In fact, the development of a proper language able to form a vocabulary of "self" was introduced later, around the 16th century, (**?**) as stated in the text by Fr. John Eudes Bamburger: "Descartes, in 1664, made the thinking self the source of knowledge and most philosophers since his time have assumed the same stance. It was shortly before this date that Locke... adopted the new word "consciousness" and defined it as "perception of what passes in a man's own mind". Coleridge was the first to use the term *self-conscious*" (Retreat conference given at St. Anselm's Abbey, Washington, DC, August 22, 2009, unpublished).

From a scientific point of view, it is interesting to wonder if we can experimentally investigate consciousness, and in case of a positive response, to understand which aspect of consciousness can we actually address and how. Some philosophers used to say that we cannot investigate consciousness because it is a *hard problem*, which means that we cannot pinpoint a specific mechanism for consciousness like in *easy problems,* where we can identify a specific function for the discrimination and categorization of stimuli. The term *hard problem*, introduced by David Chalmers (**?**), reflects the investigation of subjective experiences, the phenomenal consciousness or "qualia". The hard problem try to identify why a physical state is conscious rather than unconscious. In other words, it represents the problem of explaining why conscious mental state "light up" and why there is a "something it is like". The term hard contrasts with what it is called the easy problems, consisting in explaining how brain integrates information, categorizes and discriminates environmental stimuli. Such phenomena are considered functionally definable. For easy problems once explained the relevant mechanisms there is no explanatory work left to do.

Another American philosopher, J R Searle, tried to give a new definition of consciousness in a common sense term as follows: "Consciousness consists of inner, qualitative, subjective states and processes of sentience or awareness. Consciousness, so defined, begins when we wake in the morning from a dreamless sleep - and continues until we fall asleep again, die, go into a coma or otherwise become "unconscious"". Following this definition, the entire experience of the internal and external world would have been affected, if not caused, by consciousness. It includes everything from sensory perception, to feeling pain, states of anxiety and depression, memory, speaking, arguing and wishing. And again, he said "Consciousness is entirely caused by neurobiological processes and is realized in brain structures" (**?**).

In neuroscience, the systematic investigation of consciousness is mainly focused in understanding its neural correlates, and investigating the neural mechanisms underlying conscious experiences. At present, consciousness can be studied using different approaches in healthy subjects and psychiatric and neurological patients. Neuroscience of consciousness had proved that of all perceived stimuli, going from visual to sound perception, coming to primary sensory cortices, and of all that is further elaborated in associative areas, only a smaller portion is available to consciousness. In fact, much of information is compressed before arriving to consciousness. However, also unconscious information is an important part of experiences because it can potentially influence our actions and behaviours.

Research on neural correlates of consciousness gained importance when it was recognized that it was actually affecting real life phenomena such as visual illusion, and a huge variety of normal and pathological conditions, such as sleep (?), anaesthesia (?), disorders of consciousness (?) and brain lesions (?). This provided, together with the rise of functional brain imagining, an experimental condition frame to scientifically investigate consciousness.

To summarize, consciousness is a very variegated topic, widely investigated in different disciplines and epochs. Here we are interested in understanding its neural architecture, which gives rise to reportable conscious experiences and shapes our internal and external world perception. Which brain mechanism allows us to become conscious of some piece of information while preventing other to emerge? How exactly do these mechanisms cause conscious states? How are these processes realized in brain structures? In the next section I will give some operational definitions of consciousness before proceeding in reviewing general understanding of brain structures sustaining its emergence.

1.1 Main components of consciousness

Due to the large applicability of the concept of consciousness, it is useful to start with some definitions. As already said, "consciousness" is an ambiguous word, which can be used either intransitively (e.g. "the patient was conscious") or transitively (e.g." the subject was conscious of the perturbation"). For practical purposes, in neuroscience consciousness is classically thought as formed by two main components: wakefulness and awareness (Figure 1.1). Wakefulness (or vigilance) describes the arousal state and the potential to experience awareness and it reflects the level of consciousness. Clinically, it corresponds with an eye-open condition. Its anatomical counterparts are structures located in the brainstem and in the ascending reticular activating systems. Awareness is defined as the potential to experience phenomenal perception of self and surroundings, shaping subjectively our everyday life experiences. It reflects the content of consciousness. Its anatomical counterparts are hypothesized to be based on structures in the fronto-parietal cortex (?). Usually, this two components are linearly correlated along the spectrum of consciousness, an increase of wakefulness is accompanied by increased potential to be aware (e.g. during coma, anaesthesia, deep sleep and vigilance). However, sometimes they are dissociated, for example during vivid dreams or pathological states in which wakefulness is spared but awareness is impaired (VS or MCS), and also when somnambulism occurs (?).

The respective neural substrates of wakefulness and awareness are called full and content-specific neural correlates of consciousness (NCC).

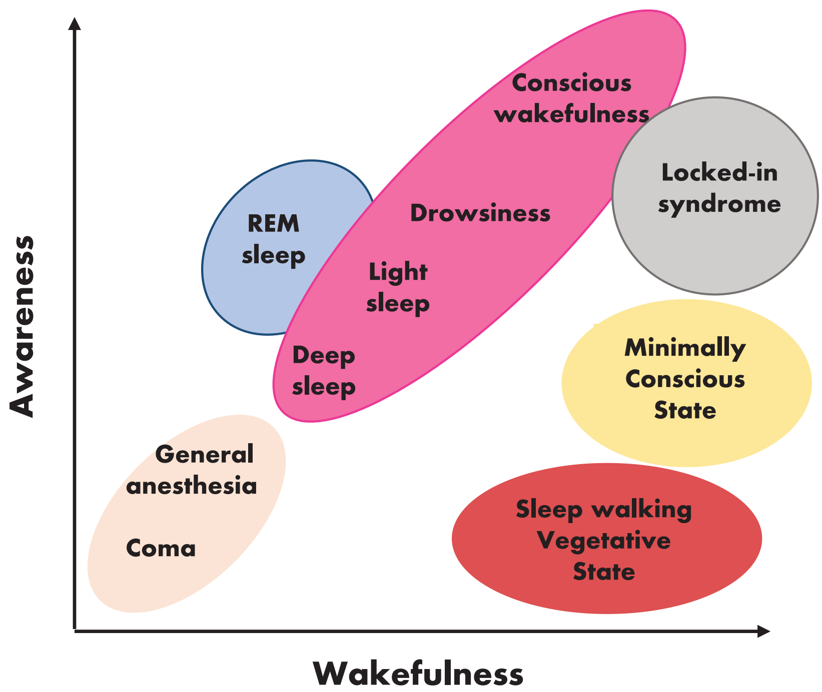

Figure 1.1: **Main components of consciousness**. The x-axis represents wakefulness while the y-axis represents awareness. Some pathological states lie outside the diagonal, such as the vegetative state, characterized by a state of wakefulness without awareness, and also some physiological states (albeit rare) like sleep walking. Adapted by Laureys et al. (?).

1.2 Full and content-specific neural correlates of consciousness

Neural correlates of consciousness (NCC) are defined as the "minimum neuronal mechanism jointly sufficient for any one specific conscious percept" (**?**). As the definition of consciousness relay on two orthogonal axes, wakefulness and awareness, also the respective neural correlates of consciousness have different substrates.

The neural correlates of wakefulness are refereed as full NCC. The state of consciousness refers to the brain ability to maintain a continuous stream of conscious content, arising from a fluctuation of vigilance and wakefulness. This neural substrate has to support conscious experiences in general, irrespective to their specificity. To identify full NCC typical experimental paradigms consist in contrasting state-base consciousness, for example comparing healthy subjects with subjects in which consciousness is diminished such as during sleep, anaesthesia or coma. Human neuroimaging studies show that loss of consciousness is frequently accompanied by a reduction in activity over fronto-parietal cortices, in particular over the posterior medial cortex (**?**). However, arousal and attention, which depend on the arousal-promoting neuromodulators, can play a confounding role in determining the physiological state of the brain. To filter these confounding factors, there are experiments aimed at contrasting conscious and unconscious conditions within the same physiological state. For example, an EEG study investigated REM and NON-REM sleep activities reported that a common network exists for the two states in a temporo-parieto-occipital region (**?**).

The second axis, related to awareness, has neural correlates reflecting a specific content of information. These content specific neural correlates of consciousness are thought to be neurons (or population of neurons implicated in a specific function), corresponding to particular phenomenal distinction (**?**). Content specific neurons are thought to fire in relation to specific stimuli, such as auditory or visual incoming information. In turn, if artificially stimulated, this NCC should trigger the same conscious content and if their activity is disrupted this particular perception should be impeded. To identify content-specific NCC, experimental conditions in which subjective experience changes while stimulation and/or behaviour remain constant are compared. Visual paradigms following this design are for example binocular rivalry (?), bi-stable perception (?) and visual masking paradigms (?). Main findings based on these report-based settings showed the involvement of a fronto-parietal network subserving this function. However recent opinions (?) based on studies in which participants do not have to explicitly report consciousness (??) argue that NCC can be identified in a more restrict zone, involving posterior cortical areas (?). These paradigms mixed explicit and implicit judgements and tried to extrapolate physiological marker of consciousness in order to filter the effect of side variables such as process occurring close in time.

1.2.1 Internal and external awareness

Consciousness refers also to our internal world, we remain conscious when we daydream and dream during sleep. Consciousness only disappears in dreamless sleep and in some artificial conditions, such as under anaesthesia, or in some pathological conditions such as disorders of consciousness. Two main functional networks can be distinguished starting from the large fronto-parietal network characterizing awareness: the external and internal awareness networks (?). The external awareness network, or executive control network, is characterized by the activity of lateral fronto-parietal regions, subserving attentional and cognitive tasks. The internal awareness network, or default mode network (DMN), is a mesial fronto-parietal network involved in self-reported process, and internally related cognitive content, such as mind wondering and autobiographical memory recall. Self-consciousness in this context can be seen as a particular instance of conscious access where the object of consciousness is turned toward the internal state. These two networks are usually anticorrelated during conscious cognition.

It is considering at the same time the neural correlates of full and content-specific consciousness that Koch et al. hypothesized the posterior cortical region as an "hot zone" for consciousness, being the exact intersection between the two (?).

1.3 Theoretical models of conscious access

Recently, theoretical models have tried to explain consciousness taking into account neurobiological main findings (?). The theoretical approach starts from phenomenal properties of experience and try to isolate important characteristics required to a physical substrate to implement them. Research on the past ten years have led to several core concepts theorized to be necessary for conscious perception (?). I will briefly report only few words for some of them to summarize the main concepts.

The first concept states that consciousness can be seen as a **supervision system**. This "supervisory attentional system" would control the activity of lower-level functions such as sensory, semantic and motor functions, occurring independently from awareness. However, when a dynamic monitoring and control is needed, conscious perception become necessary. Within this frame, the *supervision system* is proposed to anatomically correspond to the prefrontal cortex. Shallice (??) and Posner (?) were the firsts to propose this theory of conscious processing, identifying conscious percepts as the information entering an *"executive attention"*. This same concept can be recognized in the words of William James, which described consciousness as "an organ added for the sake of steering a nervous system grown too complex to regulate itself" (?).

Second, consciousness as a **serial processing system**. This concept theorizes that conscious processing has limited capacity. Although initial perceptual processing is thought to be parallel and unconscious, conscious accesses is thought to be serial, and represented as a bottleneck, which let only conscious information pass through (?).

A third core concept is the **dynamic core hypothesis**. Consciousness is associated to a coherent assembly formed by *re-entrant or top-down loops*. This theory proposes re-entry as a fundamental property of conscious perception (?). More recently, Tononi and Edelman (?), hypothesize that information reaches consciousness when it achieves two fundamental properties: *integration* (i.e. formation of a coherent and unified representation) and *differentiation* (i.e. isolation of a specific content). The dynamic core hypothesis associate the neural mechanism underlying consciousness to the thalamocortical system. While a precise definition of neural bases of the dynamic core is missing, this theory propose a quantitative index to measure consciousness: the *neural complexity* (?). This index captures a fundamental aspect of the interplay between differentiation of local areas (differing in anatomy and physiology) and integration during perception. A low index identify either completely independent or dependent components. By contrast, an high index is found when the two coexist.

The information integration theory. This theory proposed a new measure of the quantity of consciousness, phi (ϕ), measuring directed, causal interactions within a system. This index represents a sufficient condition to potentially experience consciousness (??) , for a review (?). The theory provides also some explanation of physical substrate of consciousness. For example neurons in the posterior cortex, organized following an horizontal connectivity and a converging-diverging vertical connectivity connecting neurons along sensory hierarchies, are predicted to yield high neural complexity. By contrary, independent-processing micro-zones in the cerebellum cannot generate information yielding to low neural complexity (?).

The **thalamocortical rhytms**. Several other authors proposed the thalamocortical system as generating consciousness. Lamme and colleagues suggested that to sustain conscious visual perception bottom-up processing is not sufficient but requires top-down or feedback signals forming recurrent loops (?). Llinas et al. also argued that consciousness expresses itself as a closed loop property of the thalamocortical interconnectivity, which suggested the thalamus as a hub from which any sites of the cortex can communicate with any other (?).

Finally the **Global Workspace Theory**. This theory predicts that once information reaches consciousness, the current conscious content is represented within a global workspace, able to broadcast the information to other processors. Bernard J. Baars (?) hypothesized that the neural substrate of this global workspace are localized in the ascending reticular system and midbrain, in the outer shell of the thalamus and in the set of upward projecting neurons from the thalamus to the cortex. Theoretically, the GNW model proposed a higher level unified space, interconnecting perceptual, motor, attention and memory associative areas, where conscious information resides and is broadcast back to lower level process. Dehaene et Changeux

(?) proposed the Global Workspace as a cortical mechanism, implemented by cortical pyramidal cells with long-range excitatory axons, particularly dens in the prefrontal, cingualate and parietal regions interconnecting specialized automatic and non conscious modules.

1.4 Motivation and objectives

During my PhD work I developed in parallel two projects investigating the emergence of consciousness in the human brain. In the first part of my thesis, I investigated the neural substrate of emergence of motor awareness during a sensorimotor conflict task, while in the second part I focused on the emergence of neural signatures of improved consciousness after a neurostimulation intervention in a vegetative state patient.

Part I

Motor Awareness

Chapter 2

Motor Awareness

In the first part of my PhD thesis, I investigated the issue of consciousness particularly focusing on the aspect of motor awareness emerging during a sensorimotor conflict task. The term motor awareness refers to an important part of what we call self-consciousness, responsible for building the feeling of being in control and being the author of one's movement. It is in fact the conjunction of different aspects of motor experiences which returns a feeling of control of what one is doing. This subjective experience of controlling one's own motor acts is usually referred in literature as "sense of agency". We can go through the loss of the sense of agency when normal motor experiences, which are usually fluently controlled, are interrupted by a mismatch between predicted and actual sensory re-afferent. Usually, subjective experiences can be classed as "central" and "peripheral", referring respectively to cognitive experience related to action preparation and perceptual experience of body movements. In some cases it is possible to dissociate the two, for example when involuntary movements are triggered by electrical stimulation and produce peripheral subjective experiences without eliciting central ones. Sense of agency refers to the two together.

2.1 Motor action and motor awareness

The ability to monitor and correct our movements is fundamental for motor adaptation and it is under the control of an action-monitoring system. However, motor adaptation does not always need consciousness. In fact, it is widely accepted that most of the functioning of the motor system can occur without awareness. This discrepancy between the ability to generate (or adapt) goal-directed actions and the formation of a corresponding conscious experience raises several interesting questions about the nature of motor awareness. How do we know we are moving? How do we become aware of our motor actions? In recent years, motor awareness has become a major subject of investigation for neuroscientists (???) using different approaches. Several behavioural paradigms have been developed, using psychophysics (??) and frequently introducing sensorimotor mismatches. In parallel also several technique have been developed to addresses the neural bases of motor awareness including advanced neuroimaging (??), neurostimulation (?) and brain lesion studies (?). All this literature aimed at defining a common neural substrate for the emergence of content specific consciousness. Here, I will review the literature that I consider of importance for my following studies.

One important observation to understand the dichotomy between motor action and motor awareness is to realize that at phenomenological level motor compensation can occur outside awareness (?). In fact, most of the basic functioning of the motor system can unfold without awareness. A clear example of this was elegantly provided by Goodale et al. (?). They used a phenomenon called "saccadic suppression", in which a shift of visual target can go unnoticed if it happens during a saccade. In fact, under this condition subjects did not perceive the change in target location. Castiello et al. (?) investigating the timing at which subjects become aware of an unexpected target displacement, found that subjects motor awareness emerged after (around 300ms) automatic movement correction.

It is now well established that motor adaptation of on-line movements is constantly monitored by powerful feedback loops (??). Desmurget et al. used a reaching movement paradigm without vision of the respective limb to investigate the functional anatomy of these non-visual feedback loops during large and small motor corrections. Since these loops appear to be "automatic", they should operate similarly whether subjects consciously perceive them or not. PET-scan results showed the involvement in these corrections of a network including the posterior parietal cortex, the right cerebellum and the left primary motor cortex (?).

2.1.1 Sensorimotor conflict paradigms and motor awareness

Using a drawing task Torsten Nielsen (?) designed a paradigm to quantify the limits of motor awareness. He asked subjects to draw a straight line while they were presented with a visual feedback, which could be either their hand reflected on a mirror or the experimenter's hand. In this way, he showed that subjects presented with stooge coherently moving experienced this latter as their own hand (perception of a voluntary movement). Further, he demonstrated that when the stooge hand deviated on the left subjects compensate coherently in the opposite direction, but involuntary. In this way he concluded that "the visual hand" (the visual feedback) dominates "the kinesthetical hand" (the proprioceptive feedback), however subjects were able to involuntary compensate for the proposed deviation.

Wolpert and colleagues reported similar observations during a planar point-to-point task. In this task, subjects received altered visual feedbacks trough a mirror positioned above the pointing table. Visual feedback perturbation consisted in increased perceived curvature of the movement spanning from 0cm at the ends of the movement to a maximum of 4cm in the mid-point. They showed that subjects were able to progressively restore straight paths in the visual space by compensating in the opposite direction, however without consciously perceiving the distortion (?). Surprisingly, not only subjects are not always aware about the content of their motor actions, but if they are, they can barely recall quantitative features about their movements (e.g. kinematics and joint positions). Using a similar paradigm, Fourneret et Jeannerod (?) confirmed that motor adaptation, as they said, does not always need consciousness. In their study they instructed subjects to trace a straight line on a graphic tablet without seeing their hand. Visual feedbacks were available in a mirror positioned above the graphic tablet. In some trials, subjects were presented with incongruent (deviated either to the left or to the right) visual feedbacks. When a discrepancy between the visual feedback and the proprioceptive feedback was experienced, subjects adjusted their movements, deviating from the intended trajectories. However, once asking subjects to indicate, over a possible degrees of deviations, their final hand position, they always underestimated their motor compensation, showing a poor and limited awareness about specific aspects of their intentional motor acts.

To show that subjects started to compensate even before motor awareness Knoblich and Kricher (?) investigate the emergence of motor awareness in a similar task, requiring to draw circular lines. The experimenter changed the angular velocity of subjects' movement, instructing them to rise their hand once perceived any discrepancy between what they were doing and seeing. The results clearly showed that subjects raised their hand after motor compensation.

In summary, contrary to common beliefs, these evidences suggest that we are conscious about our motor actions not because we continuously sense ourselves moving but rather because we are conscious of our initial motor intentions (?). In fact, as long as the desired goal is achieved, the system does not take into consideration the movement details. For this reason, even large discrepancies between intended movement and actual sensory signal do not emerge to consciousness. Interestingly, adaptation studies comparing progressive and abrupt presentation of sensory perturbations showed that if a certain level of distortion is introduced progressively, on-line corrective loops can handle increasing amount of errors and subjects will remain unaware about the perturbation (?).

2.2 Internal models and motor awareness

Many studies have described models of motor learning and action control, which are of fundamental importance to describe action planning, execution and adaptation. Motor control is, in ultimate analysis, a process transforming sensory inputs into appropriate motor outputs. It is now widely accepted that in order to optimize these process the central nervous system try to estimate and predicts features of one's own body movements and their consequent interactions with the external world using two types of internal models. The first type is called the "forward model" (working on causal direction) which predicts sensory consequences of motor commands by using an "efference copy" (see Figure 2.1), an internal copy of the signal producing a movement within the motor system. The idea of the "efference copy" was first proposed by Von Helmholtz, a German physician of the 19th century, which observed that passive eye movements of the eyeball generated the illusion of visual world "moving". In contrast, with the same active eye movement the world was perceived as still. The second type of internal model is known as "inverse model" (see Figure 2.1), which provides the motor commands to achieve a desired movement (i.e. transforming a desired sensory consequence into motor commands). Using these two models, the central nervous system should be able, in principle, to simulate the motor system functioning (**?**). Movement monitoring starts with the delivery of the motor command followed by the movement onset and the movement itself and finishes with the end of the movement.

The execution of complex motor behaviours requires both forward and inverse models. Forward models are critical in motor control, to solve problems involving sensory predictions, fast feedback correction and state estimation (**?**). Predictions of sensory consequences can be used to anticipate and cancel re-afference, as for eye-movements and to maintain accurate performance. Discrepancy between predicted and actual feedbacks can be used to update forward model (see Figure 2.1).

As already mentioned, although it is natural to think that we are aware about our goals and desired state, we don't have conscious access to all the details of motor commands and the relative fine adjustments and features of muscles patterns, since it would be overloading for the brain to retain all this information. So a spontaneous question arises, which components of the internal model are available to motor awareness? Blakemore et al. (**?**) speculated on this point claiming that two kinds of possible predictions coming from the forward model may be accessible to consciousness. One which precedes the movement, when the forward model generates a prediction of motor action (i.e. they predict the behaviour of a body segment in response to a motor command) and compare it with the desired outcome; and a prediction which follows the movement, when the forward model predicts the sensory consequences of a movement and compares it with sensory feedbacks.

It is hypothesized that signals we are aware of do not emerge from movement itself rather from predictions. In particular, the predictions which follow the movement can compensate for the sensory effect of movement, coding self-movement versus outside world changing. In fact, predictions of sensory consequences of motor actions might be used to label movements and their sensory consequences as self-generated (?), being important for self-motor awareness. Within this objective framework, motor awareness seems to reflect the knowledge that we are moving and that we are doing so as initially planned. During experimental conditions subjects are usually good at estimating the onset of their movement and poor at perceiving kinematic details and prone to considered their movements executed as planned (?). This implicates that the relationship between content of motor actions and motor awareness is not straightforward. We can therefore reformulate the results by Fourneret and Jeannerod (?), in this way: subjects were aware of the movement they intended to make rather than those actually unfolding.

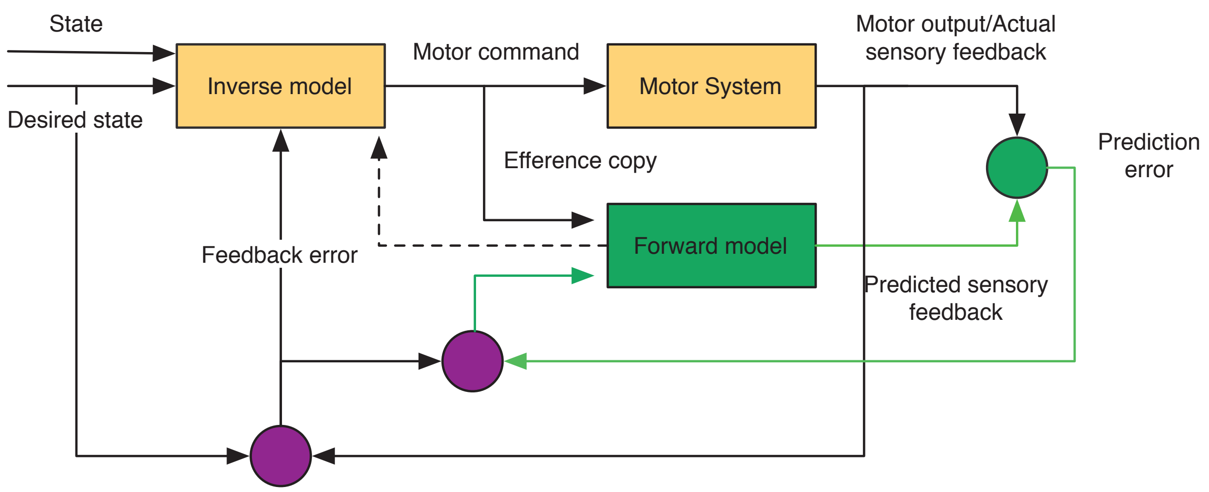

Figure 2.1: **Internal model schema**. A re-adaptation of a schematic functioning of internal models (??). The inverse model system make continuously fine adjustments to motor commands independently from awareness to achieve the desired state. In this case subjects are aware about predicted sensory feedback. It is only when the comparison between actual and predicted sensory feedback produce a large prediction error that subjects become aware of the mismatch. The prediction error and the feedback iteratively update the forward model which update the inverse model. Gaveau et al. add this new integration features because since the prediction error has a high detection threshold, it makes the loop unreliable to adaptation of small external perturbation. So for small or moderate prediction the feedback error allows a disambiguation. Inspired by Gaveau et al. 2014.

To summarize, the motor system seems to be aware of initial intentions, as long as the goal is achieved the details of motor execution do not reach consciousness even if important motor corrections are applied. So when a big mismatch between intended and actual sensory signals occurs, internal feedback loops become unable to force congruency and an error is produced, invalidating initial prediction, probably emerging from the parietal cortex. As a results motor awareness is unfolding and the subject become aware of the executed response.

2.2.1 Forward model, self-consciousness and pathological conditions

The fact that efferent signals plays a crucial role in the emergence of motor awareness is supported by three main line of evidence coming from behavioural studies on self-consciousness and on brain damaged patients. First of all, studies on self-recognition gave an insight to the relationship between the efferent signal and the capacity to attribute an action to ourselves. Tsakiris et al. (**?**) tested subjects within a paradigm in which they had either to perform a right index movement or their finger was moved passively by the experimenter through a lever. When the finger was moved passively no efferent signal was launched compared to the active condition. In the active condition, self-recognition was found to be more accurate, showing the importance of efferent output in constructing motor awareness and furthermore self-recognition.

Further evidence comes from brain damaged patients, and in particular "deafferented" patients. These patients are aware of performing a movement, for example about the movement direction, even though they are blind to any proprioceptive signals. For instance, they are unable to tell if their hand had been blocked during the movement. This dissociation has important implications as it suggests that they experience the awareness of movement even in the absence of perceptual information, therefore relying purely on efferent command. The total lack of sensory signals is also demonstrated by their lack of normal contralateral movement-evoked potentials in sensorimotor areas (**?**). However, in the study by Kristeva et al. the complete absence of sensory inputs does not prevent the patient to show a residual motor awareness. Similarly, a systematic mismatch between peripheral inputs but a preserved efferent command could also be at the origin of phantom limb pain observed in amputee patients. In this case, providing the patient with a virtual visual afferent signal can actually reduce phantom limb pain by inducing coherent efferent and afferent signals (**?**).

A quite inverse pattern is observed in patients with anosoagnosia for hemiplegia, a disturbance of motor awareness in which the patients are unable to move and still claim to do so, denying their own deficits. These patients are thought to form a normal efference copy but to fail to accomplish the motor command. These gives a strong line of evidence to the hypothesis that motor awareness has an endogenous origin. Berti and colleagues (**?**) tested anosognosic patients with lesions including the premotor areas 6 and 44, motor area 4 and the somatosensory cortex. They suggested that patients were impaired to realize that they were not moving due to a dysfunction in the comparator of intended and actual peripheral reafference (which in this case would be absent). Crucially, such interpretation is supported by the fact that these patients seem to display abnormal awareness only when required to detect active movements but not during passive movements.

In another study, Fotopoulou and colleagues proposed the rubber hand illusion paradigm to hemiplegic patients with or without anosognosia for hemiplegia. Three conditions were tested: an efferent copy condition, where patients had to raise their own paralyzed hand while seeing the rubber hand, a non-efferent copy condition, experimenter passively raised their paralysed hand and a no-movement control condition. In contrast to patients suffering only from hemiplegia, anosognosic patients with hemiplegia, disregarded visual information relative to the motionless rubber hand only when they were expected to do the movement compared to when experimenter had to do so (**?**).

All these examples on how motor perception and motor awareness can be dissociated in pathological conditions do not argue that sensory input signals are not taken into account. It is well known that passive limb displacements are easily detected, as peripheral stimulation (Carota), however in this case no expected inputs are generated, and therefore when sensory flow reaches the cortex, an error signal is produced. In fact, it is accepted that only when prediction becomes highly unreliable sensory afferent signals regain access to consciousness.

2.3 Neural candidates of motor awareness

Several brain regions have been hypothesized as potentially involved in motor awareness. Considering the literature about major abnormalities in awareness of action the posterior parietal cortex and the premotor cortex seem to be the two most probable candidates (see Figure 2.2). The posterior parietal cortex (PPC) was found to be implicated in both motor conscious intention (**?**) and motor awareness (**?**) and several studies have linked its activity with predictive computation(forward modelling).

The parietal cortex together with the cerebellum are thought to be involved in sensorimotor predictions. Following this hypothesis (**???**), the cerebellum may be part of a forward model system by providing rapid prediction of sensory consequences of actions, to be compared with actual inputs. The comparator module would have its anatomical counterpart in the climbing fibres of inferior olive, important to evaluate motor performance. These neurons have been proposed to react to unexpected and unpredictable stimuli (**?**). On the other hand, the parietal cortex would be more likely involved in prediction related to plans and goals of a movement.

The parietal lobe seems to be involved in detecting mismatches between desired and actual movement. A first evidence come from a transcranial stimulation study, delivering a single focal stimulation over the left intraparietal sulcus, at the onset of a movement correction. Using a saccadic suppression paradigm, Desmurget et al. (**?**) showed that successful corrections in response to a subliminally displaced target are linked to the PPC activity. Pisella et al. arrived to a similar conclusion testing a patient with bilateral parietal lesions and showing that she failed to correct her arm trajectory after a jump of the final target durng a saccade (**?**). They inferred that on-line control is a specific function of the posterior parietal cortex. The role of parietal cortex and cerebellum was also emphasized by a study introducing a mismatch between vision and action in normal subjects using fMRI. In fact, Giraux et al. (2002) suggested that both intraparietal sulcus and medio-lateral cerebellum were involved in the detection and adaptation during a visuomotor conflict.

Assal et al. (**?**) showed that lesions in the right parietal cortex caused the alien hand syndrome, a neurological disorder of awareness in which movements are performed without will. Further, parietal lesions were also associated with alteration of awareness of voluntary actions, and could cause the loss of "wanting to move" feeling in the initiation of a movement. Sirigu et al. (?), using Libet's paradigm on parietal patients reported that the classical delay of 206ms before EMG signalling the will to move, was abolished, suggesting that patients could not access their intentions to move. Motor awareness emerged probably 60-70ms after movement onset. In fact, in these patients motor awareness is likely to emerge from the processing of peripheral inputs rather than from (impaired) efferent command. Another study, conducted by Sirigu et al. (?), investigated impaired motor awareness in apraxic subjects, with a deficit of voluntary movement, having lesions on the left inferior parietal cortex. Subjects had to execute finger movements, while the visual feedback, presented by a video monitor, was manipulated systematically. Apraxic subjects had more difficulty in recognizing their own movements from congruent experimenter movements, mistaking a stooge hand for their own. This suggested a failure in evaluating and comparing internal and external feedbacks, highlighting the crucial role of intentions and forward processing in self-recognition (?).

Other eloquent evidence come from a neural stimulation study performed in our team by Desmurget et al (?). In this study neural correlates of motor intention and awareness were investigated by directly stimulating cortical areas likely generating this process. In particular, they found that stimulation of the right inferior parietal lobule (Brodmann areas 39 and 40) triggered the intention and desire to move the controlateral limbs, and stimulating the left side triggered the intention to move the lips or to walk. Often the patients reported "a will to move the chest" or "a desire to move the hand". Interestingly, their reports highlighted the voluntary and endogenous origin of these triggered reactions. Experimenter: "Did you move?" Patient: "No. . . I had a desire to roll my tongue in my mouth.” Experimenter: "To roll what, your. . .?" Patient: "To roll my tongue in my mouth." Always based on this study, PPC was hypothesized to store movement representations, since patients well described precise action as in the previous example. If the intensity of the stimulation was augmented from 5 to 8 mA, the intentionality switched to the certainty from the patient that he actually moved the respective body part, although no EMG activity was recorded. This implicated that a movement pre-stored representation could emerge to awareness without being accompanied by a real movement. This would account for the hypothesis that forward modelling is implemented in the posterior parietal cortex. Importantly, this study highlights a double dissociation: premotor stimulation induced movement without motor awareness while angular gyrus stimulation led to motor awareness and movement intention without EMG activity.

From previous studies, evidence had emerged that the dorsal stream provides automatic guidance to our movements to ensure to arrive at the final target as well as to avoid potential obstacle. This mechanism of automatic avoidance is severely impaired in patients suffering from optic ataxia, having lesions on the superior parietal and intraparietal sulcus, or more in general in the dorsal stream. Schindler et al. (**?**) demonstrated how these patients were not impaired in simple reaching tasks, but instead in avoiding obstacles and automatically correcting their movement towards a target.

Studies on action imagination also contribute to bring evidence of the implication of the parietal cortex in the representation of actions. For instance, using neuroimaging studies Gerardin et al. (**?**) compared imagined and executed motor actions in normal subjects. Compared with rest, these two conditions shared overlapping networks, including bilateral premotor and parietal areas, basal ganglia and cerebellum. Imagined motor actions triggered higher engagement of bilateral premotor, supplementary motor areas and left posterior and inferior parietal lobes. Conscious representations are known to be challenged once parietal lesions occurs based on knowledge of patients which are unable to maintain them and instead perceive the feeling of "losing their right arm". This was the case reported by Wolpert (**?**) of a patient with a lesion in the superior parietal lobe, presenting both sensory and motor deficits, unable to maintain an internal representation, suggesting the fundamental role of superior parietal cortex in sensorimotor integration.

Particular focus for motor awareness has been provided also for the promotor cortex, the supplementary motor area (SMA), the anterior cingulate cortex and the dorsolateral prefrontal cortex (DLPF). Main evidence supporting the role of the premotor cortex in motor awareness comes from a study by Berti and colleagues (**?**) on anosognosic patients. As already mentioned, they identified the Brodmann area 6 as frequently causing this deficit (surplus) of awareness. They hypothesized that this region is responsible for monitoring the actual movement, acting as a comparator of actual and expected sensory inputs.

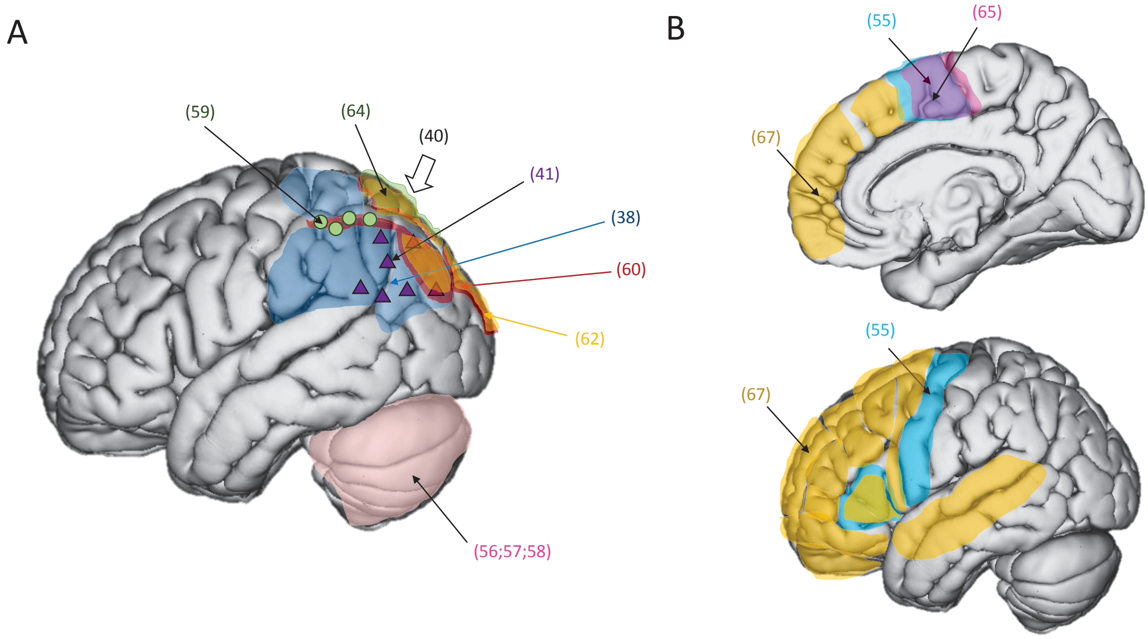

Figure 2.2: **Summary of cited literature referring to neural candidates of motor intention and awareness**. The figure shows the areas cited in the selected literature, with the respective references. Panel A refers to parietal candidates and panel B to frontal candidates of motor awareness.

The supplementary motor area and medial motor regions are also thought to play an important role in the emergence of conscious intention. Evidence of SMA implication comes from several studies (see Figure 2.2B). Stimulation studies conducted by Fried et al. (**?**) over the SMA region with subdural electrodes in epileptic patients triggered a compulsive desire to act, described as a need or a urge to move. However, subjects did not feel as they were at the origin of this urgency, instead they reported that a movement "was about to occur" and that their arm "was going to move". In this case, increasing the intensity of the stimulation triggered the actual movement. It is interesting to notice how the stimulation of the SMA evoked precise movement intentions, probably by suppressing the inhibitory signals exerted by the SMA over the primary motor cortex (**?**).

The prefrontal cortex seems to be more involved in conscious monitoring than in sensorimotor integration. In fact, it is not involved in selection of spatial target or series of action to join target. Using a similar experimental paradigm to the one already described in Fourneret and Jeannerod (?) task, Slachevsky et al. (?) showed that patients with prefrontal lesions achieved awareness of the sensorimotor conflict much later than control subjects. In fact, the amount of visual deviation had to be augmented over 24° degrees before patients realized the mismatch, showing a disturbance of conscious monitoring of action. Again Slachevsky et al. (?) using a similar paradigm, but keeping constant angular deviations at 24° degrees, evaluated whether past experiences could help behavioural adaptation. They found that patients with frontal lobe lesions were able to verbally report the correct adaptation strategy later than controls, and that this delayed awareness correlated with frontal behaviours. Moreover, subjects who reported their strategy later on time had also worst task performances. In this way, awareness was linked with the performances, which decreased during unaware trials, before that subjects were able to verbally report the correct adaptation strategy. However, also patients who never report the good strategy showed some implicit adaptation.

2.4 Brain mechanisms of conscious access

As mentioned before, several processes unfold in the brain outside awareness. However, evidence suggests that non-conscious processing can influence the entire chain of specialized areas. These mechanisms of non-conscious processing are particularly interesting in helping to highlight the difference between what is and what is not conscious. Convincingly, subliminal priming has been demonstrated at several level including visual, semantic and of course in the motor domain, as able to influence motor responses an even inhibitory control (?). However, studying non-conscious access with subliminal priming brings potential limits, such as that activity decreased with processing depth, with elapsed time, and failed to trigger lasting and flexible modifications (?).

fMRI studies on masking paradigms highlight amplification of activity in the visual cortex mostly in associative areas and the emergence of a correlated network including bilateral parietal and frontal cortex. Event-related potentials (ERPs) analysis on attentional blink paradigms revealed fully preserved visual processing on unseen trials (?).

ERPs studies using masking paradigms showed that early visual processing (200ms following stimulus) can be fully preserved in unconscious trials (e.g. trials in which the subjects deny seeing the stimulus). It is only around 300 to 500ms that neural correlates of visibility appear, as a distributed activity, called P3 or P3b. This P3b wave has to be distinguished from P3a wave, recorded also during non-conscious processing and usually attributed to highly focused continuous visual attention, localized in focal anterior regions (?).

Del Cul et al. investigated the brain activity evoked by masked stimuli using high density EEG. Using a backward masking task (50ms), they want to characterize which brain areas and what specific timing was associated to conscious access. They varied by small steps the target-mask stimulus onset asynchrony (SoA) to find ERP events showing a non-linear dependency with it. They observed that conscious reportability correlated with a late ($>$270ms) bilateral fronto-parieto-temporal network activation (?). Instead, in the preceding period (between 140-270ms) a non-linear activation proportional to SoA was observed in the posterior occipito-temporal and parietal areas, showing a non-linear amplification with neural correlates of masking threshold.

Findings from subliminal research turned out to be particularly useful to refuse previous theory on conscious processing, highlighting how specific neural mechanisms, until that moment attributed to conscious processing, were still present during unconscious conditions. For example, the believes that invisible stimuli may be coded in peripheral cortical modules, but that unconscious response does not recruit fronto-parietal network responsible for global broadcasting information through the cortex was recently challenged by Cohen et al (?). They showed how long-range synchrony after post error adaptation occurs even in the absence of conscious error adaptation. Conscious perception was also associated with the ability to hold a certain representation in time compared to unconscious stimulus perception. This theory is called Recurrent Theory and refers to the idea that an invisible stimulus may start feedforward mechanisms but not recruit recurrent processing. However, King et al. (?) showed recently how invisible information can be maintained within higher processing stage neural activity.

Finally, according to recent evidence MEG and EEG data recorded during observation of threshold-level visual stimuli showed a decrease in variability in seen versus unseen trials (subjective reports). This results showed that cortical activity for consciously perceived stimuli is more stable than for unconscious stimuli (?).

2.5 Motor awareness in children

Studying consciousness in children allows to make a link between multi-sensory development and emerging sense of self. Few studies have investigated until now the role of motor awareness in the development from childhood to adulthood. Therefore, the emergence of motor awareness in children has not yet been directly tackled using neuroimaging techniques. However, this question is of extreme interest since awareness can be crucial for the correct development of multi-sensory integration and therefore to build a stable body representation and a corresponding sense of self and agency.

It has been argued that awareness of own-body already appears during the first year of life. Filippetti et al. tested newborns using visuo-tactile synchronous and asynchronous stimuli. They presented video stimuli containing either mirror-like images of 5-month-old infant face being stroked with a paintbrush synchronously or not, called body related, and the same image but upside-down, called non-body-related stimuli. They showed how newborns preferred to look at the synchronous stimuli, but only when visual cues were body related (?). However, despite the early detection of self perception capability, it is known that multi-sensory processes underlying body representation reach maturation during the late childhood. It is in fact around 8 years old that children start to integrate different modalities in order to reduce sensory uncertainty (?).

Nardini et al., using a task in which subjects had to return an object using either visual landmarks or self-generated (proprioceptive) cues, found that children before 8 years old did not reduce their response variance when both cues were available (?). In another study, they demonstrated that sensory integration induced uncertainty reduction only after 12 years old, also if tested within a single modality, like vision. However, since children experience less sensory integration than adults, they have a more developed capability to exploit individual sensory modalities information alone, reaching greater performances compared to adults when discriminating conflicting stimuli (?). It is only during late childhood that children reach an adult-like balance between sensory cues and are able to build a correct representation of their own hand in the external space and reach a unified bodily self.

Interestingly, Cowie et al. (?) using the rubber hand illusion paradigm, and in particular an intermanual pointing task, found that children were as sensitive as adults to visual-tactile synchrony, suggesting that the visual tactile pathway is already mature at 4 years old. However, children between 4 and 9 years old were more victims of the rubber hand illusion compared to adults, perceiving their hand position closer to the rubber hand. This dissociation shows the existence of two processes with different maturing timing, where the process relying on a visual-proprioceptive information would mature later. However, the ability to identify their hand as one's own (assessed by ownership question and not by perceived hand position) had no significant development after 4 to 5 years old. In a subsequent study, Cowie et al. (?) tested the same paradigm in later childhood, considering subjects between 11 to 13 years old, comparing the new dataset with previous results. They found that perceived hand location, assessed by eye closed pointing of perceived position, reached adult-like performance around 10 to 11 years old.

A virtual reality study on bodily self-consciousness point out that touch referral was grater in synchronous compared to asynchronous condition only after 10 years old, and drift in perceived self-location was grater in synchronous compared to asynchronous condition only for adults (?). This result is of fundamental importance in suggesting that two different perception aspects, the sense of hand ownership and the actual perceived location, may develop following different timing (?).

Therefore, the bodily self is not a unitary construct developing in a unitary manner, but consists of several processes that unfold at different rates according to different time line.

It was suggested that body self-awareness may serve as a developmental bridge between the kinaesthetically based awareness and discrimination of one's own body (evident in infancy) and the more complex psychological self that develops over childhood and adolescence (?).

Body identification or self-awareness seems to mature earlier than the proprioceptivly based sense of limb location which takes a longer time to reach mature state. A more complex psychological self develops over childhood and adolescence. Adolescence sees the development of a much more complex form of self-awareness, in particular the ability to relate the self to a social environment. Sensory foundation of an adult like use of multi-sensory information is a key step to develop new conception of the self that emerges during adolescence. During early adolescence the hand reaches adult size and its perception becomes less plastic and more mature. It is at this point that children begin to combine and weight multi-sensory processing optimally (?).

The accuracy of internal hand representation was also investigated by Vidal et al. (2004) which tested 15 children aged 5 to 10 years old. They showed that both errors in the extent and direction of movement were significantly largest in younger children, which showed higher variability in the end-point reaching. They attributed this difference not only to immature control mechanisms but argued that younger children had only a partial formation of forward representation for hand localization. Efference copy and proprioceptive signals are essential to build initial and subsequent hand location, which requires a forward model formation. Internal models are generally used in two phases, for computing a movement vector linking the hand and the target, and for solving the inverse kinematic problem, finding right combination of joint angles to achieve the desired trajectories. In this sense, a noisy proprioceptive calibration or an inaccurate hand location estimates can result in a compromised planning and execution.

In adults, I discussed how neuroimaging studies had identified the recruitment of a specific set of regions in response to body-related multi-sensory integration of body modality attributed to the self. It is of great importance to test whether these same areas are activated also in the immature brain. A study by Filippetti (?) showed that cortical activations (assessed with near infrared spectroscopy) of 5-month-old infants were similar to adults in response to body-related contingencies versus non-contingent stimuli.

However, it is well documented how the human brain goes through dramatic changes of structural and functional organization during life (?). Although 90% of adult size is reached by age 6, the brain still matures throughout adolescence and young adulthood. Specifically, cortical gray matter loss occurs earlier over brain regions embedding primary sensory and motor process (until 12 months), while associative areas, controlling top-down behaviours mature later on (?). Temporal and parietal cortices associated with basic language and spatial attention skills mature next (until 10 years old), followed by prefrontal and lateral temporal cortices, integrating primary sensorimotor information seems to mature later (until 16 years old).

Chapter 3

Experimental Studies on Motor Awareness

3.1 Aim of the studies

Mismatch paradigms represent a good experimental way to introduce a discrepancy between intended movement and actual sensory feedbacks, by presenting an mismatch between visual and propioceptive cues. These paradigms are suitable to investigate how subjects integrate sensory signals to update their conscious motor perception and highlight that they are unaware about much of their motor adjustments. An ecologic way to study neural underpinnings of conscious perception is to keep sensory input constant and to study emergent differences in brain activity looking for changing contents of consciousness. Therefore, stimulus visibility is usually manipulated to create conditions differing regarding conscious perception rather than in respect to objective stimulation parameters per se. However, two experimental conditions are never strictly identical (?), and slight differences in the ERPs can account for this objective experimental bias rather than for subjective reports.

First, we focused on the neural correlates of motor awareness in healthy adults and in 7-11 year old children, trying to catch brain activity when the motor conflict becomes overt. We modified a preexisting paradigm (?), paying attention to present stimuli around each subject's perception threshold. We paired this task with high density EEG recordings (128 channels), to study the neural activity undergoing the sensorimotor conflict with a good temporal resolution.

We used blind source separation (BSS) (?) because it allowed us to reconstruct cortical regions of activity and their associated neural signal and thus to study the sensorimotor conflict task in a more precise frame. In this way, we could identify when and where the overt motor conflict was perceived.

Blind source separation can be used either for artifact rejection and neural activity reconstruction. In this work, I always used it in series, taking out the noise due to eye-blink and hand and body movements in a first step, which aimed at rejecting interfering components at a single subject level, and then I used a group BSS to find common sources to the two experimental groups (adults and children). In this way, we were allowed to compare neural signals coming from different populations but having a common cortical sources separation (for more details see Methods). We further performed analysis of sensorimotor features, as well as assessing if adults and children had the same velocity and reaction time in detecting and correcting the onset of the deviation. In this way we wanted to investigate the neural bases of motor awareness and their evolution in healthy children. To answer these questions is crucial both for adding a piece on the puzzle of motor awareness in healthy subjects and for the comprehension of normal consciousness development.

In the second section, I will present a behavioural study within which we investigated differences in behavioural performances in healthy adults and children while random deviations were proposed. Particular focus on detection theory allowed us to pinpoint fundamental differences in detection performances between adults and children.

Following, it is reported a draft version of the article which is still in progress.

3.2 Neural correlates of consciousness during a sensorimotor conflict

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3.2.1 Introduction

People strongly believe that they are completely aware about their own actions. This awareness capacity gives us a clear sense of "self", i.e. a clear sense that we exert a direct control on our movements and decisions. However, at a phenomenological level, several studies have repeatedly shown that most of the basic functioning of the motor system can unfold outside awareness (**??**). In fact, ongoing motor responses are constantly monitored by powerful feedback loops (**?**) which operate automatically and behave similarly whether or not the subject adapts consciously to the external environment. It is now widely accepted that motor awareness does not directly rely on sensory cues about the ongoing movement but rather on initial intentions and therefore on forward internal models (**?**). It is only when the comparison between predicted and actual feedbacks does not match the desired state and a certain amount of flexible control is needed that the error information gains access to consciousness (**?**).

Using a sensorimotor conflict task, it is possible to experimentally reproduce a mismatch between visual and self-generated (e.g., vestibular and proprioceptive) cues, which at times brings the sensorimotor conflict into awareness. Using this setting, in which subjects could not see their hand while tracing, Fourneret and Jeannerod showed that subjects have limited or no consciousness at all of their motor performances. In fact, not only they are not aware about the details of their own movements but they underestimate the amount of their motor corrections (**?**).

Studies on motor control and underlying cognitive functions point to the medial frontal cortex (**?**), and in particular to the rostral cingulate zone (**?**), as a "crucial node" to detect incongruent outcomes, response conflict and errors. The error detection, or the detection of a discrepancy between predicted and actual sensory outcomes is used to adjust the ongoing movement (**?**) and adapt to novel situations. Recent researches suggest that motor adaptation rely largely on parietal regions, mainly on the left hemisphere (**?**), and cerebellar circuits

(?). However, the neural substrates responsible for the emergence of motor awareness following sensorimotor conflict remain debated. Since the parietal cortex is also implicated in motor representations and online action control it is still unclear whether this activity responds specifically to awareness or encodes also movement correction features.

Nevertheless, if this question remains tricky and still unanswered for the mature brain, it has not yet been posed for the developing immature brain. The study of motor awareness development may therefore provide insights about how does motor awareness emerges when forward models are not stable yet.

Previous studies showed that not only forward model but also multi-sensory processes underlying body representation in children are not fully developed (Contreras-Vidal et al. 2004). Evidence from the rubber hand-illusion suggests that children rely more on visual than proprioceptive cues to construct the internal hand representation (?). Consequently, since proprioceptive signals and efference copy are essential to build forward model it is still unclear how motor awareness is affected or whether this mechanism can share the same neural bases with adults. However, since children are better in exploiting sensory information from a single sensory modality (?), a prediction is that ongoing visuomotor features are not optimally integrated to gain access to consciousness. This prediction, together with the fact that the parietal cortex development arrives later in the ontogenesis (?), suggests that motor awareness in children may have a different neural substrate.

The purpose of our study was to investigate the neural substrates of motor awareness disentangling specific structures or activities sustaining motor awareness from activities involved in action monitoring and to compare this mechanism in mature and developing individuals. We decided to tackle this issue by using a simple paradigm: a sensorimotor conflict (Nielsen, 1963) in which subjects were presented with a drawing task (i.e. to trace a straight line). To provide a stable background to study conscious perception around subjects' perceptive threshold we used an adaptive algorithm. In this way, we collected trial-by-trial measures of objective visuomotor features and subjective motor awareness. The purpose of our study was therefore to isolate within the functional architecture of sensorimotor conflict the specific structures or activities at the origin of the motor awareness. Using EEG recordings, we investigated the neural mechanisms encoding visuomotor features and tested how these processes varied as a function of objective motor performance and how cortical neural activity explained subjective awareness reports.

3.2.2 Methods

Stimuli and Protocol

Twenty young right-handed healthy adults (26 ± 4 years old, 11 females and 9 males) and 20 children (9 ± 1.5 years old, 14 females and 6 male) participated in this study. Subjects had normal or corrected-to-normal vision. Each experiment lasted for approximately 1 h 30 min. Subjects were unaware about the purpose of the experiment. Children were recruited through local schools and received 30 euros in compensation for their participation. All subjects and legal tutors (for children) gave written informed consent to participate in the study. The whole paradigm was approved by the local Ethic Commitee (N ID-RCB 2014-A01894-43).

Subjects sat on a chair facing a three layers table (see Figure 3.1A) composed by a graphic tablet, a mirror and a computer screen (positioned at 75, 107 and 139cm from the ground for adults and 75, 99 and 123cm for children, respectively). They were instructed to draw a straight line with their right hand on a graphic tablet (Wacom tablet Intuos 3) using an adapted stylus. To reproduce the friction sensation, a paper board was positioned between the stylus and the tablet. While subjects were tracing, they could not see their hand since they was hidden by a first surface mirror (Edmund Optics 254 x 356mm, 4-6 λ Mirror) used to reflect the visual feedback, displayed on the computer screen (HP ZR2240w 21.5-inch LED Backlit IPS Monitor, RR = 60Hz) positioned above.

We asked participants to draw a straight line from a red spot, positioned in the bottom part of the screen, to another red spot positioned 11.5 cm above on the sagittal axis, while maintaining a homogeneous velocity. At the beginning of each trail, subjects had to place the stylus tip at the starting position on the tablet, located in correspondence to the midline of their

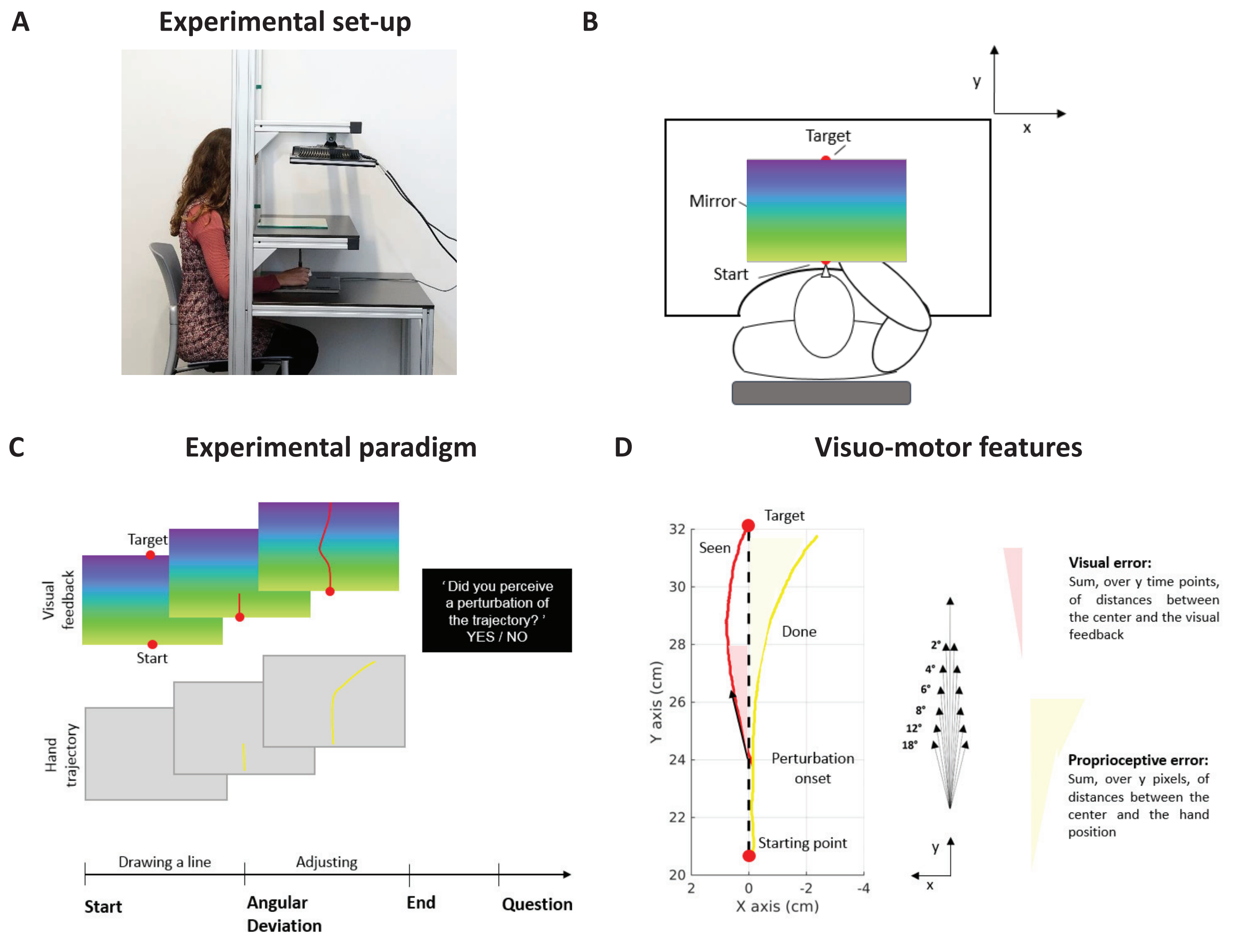

Figure 3.1: **Experimental set-up, protocol and movement trajectories.** (A) Lateral view of the experimental set up. The subject's hand was hidden by a mirror. The hand movement was recorded by a graphic tablet and reflected in the mirror as a cursor. The mirror reflected the cursor, the starting point and the target (red dots). (B) Top view of the experimental set-up. (C) Experimental protocol. Both hand trajectory and visual feedback are presented (bottom and top respectively). Subjects had to trace a straight line from the bottom to the top of the graphic tablet (yellow line). At a certain jittered location, an angular deviation was introduced, and the visual feedback was rotated by a corresponding angle. The subject started to compensate in the opposite direction to reach the visual target.(D) Example of movement path for one perturbed trial. Red line: visual feedback. Yellow line: real hand position. Visuomotor features are schematically represented, including visual error, related to the apparent direction of the movement ("seen") compared to the expected one (straight ahead), and proprioceptive error, related to the actual direction of the hand ("done").

body. Subjects had to reach the final target without directly seeing their hand and to use the computer-generated visual feedback reflected on the mirror (defined as the "seen trajectory") to assess their ongoing position. The visual feedback consisted in a dark spot corresponding to the actual position of the hand. The position of the stylus tip (defined as the "real trajectory") was presented as a dark spot overlaid on a rainbow background (see Figure 3.1C). Stylus positions were sampled at 60Hz and displayed on the computer screen.

In 80% of the trials, an angular bias (ranging from 1° to 15° degrees) was introduced on the visual feedback, either to the right or to the left. In these trials, in order to see a straight line on the screen and to reach the target as instructed, subjects had to adjust their actual movement direction. To reduce anticipatory reaction, learning and adjustments from participants the deviation onset was jittered in a random way within a window of 2 cm, going from 2 cm after the starting point until the center of the path. Trials maximum duration was set at 5s for adults and 10s for children. Trials ended when subjects reached the target spot. Subjects were then asked to answer to the following question: "Did you perceive a perturbation of the trajectory? "and they had to report (adults) or to say (children) "yes" or "no".

Subjects did 10 min of training before doing the EEG to make sure they understood the instructions and were familiar with the set-up. Adults and children performed a total of 480 trials. Twenty percent of the trials were presented without deviations (and they were called "neutral"). An additional block, called the source block, of 192 trials (with 32 neutral trails), was performed by adults after the training and before the experiment. This block was designed to present big deviations (ranging from $-15°$ to $15°$) in order to optimally extract task-related sources and prevent to base the source separation only on trials with small deviations. Since children had less resistance to fatigue they did not perform this additional block.

A staircase algorithm was implemented to ensure to present stimuli around each subject's detection threshold. This procedure is described by Levitt et al. (?) and consists of adjusting the deviations in each trial, according to the subject's previous response. In particular, to reach 50% level of aware and unaware trials, an up-down adaptive staircase procedure was applied, where the stimulus level was decreased after a positive response (or increased after a negative one). Every subject completed 4 staircases of 100 trials, 2 with left deviations and 2 with right deviations, starting either from 0° or 15° degrees (implemented using the Matlab toolbox - Arthur Lugtigheid 2009). Different step sizes were used throughout the paradigm. Staircases steps changed by 2° degrees for the first 5 reversals, by 1° degree for the second 10 and by 0.5° degrees for the remaining ones. The 4 staircases were randomly interleaved. The online estimation method had the advantage to be robust to perception drift, in fact if a drift of perception threshold occurs during the task also the stimuli intensity placing will follow this drift.

Preprocessing

Electroencephalographic (EEG) signals were recorded using the Brain Product™ actiCHamp system with 128 active electrodes (actiCAP 128Ch Standard-2) mounted in an elastic cap at 10-5 system standard locations (**?**). All electrodes impedances were kept below 50 kOhms. EEG data were recorded at a sampling rate of 5kHz without an online reference, then all data post-processing was done offline using the MatlabTM software. Subjects were seated in a darkened, shielded room. Offline, data were band pass filtered using zero-phase Chebychev type II filters (Low pass - cutting frequency: 45 Hz, transition band width: 2 Hz, attenuation: 80 dB; | High pass – cutting frequency: 0.75 Hz, transition band width: 0.2 Hz, attenuation: 80 dB) and re-referenced to common average. Then, data were epoched from -0.5ms before to 1.5ms after the stimulus onset. Corrupted epochs were automatically detected and rejected using EEGLAB (**?**) and the FASTER toolbox (**?**). Finally, for each subject, non-brain artifacts (eye movements, ballistocardiac noise, sensors movements and other electrical noises) were detected and rejected using independent component analysis (ICA)/blind source separation (BSS) with UW-SOBI (1001 times delays). The UW-SOBI algorithm (**?**) is an adaptation of the well-known SOBI algorithm (**??**) reformulated as an uniformly-weighted nonlinear least squares problem to avoid the common "whitening" phase which is known to limit the performance of BSS/ICA algorithms in noisy conditions (**?**).

Behavioural Data Analysis

Staircases Thresholds. For every staircase a convergence threshold was computed as the mean of deviations of trials where a reversal occurs (where reversal is defined as a trial in which the subject switches his answer from "yes" to "no" or the contrary). The threshold of conscious perception was estimated using reversals occurring after the fifth trial.

Trials discarded during the EEG preprocessing were not included in further analysis. Raw data of x and y coordinates (horizontal and vertical positions) of the dot displacement corresponding either to the real stylus trajectory and seen black dot trajectory were further analysed using MATLAB (The Mathworks, Natwick, USA). Few pixels before the final target (at 645 pixels from the top of the screen) were not considered systematically in the trajectories analysis, being possibly contaminated by artefacts due to reduced velocity. All x trajectories (defined by x positions sampled each 60Hz) were realigned to the onset of deviation (due to the jittered procedure previously described). A window of 2s, starting 0,5s before and ending 1,5s after the deviation onset was extracted. Trials shorter than 2 seconds were filled up with empty values. For every subject, their adaptation threshold was computed using Matlab toolbox - Arthur Lugtigheid 2009. For every trials, the onset of correction and mean velocity were computed as follow.

Correction Onset. Y and X coordinates were interpolated to be sampled every 10ms. The first derivative of the x coordinates (horizontal position) of the hand trajectory was computed. Since to adapt to stimuli subjects had to drift away from the center of the screen, the correction onset was defined as the moment at which the first derivative of x started to stray from the straight direction. To minimize the effect of different deviation intensities and orientations (left/right) the x coordinates of each trajectory were normalized in respect to their distance from the ideal line at 700ms. This timing was chosen as a trade-off between finding a timing in which trajectories were already biased from the stimuli intensities and a timing for which all trials had valid (and not empty) coordinates. The mean over the baseline was computed and systematically subtracted from the trajectory. Further, to remove any natural directional tracing bias, for each subject a median trajectory was computed in the aware and unaware conditions for left and right deviations. The left median trajectory was then subtracted from the right median trajectory.

Cleaning Procedure. Trials in which subjects did not perform correctly the task were discarded. A trial was considered valid when the hand trajectory crossed an imaginary starting line (settled at 1072 pixels from the top of the screen, 2 pixels after the starting spot) and arrived first at the deviation onset and second to the final target. Only trials deviated more than 1° was considered since smaller deviations were unlike to provide a visual feedback appreciably different from the neutral one.

Velocity of Execution. The velocity was computed as the ratio between the length of the trajectory traced by the hand and the relative execution time. The data were filtered as follow for every subject: only trials in the range of 3 standard deviations from the mean value were considered for further analysis, for all the following features.

Two movement features were selected as reflecting sensorimotor conflict, called visuo-motor features: the visual error seen until 400ms and the proprioceptive error.

Visual Error at 400ms. For each trial, we quantify how much the visual feedback drifted from the midline until 400ms. Four hundred milliseconds was chosen considering that the onset of correction should occur at 350 ms on average. Because we wanted to capture the amount of visual feedback deviation from the straight planned movement from the onset of deviation until an effective correction took place, we considered 400ms after deviation onset. The visual error was computed as the root mean square error between the seen trajectory (x position integrated over time points) and the ideal central line (relative to the beginning of the movement) taken as the x position at the onset of the perturbation.

Proprioceptive Error. To quantify the hand correction, for each trial, we computed the root mean square error between the real trajectory (x position integrated over pixels) and the ideal central line after the onset of the perturbation, normalized by the same index computed before the onset.

Statistics

For all following measures, the normality of the distributions (Kolmogorov-Smirnov) was tested prior to apply the ANOVA, if this condition was not respected, non-parametric tests were applied. A mixed ANOVA was performed on perception thresholds with group (adults/children) as independent variable and laterality (left/right) as dependent variable to test if children have higher perception threshold than adults. Because no significant difference was noticed between deviations applied to the left or to the right side ($F(1,38) = 3.042$, $p = 0.089$, $\eta_p^2=0.074$), further analysis were run without distinction between left and right trials. To test the onset of correction and velocity, a mixed ANOVA was performed on correction onsets with group (adults/children) as independent variable and awareness (aware/unaware) as dependent variables. For visual and proprioceptive errors, an ANCOVA was performed with group (adults/children) as independent variable and awareness (aware/unaware) as dependent variable and mean deviations and difference between deviations presented in the aware trials and unaware trials as co-variable.

Sources

Group blind source separation (gBSS) offers a straightforward and computationally tractable solution to the problem of multi-subjects analysis by creating aggregate data containing observations from all subjects. By providing a single estimation of the mixing and the demixing matrices for the whole group, this strategy allows direct estimation of the components that are consistently expressed in the population. We employed UW-SOBI, a Second Order Statics (SOS) based BSS algorithm based on the approximate joint diagonalization of lagged-covariance matrices. This method is robust with respect to anatomo-functional inter-subjects variability and can separate group specific sources with non-proportional power-spectra without deleterious prior dimension reduction. The other potential benefits of SOBI methods are first, their ability to separate correlated sources (**??**) and second, a better sensitivity for the detection of critical sources that are often occulted by the most energetic phenomena (**?**). One thousand one lagged-covariance matrices with time delays from 0/1000s to 200/1000s were calculated on each epoch. Then, lagged-covariance matrices were averaged across the dataset epochs first and then across subjects, resulting in 1001 averaged lagged covariance matrices. Finally, adults and children lagged covariance's matrices were averaged and approximately joint-diagonalized with the UWEDGE algorithm (**?**) leading to the identification of 128 independent components (ICs). Adults and children were considered together in the group analysis, in this way signals coming from the same cortical sources became comparable.

ICs Localization

The sLORETA software (?) was used to estimate the intracerebral electrical sources separated by the BSS algorithm. The sLoreta solution of the inverse problem was computed using an amount of Tikhonov regularization optimized for an estimated Signal/Noise Ratio of 100.

Sources Selection

Among the first 20 independent components (ICs) obtained from the gBSS, only ICs showing an evoked activity time locked with the onset of the deviation were further considered (one-sample Student t-test, Bonferroni corrected for the number of time points).

Encoding and Decoding Models

Cortical sources showing significant evoked activity during the sensorimotor conflict task were selected for further analysis. Two linear regression models were used, one to test the encoding of visuomotor features in the cortical sources, and the other one to decode subjective awareness reports from neural signals. Our goal was to assess weather cortical sources activity was modulated by visuomotor features and then to isolate cortical activity related specifically to subjective awareness. In the encoding model, the sources activity was modelled using the proprioceptive and visual errors as explanatory variables.

$$EvokedActivity = \beta_1 PE + \beta_2 VE \tag{3.1}$$

Where the *Evoked Activity* is the activity reconstructed for every task-reactive source, β_1 and β_2 are the regression coefficients and PE and VE the proprioceptive and visual errors, respectively. In this case, the encoding model worked in a *causal* direction for VE (encoding in a stimulus-based setting), and in an *anti-causal* direction for PE (encoding in a response-based setting) (?). Since PE was computed at the end of the trial it can be considered as reflecting the motor correction. In this way, only results coming from the causal encoding support causal interpretation. In the decoding model, the subjective awareness was modelled using cortical sources activities as explanatory variables. The decoding model worked in a causal direction; it models the effect (subjective awareness) of a cause (brain activity) (?). The decoding model allowed us to test whether task-related sources activities potentially caused (among other missed causes) the observed response. Only cortical sources previously selected (see Sources Selection) were tested in the decoding model. Task-related cortical sources were different for adults and children. Moreover, the decoding model gave us the opportunity to characterize precisely on time when the different sources contributed to the emergence of consciousness.

$$MotorAwareness = \beta_1 S_1 + \beta_2 S_2 + \beta_3 S_3 ... \beta_N S_N \tag{3.2}$$

Where *Motor Awareness* refers to the subjective responses, β are the regression coefficients and S1,S2,S3,. . . ,SN the sources activity.

SAM Filter

gBSS generate spatial filters optimized for group level analysis. Mainly for representational purpose, we adapted the gBSS spatial filters to be optimal for single subject. To switch from one to the other it is sufficient to consider the same location found with gBSS while filtering not for the group interfering source but for the interfering sources estimated at the trial level. To estimate the interfering sources at the trial level, spatial information are used in combination with beamformers techniques, such as Linearly Constrained Minimum Variance (LCMV) (?) and Synthetic Aperture Magnetometry (SAM) (?). The spatial filter used for individual analysis at each trial j:

$$Wi(j) = Crj^{-1} Ai [Ai^T Crj^{-1} Ai]^{-1} \tag{3.3}$$

Where Crj is the regularized data covariance matrix, computed for the trial j:

$$Crj = Cj + \mu.diag(Cj) \tag{3.4}$$

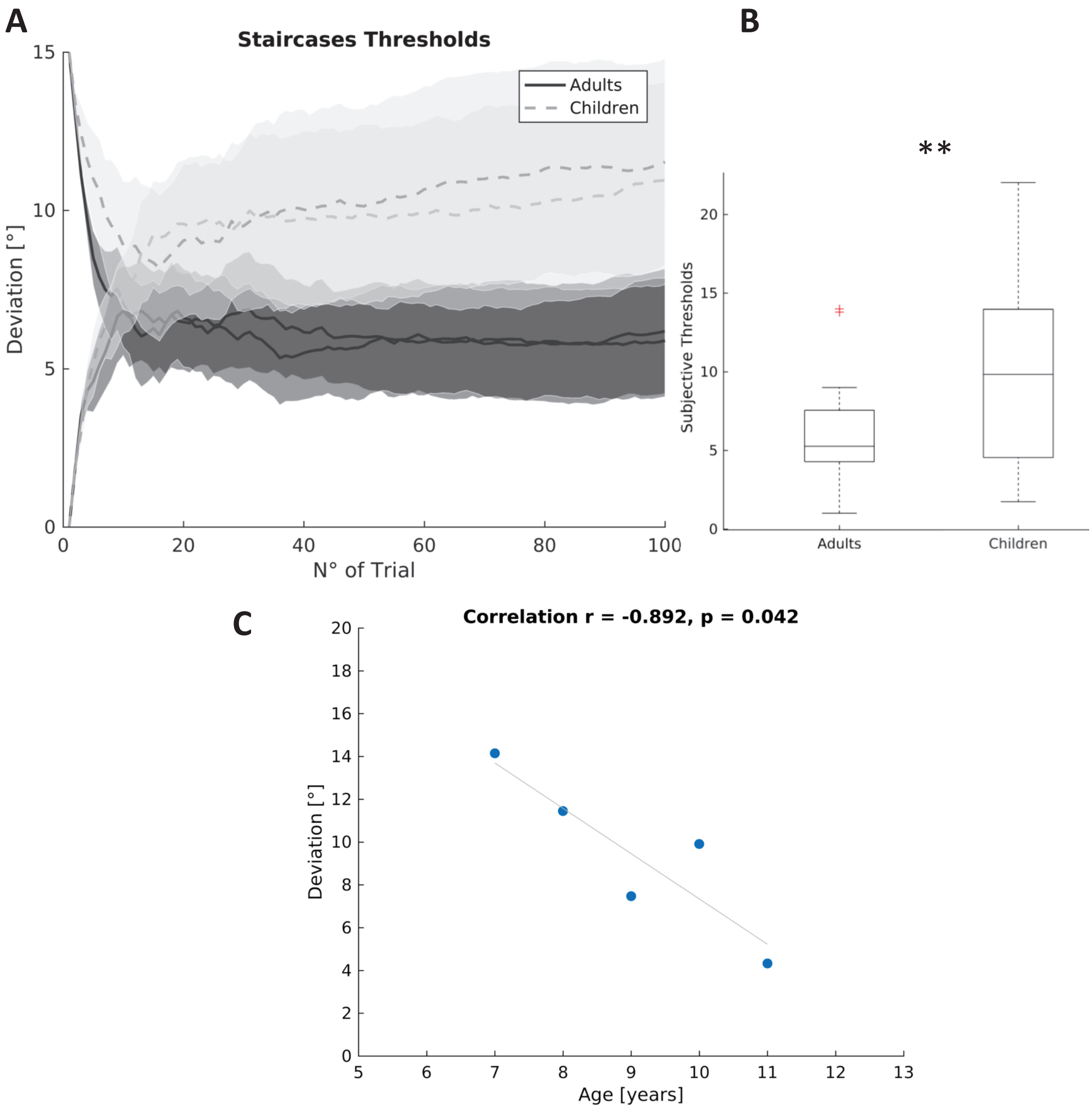

Figure 3.2: **Behavioural results.** (A) Staircases are averaged across children (light gray) and adults (dark gray).(B) Boxplots of perception thresholds in adults and children.(C) Correlation between subjective thresholds and age in children.

Where *Cj* is the data covariance matrix computed for the trial j, μ the Backus-Gilbert regularization parameter (=10) and *diag(Cj)* the matrix of the diagonal elements of *Cj* (the diagonal matrix of sensor noise).

3.2.3 Results

Behavioural

*Subjective Awareness.*The forced-choice adaptive staircase algorithm, which provided for every subject stimuli distributed around its perception threshold, converged to a stable asymptote after about 10 trials in adults and 15 trials in children (see Figure3.2A). As expected, an analysis of variance (ANOVA) on detection thresholds, with factor of group (adults and children), revealed a significant difference between children and adults ($F(1,38) = 7.043$, $p=0.011$ or $p<0.05$, $\eta_p^2 = 0.156$), showing that children had higher detection thresholds (10.04 ± 5.79 degrees) compared to adults (6.05 ± 3.34 degrees) (see Figure 3.2A). No significant difference was noticed between deviations applied to the left or to the right in any group ($F(1,38) = 3.042$, $p = 0.089$, $\eta_p^2 = 0.074$). For this reason, further analysis were run without distinction between left and right side. This effect was replicated intra-group: younger children had higher detection thresholds (correlation for age bin 7 8 9 10-11 years old were grouped: $r=-0.892$, $p=0.042$). Stimuli distribution around adaptive thresholds had higher standard deviation in children compared to adults, indicating wider range of stimuli presentation ($F(1,19) = 6.962$, $p = 0.016$, $\eta_p^2=0.2681$).

Amount of deviations over different conditions. To verify that staircases presented similar stimuli in the aware and unaware conditions, a mixed ANOVA was run over the deviations presented with factors of group (two levels, adults and children) and conscious perception (two levels, aware and unaware). Apart from an evident group effect ($F(1,38)=6.000$, $p<0.019$, $\eta_p^2=0{,}136$), explained by the difference in detection thresholds between adults and children, an effect of awareness was also found. Deviations presented in the aware condition were significantly higher (8.9° ± 0.7° degrees for aware and 8.0° ± 0.7° for unaware) ($F(1,38)=55.515$, p<0.0001*, η_p^2=0,593). Considering that stimuli came from a staircase procedure this difference was not surprising and not relevant as well, being really small and probably undetectable. Nevertheless, it can influence further ANOVA analysis and results in motor awareness effect.

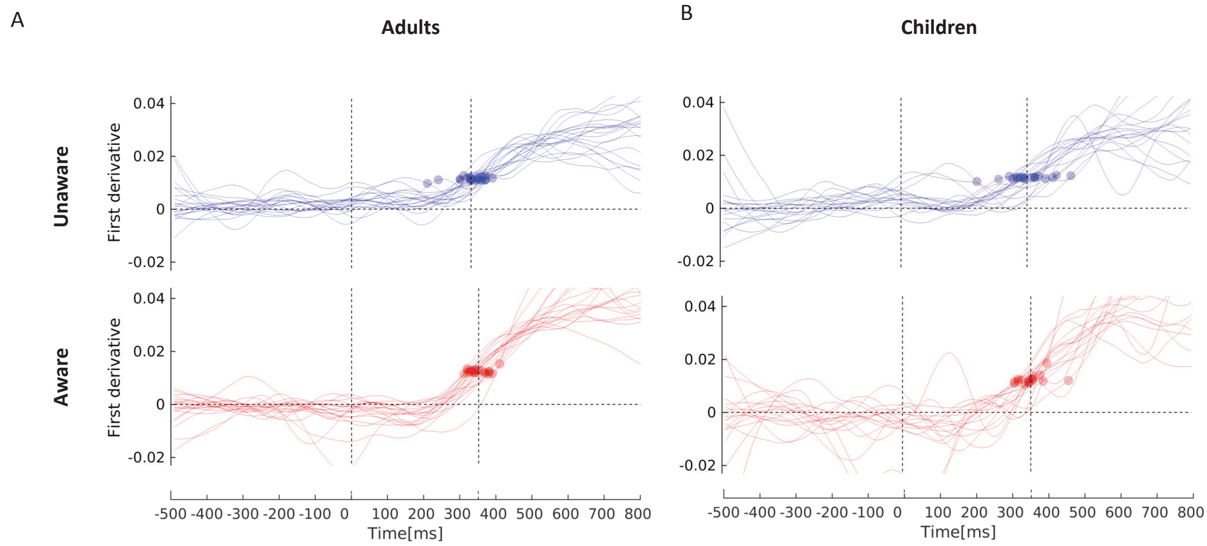

Figure 3.3: **Onset of correction.** The onset of correction (computed over the first derivative over time) are presented in adults (left) and children (right) for unaware (blue) and aware (red) conditions.

Onset of correction. Adults and children started to correct their trajectories initiating at the same time, regardless of the specific condition and group (around 341 ± 48 ms) (see Figure 3.3). In fact, a mixed ANOVA on onsets of correction showed no significant effect of group or consciousness (group: F<1, consciousness: F(1,38)=1.327, p=0.256, η_p^2=0,033).

Velocity. A mixed ANOVA demonstrated that adults and children had the same velocity of execution (F<1), however a small (but significant) effect was noticed for awareness (F(1,38)= 5.095, p=0.029 η_p^2=0,118). Subjects performed movements faster in the unaware trials (217 pixels/s) compared to aware ones (213 pixels/s). Once controlled for deviation differences between aware and unaware conditions, the ANCOVA demonstrated no effect of group and awareness (F<1).

Visual errors. As expected, visual errors increased as a function of the amount of deviation presented. Thus, subjects with higher perception threshold were presented with higher deviations and showed also higher visual errors. An ANCOVA revealed an effect of mean amount of deviations presented on the visual error at 400ms (F(1,36) = 96.852, p<0.001, η_p^2= 0.729) and an effect of difference between deviations presented for aware and unaware trials on the visual error at 400ms (F(1,36) = 15.558, p<0.001, η_p^2= 0.301). Once corrected for the amount of deviation and for the difference of amount of deviation in the two conditions (aware/unaware) awareness and group has no effect on visual error (awareness: F<1, group: F(1,36)=2.588, p = 0.11). An interaction effect was found between awareness and intensity of deviation (F(1,36) = 8.511, p=0.006, η_p^2= 0.191) and difference of amount of perturbation (F(1,36) = 35.675, p<0.001*, η_p^2= 0.498).

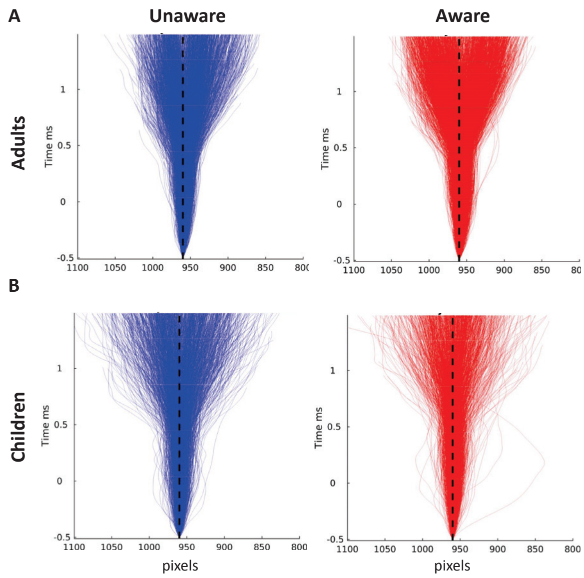

Figure 3.4: **Examples of hand trajectories.** Hand trajectories are shown for adults (top) and children (bottom) for unaware (blue) and aware (red) conditions.

Proprioceptive error. An ANCOVA revealed that the amount of deviation had an effect on the proprioceptive errors (F(1,36) = 760.745, p<0.001*, η_p^2=0.955). No significant group (adults/children) or awareness effect was observed (F<1). Once corrected for the amount of deviation and for the difference of amount of perturbation in the two conditions (aware/unaware) the awareness and group had no effect on proprioceptive error (F<1, F(1,36)=2.108, p = 0.155). An interaction effect was found between awareness and difference of amount of perturbation (group: F(1,36) = 14.246, p<0.001*, η_p^2= 0.284).

Partial correlation results. Before using visual (VE) and proprioceptive (PE) errors to explain EEG activity, we test whether these two variables were independent. At first sight, they were correlated, but both of them increased with higher stimuli. Once corrected for this effect, PE and VE resulted to be still dependent but anti-correlated (adults: partial correlation, r = -0.3275, $p < 10^{-308}$, children: partial correlation, r = -0.3275, $p < 10^{-308}$);

EEG results

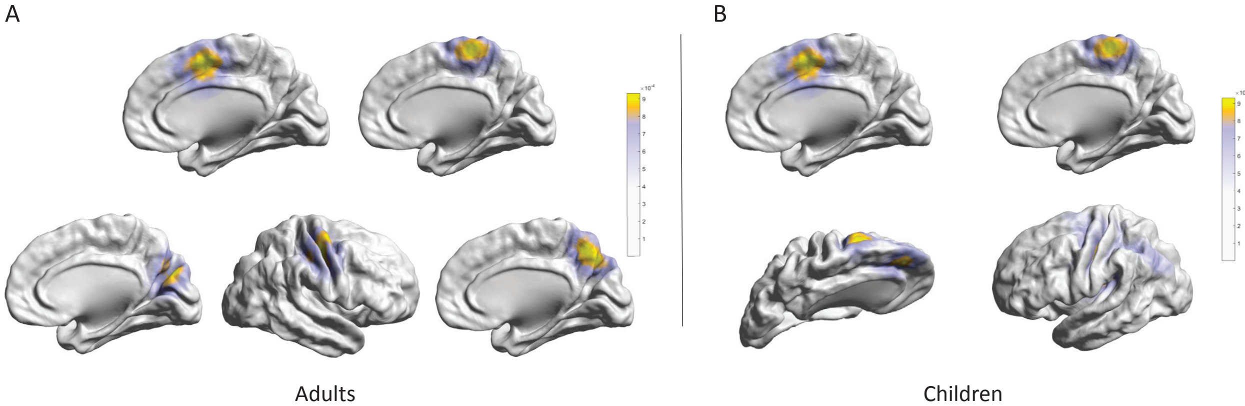

Figure 3.5: **Task-related sources in adults and children.** sLORETA reconstruction of task-related sources eliciting an evoked potential time locked with the onset of the deviation. In adults the sources network included medial fronto-parietal regions, sensorimotor cortex and associative visual cortex. "Task-related sources network" in adults were localized, in order of explained variance, as following: in the supplementary motor area (SMA, BA 6, Medial Frontal Gyrus, MNI coords X= 0 , Y= -10 , Z= 55), in the paracentral lobule (BA 4 MNI coords X= 0 , Y= -40 , Z= 65), one over the precuneus (BA 7, MNI coords X= 5 , Y= -70 , Z= 50), in the right pre-central gyrus (BA 6, MNI coords X= 35 , Y= -15 , Z= 65) and over the cuneus (BA 19, MNI coords X= 5 , Y= -85 , Z= 35). In children, two sources were in common with adults' network: the supplementary motor area (SMA) and the paracentral lobule. The other two sources were a frontal source (BA 6, MNI cords X= -20 , Y= 0 , Z= 60) and a source in the left inferior parietal lobule (BA 40, MNI cords X= -35 , Y= -50 , Z= 40).

We first identified at the cortical level all neural sources reacting to the task and we tried to identify, among them, the neural components specifically related to consciousness. In adults, we found cortical activations over five main regions, including medial fronto-parietal regions, sensorimotor cortex and associative visual cortex (see Figure 3.5A).

We next sought to define the neural mechanisms underlying the behavioural dissociation between conscious and unconscious motor actions. Therefore, task-reactive sources activities were used in a decoding model to explain the subjective awareness in adults. All task-related sources were found to partially explain the motor awareness (multiple linear regression, Bonferroni corrected) however with different timing. In particular, between 300ms and 400ms, the precuneus was the first source to significantly explain motor awareness in adults, simultaneously with another source, localized in the visual cortex (V3A), which showed a smaller window of significance (multiple linear regression, Bonferroni corrected). Then, between 400ms and 500ms after deviation onset, the supplementary motor area (SMA) participated in the decoding of motor awareness, followed by the paracentral lobule and in the sensorimotor cortex, which was active around 500ms.

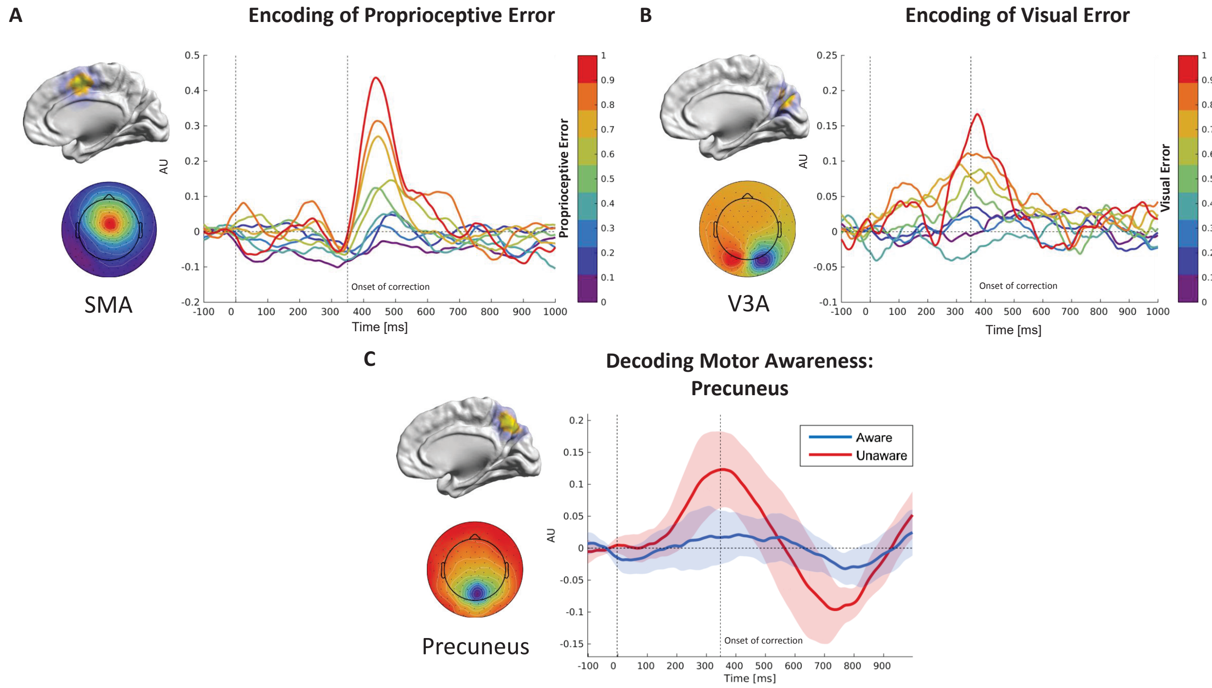

Figure 3.6: **Neural correlates of motor awareness and visuomotor variables in adults.** (A) Topography and evoked cortical activity of supplementary motor area (SMA) source. Different ERP amplitudes, peaking at 450ms, correspond to different amount of proprioceptive errors (PE) across trials. PEs were sorted in increasing order and grouped into 10 classes considering 10 homogeneous subdivisions of the PEs spectrum of values, obtaining 10 sources ERPs, from violet (small PE) to red (big PE). High PEs values (either due to big deviations or bad adaptation) corresponded to high ERPs amplitude. (C) Topography and evoked cortical activity of area V3a during the sensorimotor conflict. This area maximal activity was reached at 400ms. The peak intensity was modulated by the intensity of visual error.(B) Topography and evoked cortical activity of the medial parietal source (precuneus), decoding motor awareness. Evoked activity (extracted by SAM filter) was reconstructed, for illustrative purpose, as a mean across single subject activities at the precuneus level using a SAM filter (see Materials and Method). An evoked activity around 350-400ms was observed only when subjects were aware of the conflict.

We next asked whether this emerging neural activity was specifically related to motor awareness rather than to visuomotor features, linked to the sensorimotor conflict. Thus we wanted to determine neural sources specifically related to consciousness, without considering potential confounding factors. Therefore, the encoding of visuomotor variables was tested for each task-related source. Within this network of cortical activity sustaining consciousness, two sources were found to react to visual or proprioceptive errors, related to external visuomotor features. In particular, the SMA activity increased coherently as the proprioceptive error increased (see Figure 3.6A). Since the proprioceptive error was estimated at the end of each trial, the supplementary motor area (SMA) contribution was potentially linked (in an anti-causal direction, see Methods) to the subsequent motor adaptation, that the subject had to execute in order to reach the final target. The encoding of PE in the SMA was significant from 400 to 500ms (multiple linear regression, Bonferroni corrected) after stimulus onset. Further, the associative visual areas (area V3A) in the occipital cortex encoded the visual error from 300 to 450ms (see Figure 3.6B). All the sources located in the parietal cortex, or in its proximity, such as the paracentral lobule, did not show any encoding of visuomotor features, remaining specific in their role of decoding motor awareness (see Figure 3.6C).

Since subjects were presented with stimuli around their personal subjective threshold, they worked constantly in a "transition phase" characterizing the shift between unconscious to conscious perception, which was reached independently from stimuli intensity. Single subject activity over the precuneus is shown in Figure 3.7 for three different subjects experiencing respectively big, average and small deviations (4°, 6° and 8° degrees), according to their own perception threshold. Activity in the precuneus clearly distinguished between aware and unaware conditions also at the single subject level (Student t-test, p cluser).

Comparing the network of task-related sources in adults and children, we found that in children the sources implicated in the sensorimotor conflict resolution were different (see Figure 3.5B). In fact, adults and children had only two common sources, notably the SMA and the paracentral lobule sources. Moreover, children showed an additional activation of the inferior parietal regions, contralateral to the hand movement (on the left hemisphere) and a frontal source.

In children, subjective awareness was related only to a single source, localized in the supplementary motor area (SMA) (multiple linear regression, Bonferroni corrected over time

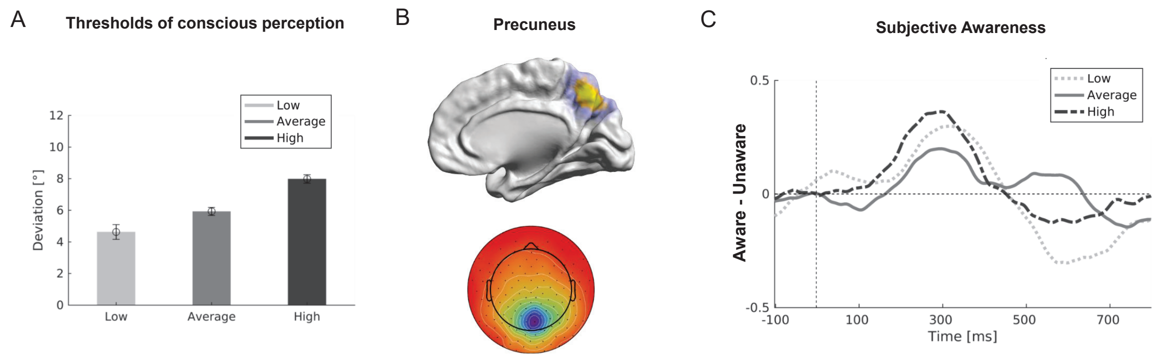

Figure 3.7: **Examples of precuneus activity in aware vs unaware conditions, in three sample subjects with low, medium and high perception thresholds.** (A) Histograms of angular deviations for three subjects, having respectively low, median and high detection thresholds. (B) Topography and sLoreta localization of the medial parietal source. (c) A contrast between the aware and unaware conditions is provided for the three subjects with different line styles.

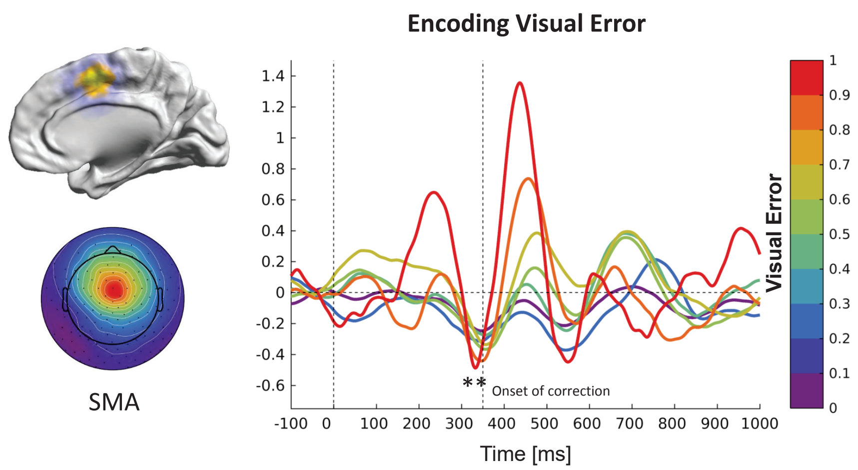

Figure 3.8: **Decoding motor awareness in children.** The figure represents the topography, the sLORETA localization of supplementary motor area sources and encoding of visual error in the SMA activity at 350ms.

points and sources tested), which was found to decode the subjective awareness at 350ms, earlier compared to adults. As previously observed, also in children the SMA activity significantly encoded a sensorimotor variable at 350ms. However, the SMA activation was no longer related to proprioceptive information but rather with the visual error. Importantly, the proprioceptive error have never been found to be encoded in the children pre-selected sources involved in the sensorimotor conflict (see Figure 3.8). In fact, the inferior parietal lobule, the frontal cortex and the paracentral gyrus, all showed causal encoding of visual error around 350ms, 400ms and 1s, respectively.

3.2.4 Discussion

To solve a sensorimotor conflict task requires the recruitment of several cognitive and action monitoring processes to integrate cues coming from different modalities (vision and proprioception) in order to produce an appropriate motor response. The task correct execution is a puzzle of good estimations of sensory mismatches and re-adaptation to them. Here, we wanted to investigate the neural correlates of emergence of motor awareness once controlled for neural activities related with objective visuomotor features.

First, we identify the spatial localization and the temporal occurrence of cortical neural activity recruited to overcome the mismatch. A widespread and different networks emerges for adults and children. Our results show that the parietal cortex sources were critical for bringing the sensorimotor task into awareness, and highlight in particular the role of the precuneus. In fact, the precuneus activity around 350ms was specifically related to motor awareness reports. In fact, this region did not encode objective visuomotor features, coming from individual modalities, but was specifically activated by subjective responses and therefore it can be considered a marker of motor awareness. This activity is likely to signal the discrepancy between intentional and actual feedback, eliciting the emergence of a mismatch at a conscious level. However, since subjects were working around their perception thresholds the awareness of the conflict did not influence their effective motor performances. However, one could expects that in everyday life situations the raise of awareness to the sensory-motor conflict allows to access different levels of motor control.

The fact that the parietal cortex and the precuneus sources reacted mainly to subjective awareness was not surprising since this region is an associative cortex, highly interconnected with structures mostly elaborating integrated information at both cortical and subcortical level (?). Noticeably, the precuneus is well known to be involved in execution of spatially guided behaviours (?) and in the elaboration and the storage of information about targets and body schema (?). The precuneus was also implicated in studies investigating the re-configuration of stimulus-response mapping, however likely reflecting visual attention to increased task demands (?). Arrighi et al. showed that theta oscillations over the precuneus in high-error level condition during prism adaptation compared to low-error condition significantly increased (?). Finally, the precuneus activation was found in specialized neural network of error-processing, notably the error positivity a neural signal specifically associated with error that were consciously perceived (??).

The other cortical sources participating in the decoding of motor awareness encoded visuomotor features as well, suggesting to reflect neural mechanisms underlying action monitoring and motor adaptation. The associative visual cortex (V3A) activity reacted to the amount of visual error, at the same time as the precuneus activity. Our findings are in line with previous studies showing that tracking error were efficiently classified using visual cortex as a region of interest (?). On the other hand, the supplementary motor area (SMA) reacted around 450ms encoding the proprioceptive error and therefore the amount of motor correction. The SMA is well known to be involved in action selection, performance monitoring (?) and in the emergence of motor intention (?). In this task, the SMA activity seemed to play a predictive role, since its activity temporally preceded the end of the movement. (However, at the timing in which SMA activity appeared 450ms, subjects probably already started their automatic correction). Therefore, this area seems to code the change of the motor plan, encoding the amount of motor correction needed to achieve the target. Activity in this area was already related to switching of intended reaching path in monkeys (?).

Further, we tested the same protocol in children, hypothesising that in absence of a reliable forward model sensory inputs would play a predominant role in the access to consciousness. We found that thresholds of motor awareness were shifted in children of about 4° degrees of deviation. Thus, children needed much more sensory cues to become aware about the visuomotor mismatch. However, once controlled for the different intensity of the stimuli proposed in children and adults, children were as fast as adults to trace lines and they started to automatically compensate at the same timing.

In parallel with increased thresholds of motor awareness compared to adults, children showed a different network of cortical activations. In fact, the emergence of motor awareness does not elicit any activity in the precuneus, but it was instead decoded by the supplementary motor area. However, the activity of the SMA was not specific for motor awareness since it was shown to encode the visual error. This result confirmed that children do not use the same cortical regions, probably involved with forward model computation, to solve the sensorimotor mismatch. Thus to build their motor awareness they may rely more on sensory signals, and in particular to vision encoded in the SMA.

The timing of SMA decoding in children preceded the adult of about 100ms, suggesting that the SMA activity was elicited in advance, maybe lacking the integration of proprioceptive signals observed in the adults. Further, we found that children cortical activity related to the task recruited different cortical sources, including the paracentral lobule, the inferior parietal regions, contralateral to the hand movement (on the left hemisphere) and a frontal source. However, this different activation network was mainly driven by visual stimuli input, being therefore unspecific to motor awareness. This confirm previous study of sensorimotor integration in children, claiming that balance between vision and proprioception is unstable at this age and highly influenced by vision (**?**).

3.3 Behavioural study on motor awareness

3.3.1 Introduction

We wanted to test adults and children using the same set of stimuli for both groups, in order to asses their detection performances using a classification function.

3.3.2 Methods

Subjects

Twenty young right-handed healthy adults (27.45 $\pm$ 5.62, 10 females and 10 males) and 21 children (8.52 $\pm$ 1.12 years old, 14 females and 7 male) participated in the study. Subjects had normal or corrected-to-normal vision. Each experiment lasted for approximately 1 hour. Subjects were unaware about the purpose of the experiment. All subjects gave written informed consent to participate in the study, and the whole paradigm was approved by the local Ethic Committee.

Paradigm

Subjects were presented with the Nielsen task, using the same apparatus described in the method. However, stimuli were the same for all subjects, and angular bias were presented randomly. Subjects had to perform a total 220 trials: 180 were deviated trails, ranging from $-20°$ to $20°$ degrees, 40 were neutral trial (with $0°$ degrees of deviation). In deviated trials angular bias were presented as follow: between $1°$ to $5°$ and $15°$ to $20°$ with a gap of $1°$, between $5°$ and $15°$ with a gap of $0.5°$, in this way each deviation was presented three times. Half trials were deviated to the right and half trials were deviated to left and they were mixed randomly with neutral trials. Six sessions were performed, with alternated period of pause.

ROC Analysis

The Receiver operator characteristic was used to represents the performances of a binary classifier (the subject's capability to distinguish different type of trials) when its discrimination threshold is varied. Sensitivity (true positive/positive responses) and 1-Specificity (1- (true negative/negative responses) considered, representing the relationship between true positive (a deviated trail detected by the participant) and false alarm (a neutral trail detected as deviated). In the end, it represents the detection as a function of fall-out. The probability distribution was fitted for the two groups starting from the area under the curve (AUC). The AUC was used to perform a comparison across groups using a parametric Student test (t-test), after normality check.

3.3.3 Preliminary results

Adults and children differed in their detection performances. In fact, adults could better discriminate deviated trials than children, keeping their false alarm rate low (Student T-test, $p = 0.0038$). The subjective threshold to perceive a deviation was higher in children compare to adults using the same stimulation in both groups. In other words, adults had a subjective awareness shifted toward lower deviations, allowing them to consciously perceive smaller deviations than children.

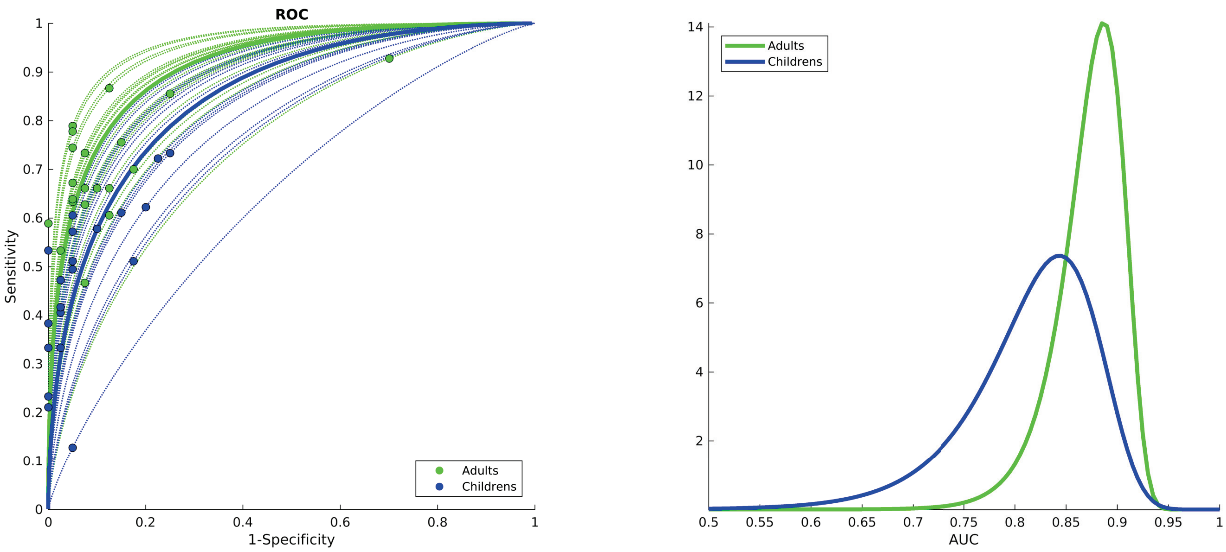

Figure 3.9: **Receiver operator analysis and probability distribution over adults and children.** (A) Receiver operator analysis (ROC) curves fitted over two groups: adults (green) and children (blue). The two axis represent the sensitivity and 1-specificity. The area under the curve (AUC) represents the probability to discriminate between deviated and non-deviated trials. Probability distribution for adults (green) and children (blue).

Part II

Vagus Nerve Stimulation

Chapter 4

Disorders of consciousness and vagus nerve stimulation

A fascinating way to study the neural substrate of consciousness is to consider lesional cases in which lost or reduced consciousness is a prominent clinical sign (?). The vegetative state, a condition in which arousal appears without awareness, gives us an insight on disrupted mechanisms causing disorder of consciousness (DOC) and sustaining awareness. Further, focusing on clinical research allows us to try to find new methods and solutions to modulate, and possibly restore, conscious state. In this background part, I will recall some basic definitions about disorders of consciousness, briefly describing existing theories and methods to study and treat this pathological condition.

4.1 An overview on disorders of consciousness and traumatic brain injuries

Patients who survive brain damages and awake from coma but remain without behavioural sign of awareness are left with a chronic disturbance of consciousness. Disorders of consciousness can arise after both focal or global brain injuries. They can result from direct perturbation of specific neural system regulating arousal or awareness, but also indirectly if the lesion causes a disruption of the connections between these systems (?). Vegetative state can both arise gradually, as a consequence of neurodegenerative disorder, or suddenly when it is due to non-traumatic (e.g. hypoxia or anoxia, infection or haemorrhage) or traumatic brain injury. Here, I will focus on literature regarding disorders of consciousness related to traumatic brain injury.

Traumatic brain injury (TBI) is a major public health and socioeconomic problem throughout the world, not only causing severe disability but also leading to high cost for the public health system (?). In the United States (US), the Centers for Disease Control and Preventions estimated that 1.7 million people sustain a TBI, of them 1.365 million are treated in emergency departments, 282 000 are hospitalized and 52 000 die (?). In Europe (population 508.5 million) TBI related deaths in the year 2012 were estimated at 56 946 and hospital discharges at 1 445 526 (?). Every year in France, there are about 155 000 hospitalizations for TBI. As a results of these injuries, 8 500 people experience the onset of long-term disability (?). Road accidents are the leading cause of head traumas (60% of TBI hospitalizations, 70% cause of TBI deaths) with a major prevalence between 15 and 30 years old. TBI causes the 30% and 37% of all injuries related deaths in US and Europe, respectively (?). Older adolescent, older adults, and male across all groups reflect the main victims.

The incidence of TBI in the US has risen from 2001 to 2010, arriving in 2010 at 824 per 100 000 (Centers for Disease Control and Prevention [CDC], Congress on Traumatic Brain Injury Epidemiology and Rehabilitation, 2014) (?). While emergency department visits rose of about 70%, the hospitalization only increased of about 11%, and deaths decreased of 7% (?). These results have been achieved by increased automobile safety, the use of seat belt, and improvements in treatment of severe TBI, all this contribute to a reduction of mortality. None-the-less, according to the World Health Organization, TBI will surpass many diseases as a major cause of death and disability by the year 2020 (?).

TBI consequences happen at two different temporal stages: a first stage involving the cellular destruction due to the initial trauma (usually a bump, blow or jolt) producing an unavoidable damage. A second stage corresponds to the post-trauma and involves cellular excitotoxicity, inflammation, release of oxygen free radicals and brain oedema, leading to an increase of intracranial pressure (?). While some patients recover quickly, going through different altered states of consciousness, others take longer or never recover. In the US alone, between 3.2 and 5.3 million persons (1.1% and 1.7% of the population) live with long term disability resulting from a TBI (?). To understand the mechanisms and pathophysiology that underlie alterations of consciousness is therefore a major issue in current clinical research and it is fundamental to develop appropriate diagnosis and treatments.

Different behavioural features and clinical symptoms are used to classify patients into corresponding states of consciousness, and thus formulate a diagnosis and prognosis. However, since behavioural features are often fluctuating across diagnostic boarders, behavioural signs are not always sufficient to evaluate conscious state and consequently the classification of this patients is not always precise. In the next section, I propose some basic definitions of DOC, briefly describing the main traits for each category.

4.1.1 Definitions

Coma

Coma, from Greek "deep sleep or trance" is a state of unresponsiveness, clinically characterized by a complete loss of spontaneous or stimulus-induced arousal. Patients in coma have completely lost their sleep-wake cycle, their eyes remain continuously closed and they show no evidence of motor activity or speech. Although, patients may respond to painful stimuli with a grimace or stereotyped withdrawal reflex. Coma itself is considered as an acute altered state of consciousness and typically does not last for more than 2 weeks usually evolving into either vegetative state (VS) or minimally conscious state (MCS) (?).

Vegetative state/unresponsive wakefulness syndrome

The vegetative state (VS) or unresponsive wakefulness syndrome (UWS) as proposed recently by Laureys et al. (?), is a subacute altered state in which arousal is preserved but there is no evidence of purposeful behavioral response to stimuli. It is a peculiar disorder in which patients seems to be *awake* but *unaware* about their surroundings. A spontaneous eye-opening re-emerged, signalling the recovery of the reticular activation system and determining a condition of wakeful unconsciousness. However, the presence of alternating cycles of eyes-open/eyes-closed periods do not imply the preservation of a physiological sleep architecture (?) . Other specific classifications are added following the temporal outcome. If the patient stays in this condition for at least 1 month, the vegetative state is referred as *persistent* (PVS). Further, PVS is defined as *permanent* 3 months following non-traumatic brain injury and 12 months following traumatic brain injury (?).

Despite the heterogeneity of lesions causing disorders of consciousness (DOC), the neuropathology of the vegetative state (VS) due to traumatic brain injury has some classical common features. In fact, it is characterized by diffuse cortical or axonal injury, implying a deep damage to the subcortical white matter coupled with damage to the major nuclei of the thalamus, rather than brain stem lesions (?). On the other hand, VS caused by non-traumatic lesions is associated with extended necrosis in the cerebral cortex, always accompanied by thalamic damage (?). These lesions are likely to cause a disconnection between cortical areas and thalamic nuclei, which can clearly influence the functioning of the remained intact cortex (?). For this reason, VS/UWS is also referred as a "disconnection syndrome", modifying the architecture of brain connectivity over long-range cortico-cortical (between latero-frontal and middle-posterior regions) and cortico-thalamo-cortical connections (between non-specific thalamic nuclei and lateral and medial frontal cortices) (?).

Minimally conscious state

The minimally conscious state (MCS) condition is characterized by inconsistent (fluctuating) but discernible behavioural evidence of awareness, signalling the presence of self and environmental awareness (?). These evidences must be reproducible, and should include one or more of the following behaviours:

- Following simple commands
- Gestural or verbal yes/no responses
- Intelligible verbalization
- Purposeful behaviour
  - appropriate smiling or cry
  - appropriate vocalization
  - reaching for objects
  - appropriate objects manipulation
  - pursuit eye movement or sustained fixation

(?)

All these signs are usually inconsistent, making necessary multiple examination to fully capture behaviours that might occur infrequently and might be ambiguous or masked. MCS patients pathophysiology consists with grade 2 and 3 diffuse axonal injury (DAI), sometimes coupled with thalamic involvement, which are less prevalent than in VS (?).

A further categorization exists inside this class, MCS- (i.e. patients showing reactions to contingent stimulus, such as visual pursuit and localization of noxious stimulations) and MCS+ (i.e. patients showing command following). This distinction is based on presence or absence of language comprehension. Using glucose metabolism, Bruno et al. try to define the neuroanatomy underlying these two different behaviours. MCS- patients presented more disconnections between Broca's region and the language network, mesiofrontal and cerebellar areas. On the other hand, MCS+ patients showed higher metabolism in left-side areas, comprising higher activations over language network, sensory cortices and premotor and supplementary motor areas (**?**).

Akinetic mutism

Akinetic mutism manifests as state of avolition involving a lack of the desire to think, to speak or to move. This syndrome is characterized by a reduction of all motor gestures including facial expressions and verbal flow. These patients presents a severely reduced drive rather than arousal, being explicitly observable in the failure to follow commands or engage in goal directed behaviours. Like in MCS patients, these behaviours can still be triggered by intense sensory or personally salient stimuli (**?**). This phenomenon is known as the ”telephone effect”, implying that such patients, in principle mute or unresponsive, are able to initiate a likely ”normal” conversation when presented with a phone (**?**). Akinetic mutism can follow stroke involving critical areas such as the frontal (cingulate gyrus, supplementary motor area and dorso-lateral border zone), basal ganglia (caudate, putmen), the mesencephalon and thalamus.

4.1.2 Pathophysiological mechanisms of DOC

As already mentioned, disorders of consciousness are a neurological model to study impaired consciousness and its underlying mechanisms over at cortical and subcortical level. After a traumatic brain injury (TBI), a common pathophysiological consequence is a widespread "deafferentation" of cortical areas involved in the lesion, and therefore a reduction of inputs to neurons across the corticothalamic system (**?**). In the most severe cases, if all inputs to the cerebral cortex are removed, the "deafferentation" provokes the establishment of a diffuse low-frequency electrical activity. Most of the time lesions provoke only partial disconnection of the corticothalamic system, however this background alteration can have important consequences in the global firing pattern and excitability of the cortex.

Conscious awareness seems to require a sufficient number of connected neurons, necessary for local processing and synchronization between distant regions. In this frame, to explain the continuum of the DOC spectrum, a common mechanism of down-regulation has been proposed called the *meso-circuit hypothesis*

Several studies on coma patients allowed us to identify different structure which can participate to the maintenance of a conscious state. The etherogenity of lesions at the base of DOC, suggest that no specific regions are unequivocally related to consciousness. Observation done during epileptic seizure show how transient unconscious state is linked with brain function rataher that macroscpoic structures (**?**). Recent studies have shown how a common characteristic of loss of consciousness is a multifaceted dysfunctional connectivity pattern which seems to be reduced in a widespread frontoparietal network and thalamic connectivity **?**.

Usually, fronto-parietal midline structures (or default mode network (DMN)) are more often associated with *internal*, stimulus independent, "self" consciousness, whereas lateral fronto-parietal cortices are associated with *external* or sensory awareness (**?**). Both these networks can be impaired in disorders of consciousness. In particular, changes in the effective connectivity have been described in the midline part (including anterior cingulate/mesiofrontal and posterior cingulate/precuneus) and lateral structures (prefrontal and posterior parietal regions)(**??**). Because the midline part is associated with internally oriented cognitive content, the DMN has been investigated during DOC, showing reduced connectivity mostly over the precuneus, a critical hub with high interconnectivity (**?**). Further, disconnection between primary sensory areas and higher-order associative cortices in VS/UWS can also be considered as traits in connectivity patterns in VS/UWS patients, which cannot perceive, or are impaired in perceiving, auditory or noxious stimulation (**??**).

Evidences from recovery of consciousness

To support the importance of the frontoparietal network and long-range connectivity in sustaining consciousness a study by Laureys et al, of a spontaneous recovery from in a patient after carbon monoxide poisoning showed the restoration of frontoparietal network, (?). Another study on a vegetative state patient recovering from VS, showed augmented functional connectivity between intralaminar thalamic regions and right prefrontal cortex (?). This observation reinforced the idea that thalamic projections are of fundamental importance in supporting cortico-cortical connections (?). Further, this result highlights a second fundamental structure, candidate for sustaining consciousness: the central thalamus.

Another remarkable result was reported by Schiff et al., who attempted (and succeeded) to restore lost connectivity in a MCS patient, acting on preserved large-scale cerebral networks which were silent but still functioning. Following the hypothesis that residual spared functions can be reactivated using therapeutic intervention, they electrically stimulated the central thalamus using bilateral deep brain stimulation (DBS) following a 6-month double blind alternating crossover paradigm. After being in MCS for 6 years, the patient improved in eye-opening and command following functions, significantly increasing his CRS-R scores in arousal, oral feeding and limb control over the ON compared to OFF stimulation periods. Importantly, supplemental results shows that deep brain stimulation (DBS) activated the midline posterior central cortical regions, consistent with the anatomy of the projection from thalamus to medial parietal cortical regions (?). The mesocircuit model fits with this last observation, predicting that thalamic stimulation would bypass the thalamic inhibition operated by the globus pallidus (?).

4.1.3 Detection of awareness in DOC

Functional neuroimaging techniques represent an important mean to investigate the neural correlates of consciousness by giving the possibility to to observe directly impaired brain structures and mechanisms. They represent an objective method to quantify residual cognition in the human brain and therefore an important tool for helping correct diagnosis. In fact, in severely brain damaged patients, the bedside examination of consciousness is frequently challenged by the delicate state in which these kind of patients lie. Small movements, inconsistent and rapidly exhausted behaviours and responses, maybe due to concomitant deficits both in the verbal and non-verbal communication, potentially lead to misdiagnosis. In VS/UWS the rate of misdiagnosis is around 40% by using clinical consensus methods and this is due to insufficient sensitive standardized scale for behavioural assessment (**?**).

Detection of residual consciousness using fMRI: mental imagery

Neuroimaging techniques are particularly useful not only to confirm behavioural examinations but also to improve detection of residual awareness in patients that cannot communicate. An important advance is the development of paradigms investigating volition and awareness without ambiguity. Notably, Owen et al. first assessed residual brain functions in a vegetative state patient with peripheral motor system impairment using fMRI. They demonstrated that their patients could follow some mental imagery commands, as healthy subject can do. For example, when asked to "imagine playing tennis", a correspondent activation of the supplementary motor area was observed, and when asked to "imagine moving around her house", there were a correspondent parahippocampal activation (**?**).

Following this example, the mental imagery paradigm was extended to other techniques, such as EEG event related potentials and electromyography (EMG), and it was also re-adapted, by Monti and colleagues (**?**) to use different mental images as basic communication language. Unfortunately, mental imagery-based communication paradigms were proved not to always be reliable and consistent (**?**). The paradigm was nonetheless extended to activate different cortical networks, for example asking participants to name an object presented on the screen, and triggering a correspondent activation of the language network (**?**).

Detection of residual consciousness using fMRI: resting state

Although establishing communication with non communicative patients is an interesting topic with wide clinical application, doing so using fMRI is considered too expensive and not efficient. The potential of this technique in producing an objective diagnosis on disorders of consciousness was also evaluated by studies on spontaneous brain activity fluctuations, using BOLD signals (fMRI) during ”resting state” protocols. They end up with the identification of multiple functional networks. Among them the default mode network (DMN) has been the most investigated in consciousness literature, and it has been proposed as the locus of conscious awareness (**?**). This network is often associated to *internal awareness*, self-consciousness, mind-wondering and general task-independent thoughts. The default mode network anatomy includes the posterior cingulate cortex/precuneus, which is an highly interconnected area considered a critical hub for his massive degree of connectivity, the anterior cingulate cortex/mesiofrontal cortex and the temporo parietal junction. Several studies demonstrated that DMN disappeared in brain death and decreases in VS (**??**) . Vanhaudenhuyse et al. observed that unresponsive patients showed a decreased pattern of functional connectivity in the posterior cingulate/precuneus area compared to MCS patients. Therefore, they proposed that that "default mode" connectivity seems to reflect the level of consciousness in non-comunicative patient with DOC (**?**).

Detecting consciousness using PET: resting state

Neural activity of brain regions involved in consciousness can also be investigated using 18F-fluorodeoxyglucose PET. 18FDG-PET is a neuroimaging technique which allow to study glucose metabolism and to estimate the rate of cerebral energy turnover. In clinical neuroimaging, PET-scan is an established tool to investigate disorders of consciousness (**??**). It was first using this technique during resting state conditions that a massive decrease in brain metabolism was observed in DOC. In particular, disorders of consciousness causes specific metabolic decreases in the frontoparietal associative areas. Patients in MCS maintain partial metabolism, although severely reduced compared to normal subject while VS/UWS patients show severe bilateral frontoparietal dysfunction (**?**). Experiments on FDG-PET reported that cerebral metabolic rate fall to 50% of normal or less during loss of consciousness, being 40-50% in the acute phase and 30-40% during subacute or chronic phase. It remains unclear if this reduction directly correlates with the level of consciousness or with lesions extension. Shulman et al., based also on previous evidence, argue that metabolic phase transitions are linked with transition between level of consciousness, proposing high level of brain energy as a necessary property to sustain consciousness (**?**).

A recent study by Stender et al. (**?**) aimed at quantifying the metabolic differences between patients in UWS/VS and MCS patients, showed that global cortical cerebral metabolic rate of glucose (CMRglc) averaged 42% of normal in VS/UWS and 55% in MCS compared to healthy subjects, making possible to reliably distinguish between the two groups. In fact, metabolism was lower in patients than in controls but sufficiently higher in MCS patients to distinguish them from VS patients, corroborating the idea that metabolism is a determinant of conscious state. Subsequent regional variations were also reported, supporting theory claiming from specific anatomy of DOC. The first remarkable finding being a 60-70% reduction of metabolism over the the brainstem, which can explain impaired and fluctuating level of arousal. Differences between patients were found in a region including front-, temporo- and occipito-parietal junction, encompassing primary sensorymotor areas, frontoparieetal regions and precuneus. Instead, no significant differences were reported for the thalamus and brainstem. This further supports the role of frontoparietal network in supporting consciousness, where the precuneus and the cingulate cortex may play a key role in promoting frontoparietal awareness network. However, preserved metabolic rate in these regions cannot be regarded as a discriminant feature for awareness since several patient in MCS has low value of metabolism in this area (**?**). These studies had a key role in establishing brain regions strongly related to consciousness including lateral and medial frontoparietal network.

In a more recent article, Stender et al., after having measured the "resting state" brain glucose metabolism in 131 DOC patients, found that the minimal energetic requirement to sustain conscious awareness is 42% of normal cortical activity (**?**).

Other PET studies, using a different radioligand, H215O-PET further suggest that UWS/VS is a global disconnection syndrome, in which a possibly preserved awareness network remains functionally disconnected from primary cortical areas (**?**).

Detecting consciousness using resting state EEG

Although fMRI and PET have been shown to better classify different states of consciousness in respect to EEG, they have complex and difficult access. Therefore a quite extended EEG literature have been developed in the last 5 years, focused on investigating the potential of EEG-based techniques in the evaluation of cognitive function (diagnosis), detection of residual consciousness and prediction of possible outcome (prognosis). This gave rise to a flourish production, including studies validating their methods over a large cohort of patients, and aimed at reviewing current markers of consciousness to establish which of them can better discriminate among different states of consciousness.

Several types of analysis have been used, spanning from time and frequency domain classical indexes, such as relative and absolute power spectrum, to connectivity and graph theory metrics, entropy and bispectral index, which in some cases offers single-patient level solutions. I will briefly review the most recent advances (and in my opinion relevant studies in this field) since I believe that this background constitutes a strong basis for investigating DOC.

Of remark in EEG literature, the work done by King and colleagues (**?**) introduced a functional connectivity measure called weighted symbolic mutual information (wSMI), providing a sensible index of the state of consciousness. This measure estimates the amount of information sharing across EEG electrodes pairs capturing medium and long-distance connectivity over the scalp. They found that wSMI values increase as a function of consciousness and are able to separates VS/UWS patients from MCS and control patients, where the best classification results were obtained considering the activity in the theta band. Moreover, maximum increase of wSMI was observed over centroposterior regions mostly for medium to long connectivity patterns. This result was consistent with the theoretical notion that loss of consciousness is accompanied by a corresponding reduction cortico-cortical connectivity, and states how brain-scale information sharing provides a robust signature of conscious processing. Importantly, although it can appear in contradiction with theory claiming the fundamental role of frontoparietal network compared to a centroposterior regions, frontal areas were less impaired.

Sitt et al. compared the efficiency of different electroencephalography markers of conscious state on a dataset of 113 high-density EEG recordings acquired during a 30 minutes protocol. Main findings included that power spectrum measures are good markers of consciousness, in particular normalized delta (1-4Hz) decreased and normalized theta (4-8 Hz) and alpha (8-13Hz) band power increased from VS to controls. Importantly theta and alpha band power increased over parietal regions. Moreover, using a multivariate classification, they claim that using the entire set of measures, the area under the curve (AUC) was significantly higher then when use the best (single) measure, going from AUC = 71% $\pm$ 4 to 75% $\pm$ 4 (**?**).

At the same time, Chennu et al. (**?**) investigated spectral signature of reorganized brain networks in a cohort of 32 patients suffering from chronic disorders of consciousness. In particular, they focused on graph-theory based measures over different frequency bands. Graph theoretical quantification showed that a typical configuration of alpha network in a healthy brain is a combination of strong long interactions and robust inter connectivity features. The presence of prominent modules of long-range synchrony, linking occipito-parietal and frontal electrodes, seemed to be absent in patients. They described main pathological patterns as reduced local and global efficiency and fewer hubs in the alpha band. Moreover, alpha network efficiency correlates with the degree of awareness, although in this first study they could not observe a significant distinction between VS and MCS patients.

Another interesting finding came from the analysis of modular span, a measure of modularity normalized by topographic information. As expected, in patients alpha network modules lacked long distance interaction structure. In the theta band, they reported an inverse patter, saying that connectivity was higher in patients compared to controls. This effect is probably due to pyramidal neurons in layer V of partially deafferented cortex, which tends to synchronize on these rhythms, however, modular span remains rather low.

In a consequent paper, Chennu et al. (**?**) used recent technologies for elaborating diagnosis and prognosis of unresponsive wakeful syndrome. They analysed data from 104 patients, using classical and connectivity metrics to classify patients into different categories including: UWS, MCS- and MCS+, emergent from MCS, locked-in syndrome and controls. From a diagnostic point of view, connectivity measures outperform alpha power in discriminating awareness. Their results show progressive increase in strength of EEG connectivity on alpha band, computed as debiased weighted Phase Locking Value (dwPLI) during resting state, corresponding to increased level of consciousness. Alpha band connectivity measured with participation coefficient topographies, shows a re-emergence of brain connectivity hubs corresponding to frontal and parietal areas. In particular, alpha participation coefficient discriminates between UWS and MCS- with an accuracy of 79%. MCS+ shows a more prominent frontoparietal network activation, which further increases in patients with higher level of consciousness. These two results together show that main divergences among brain activities belonging to different states of consciousness is the level of connectivity between different hubs, which became stronger with the emergence of consciousness. The delta band power better distinguishes between MCS- and MSC+. Another central issue was to determine whether two intrinsically different neuroimaging techniques, such as EEG and PET could predict each other. They demonstrated that positive metabolism (spared but still pathologically reduced bilateral metabolism in frontal and parietal cortices) correlated with EEG connectivity over frontal, parietal and central hubs. This highly interconnected network requires higher energetic demands. Further, they wanted to assessing the prognostic value of EEG, comparing brain signals of patients with positive and negative outcome prediction. Delta band connectivity was diminished for positive outcome prediction, showing once again as delta reflects a pathological rhythm. Further, patients with positive outcome after traumatic brain injury have higher clustering coefficients in delta networks, suggesting local topological connectivity. Graph theory metrics such as local modularity and clustering coefficients on delta band predicts future outcome. The classification method developed within this article, taking into consideration both connectivity in hub nodes in alpha network and delta band power, was shown to correctly classify patients in MCS+ status, which were previously misdiagnosed.

This confirm EEG is a reliable tool for detecting consciousness at the bedside. Other studies on detection of awareness include evoke potential protocol and the use of TMS to assess how signal spared into the brain. Although this topic is very interesting and also introduced important advances, I would not discuss it further here.

4.1.4 Neuromodulation of the conscious state in DOC

Clinical management has usually two main objectives: to prevent secondary medical complication and to restore cognitive-behavioural functions. Despite the fact that a wide spectrum of treatments are available ranging from behavioural to pharmacological and rehabilitation-oriented interventions, only a few have been demonstrated to effectively enhance functional recovery.

Pharmacological interventions

Randomized clinical trials have proved the effectiveness of two medications in the modulation of key neurotransmitters systems mediating arousal, attention and drive functions: amantadine and zolpidem.

- **Amantadine**: is a drug approved both as antiviral and as antiparkinsonian. It is one of the most commonly prescribed medication in patients with TBI. In a cohort of 184 patients, either in VS or MCS, Giacino and colleagues administrated amantadine or placebo for 4 weeks in patients enrolled early or late post-injury (28-70 days or 71-112 days). They reported that patients treated with amantadine recover significantly faster the ability of following commands consistently, answering to yes-or-no questions accurately, using objects and speaking intelligibly. Although the gain was usually well maintained after the washout period, the rate of recovery was attenuated and at 6 weeks follow-up the Disability Rating Scale (DRS) scores were not different for the amantadine and placebo groups (**?**).
- **Zolpidem**: is an omega-1 specific indirect GABA agonist, used for insomnia, and paradoxically employed for the treatment of TBI. It was previously reported to produce consis-

tent improvements in augmenting the complexity of behavioral responses in some patients suffering from DOC. In a placebo-controlled, double-blind, crossover trials in 15 patients, in VS or MCS for at least 1 month was effective in just one. The patient regained visual pursuit, command -following ability and automatic social gestures (?).

Neurostimulation

Neuromodulation is a therapeutic technique, consisting in carrying energy into the nervous system in order to achieve excitation, inhibition, or otherwise modifying neural activity. Neurostimulation methods have recently been applied in the treatment of functional and motor deficit in TBI animal models, stroke and few TBI patients (?).

tDCS

Short duration transcranial direct current stimulation (tDCS) applied for 20 minutes on dorsolateral prefrontal cortex in DOC patients has been demonstrated to improve CRS-R scores, mainly in MCS patients (?). Following a double-blind-controlled crossover design, 13 MCS and 2 VS patients showed behavioural signs of consciousness after tDCS.

Deep Brain stimulation

Deep brain stimulation is an invasive technique used as a gold standard to treat motor symptoms in Parkinson disease (PD) patients for whom medications effects begin to fade. Currently DBS has been approved by Food and Drug Administration (FDA) to treat essential tremor, dystonia and obsessive compulsive disorders (?). Its mechanism of action is thought to be the disruption of abnormal neural synchrony between affected brain regions. DBS seems to alter the activity patterns and moderate abnormal function related to symptoms. Despite the numerous application of DBS in neurological diseases there are only a few studies reporting the use of DBS in human TBI patients. These studies aim at restoring consciousness by changing the arousal state.

Tsubokawa et al. first reported an increase in arousal after using DBS in TBI patients. They started a clinical trial involving either 8 cases of persistent vegetative state, stimulating the mesencephalic reticular formation or non-specific thalamic nucleus. Patients have been chronically stimulated for 6 months. Four patients recovered verbal communication, a modified spectrogram showing desynchronization of EEG, increased cerebral blood flow in the whole brain tissue and after 3-6 months transmitter substances and their metabolite increased in the cerebrospinal fluid (**?**).

As mentioned in the previous paragraph, in a a more recent study, Schiff et al. (**?**) uses bilateral CT-DBS over central thalamus. The location of the stimulation targeted the anterior intralaminar thalamic nuclei and adjacent paralaminar regions of median dorsalis and the posterior-medial aspect of the centromedian/parafascicularis nucleus complex. This placement maximizes the coverage over regions projecting to the supragranular cortcial regions, since they are thought to have a parallel role in cerebral activation as projections form the brainstem arousal system. After a titration phase in which intensity frequency, cycles duration and other parameters were set, a six-month double blinded alternating on/off crossover phase began using optimal selected parameter. With this study they demonstrated that chronic thalamic central electrical stimulation may promote recovery also after many years.

4.1.5 Neuromodulation using VNS

Here we want to propose another method for the treatment of TBI: the vagus nerve stimulation. Vagus nerve stimulation (VNS) is a neuromodulatory technique extensively used as a therapy in patients presenting pharmaco-resistent epilepsy who are suitable candidate for resective brain surgery or for whom surgery has failed (**?**). More recently, there has been an increasing interest in using VNS to treat depression following report that epileptic patients, suffering also from depression, improved their quality of life regardless of seizure outcome (**?**).

Recently, it was hypothesized that VNS could be efficient in treating TBI. Shi et al. (**?**) after evidences of positive results on TBI rat model, obtained the approval for a pilot study on TBI patients. They hypothesized that post VNS, patients should show increased metabolism in the forebrain, thalamus and reticular formation, which should help the restoration of arousal and consciousness. However, until now, they didn't report their results yet.

A a single case study by Yu et al. (?) investigated transcutaneous VNS in a patient suffering from cardiac and respiratory arrest, in VS since 7 weeks. They demonstrated, using fMRI data, that VNS can in principle augment the activity over the DMN, increasing connectivity between the precuneus and hypothalamus, thalamus, ventral medial prefrontal cortex and superior temporal gyrus. These neuroimaging results were coupled with positive clinical outcome including CRS-R score increasing from 6 to 13.

Before entering in the details of my second study, I will present here some literature on vagus nerve stimulation which I consider interesting and useful to further understand the second part.

4.2 An overview on vagus nerve stimulation (VNS)

In this section I will describe the vagus nerve anatomy supporting the different effects of vagus nerve stimulation, both in animal models and for different clinical and therapeutic applications.

The vagus nerve is a major component of the autonomic nervous system (ANS), which through its sympathetic and parasympathetic parts, regulates the functions of different organs, glands and involuntary muscles (e.g. including heart rate, respiration, swallowing, vocalization, gastric secretion and intestinal motility). The vagus nerve plays an important role in metabolic homeostasis regulation and also a key role in the neuroendocrine-immune axis (?). The term *Vagus Nerve Stimulation* (VNS) refers to different techniques aimed at stimulating, either electrically or manually, the vagus nerve. The most used device consists in a programmable pulse generator implanted in the chest, delivering chronic intermittent electrical stimuli. The use of VNS is widely approved for the treatment of refractory epilepsy and depression and recently it is under investigation for several other neurological disorders, including Alzheimer's disease, migraine, multiple sclerosis and either eating disorder (??).

4.2.1 Vagus nerve anatomy

The vagus nerve, from latin *vagus* "wandering, straying", is the tenth cranial nerve, so called due to its widespread function and complex anatomical distribution. In fact, it has the most extensive distribution among all the cranial nerves. The vagus is a mixed nerve carrying both general and special, visceral and somatic efferent and afferent fibers (**?**).

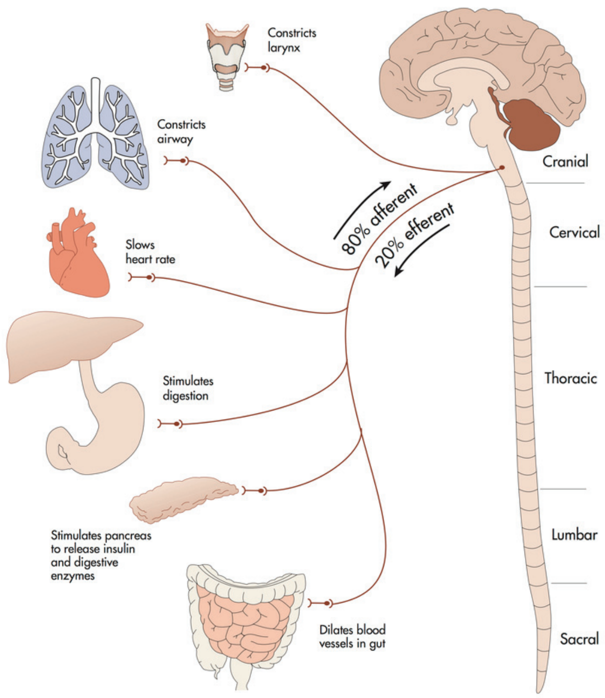

Figure 4.1: **Parasympathetic innervation of the vagus nerve.** Due to its widespread innervation, the vagus nerve is involved in several tasks such as heart rate, gastrointestinal peristalsis, sweating, muscle movements in the mouth. Credits: http:brainstimulationclinic.squarespace.com-vagus

It is mainly composed (80%) by "afferent" (sensory) fibres (carrying information from the body to the brain), mostly of visceral type, originating from receptors in the lungs, aorta, heart, oesophagus, gastrointestinal tract and aortic chemoreceptors (see Figure 4.1). Small myelinated sensory afferent fibres carry information from the concha of the ear **?**. This afferent inputs play an important role in the regulation of reflexes such as the respiratory, digestive and cardiovascular ones **?**. In fact, an important function of the vagus nerve is transmitting sensory information coming throughout the body to the brain. The remaining 20% are "efferent" fibres (sending signals from the brain to the body) (see Figure 4.1). The efferent fibres provide parasympathetic innervation to heart, lungs, gastrointestinal tract and other visceral organ of the abdomen and innervate the voluntary striate muscles of the larynx and pharynx.

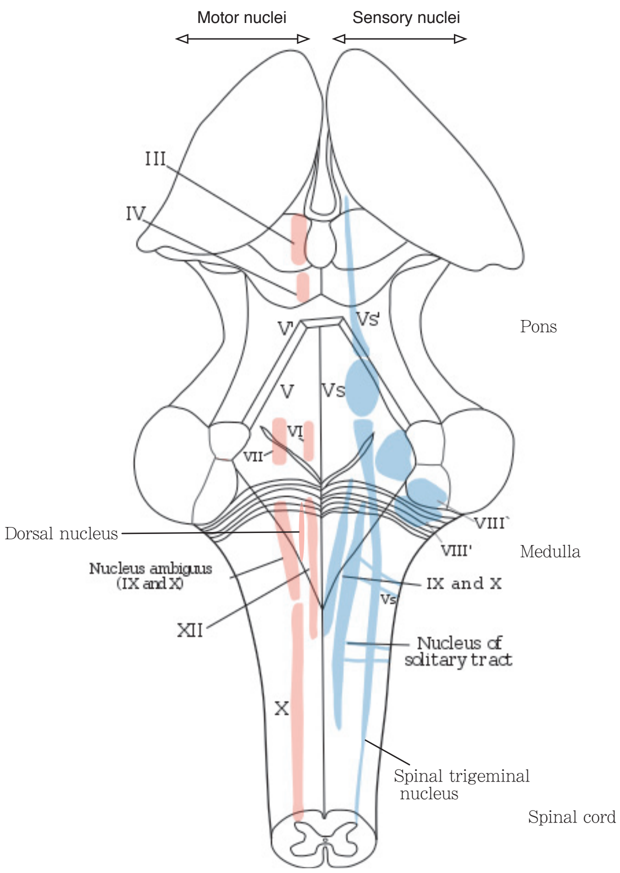

Figure 4.2: **Origins of the vagus nerve.** Schematic representation of the dorsal nucleus, the nucleus ambiguus, the nucleus tractus solitarius and the spinal nucleus of trigeminal nerve.

The vagus nerve originates from four nuclei in the medulla oblungata (see Figure 4.2): the dorsal nucleus, the nucleus ambiguus, the nucleus tractus solitarius and the nucleus of trigeminal nerve (?).

- The dorsal nucleus: from here originate the pre-ganglionic parasympathetic visceromotor fibres.
- The nucleus ambiguus: cells containing motor neurons, send parasympathetic output to the heart.
- Nucleus tractus solitarius: receives viscerosensory information from gastrointestinal tract,

respiratory system and taste information. Via the NTS the vagus extends mainly to the locus coeruleus and the dorsal raphe nuclei.

- Spinal nucleus of trigeminal nerve: receives somatic sensory afferent.

The right and left vagus nerves continue to spread, as already mentioned, through the neck to the upper and lower chest and diaphragm to arrive into the abdominal cavity. The activation of this efferents causes various visceral consequences such as the slowing of the heart rate (**?**). In the brainstem, sensory "afferents" fibers terminates in the nucleus tractus solitarius (NTS), to continue directly or indirectly to different brain regions (see Figure 4.3). From the NTS widespread projections travel throughout the central nervous system (CNS). Vagal projections arrive in the dorsal rephe nuclei and locus coeruleus, the first one being the major source of serotonergic neurons, and the second one containing noradrenergic neurons. Further projections reach the hypothalamus, amygdala nucleus, the nucleus ambiguous, the dorsal motor nucleus, the parabrachial nucleus, the thalamus which projects to the insular cortex (**?**).

Different fibers types (A, B and C fibers) are classified in accordance to their conduction velocities which, in myelinated fibres, are proportional to their size. Different fibres play different physiological roles: "A-fibres" are large and myelinated and carry visceral information and motor input, "B-fibers" are small and myelinated fibres carrying parasympathetic input, "C-fibers" are small and unmyelinated and carry afferent visceral information. 90% of the "afferents" and 70% of efferents are unmyelinated C-fibres (**?**). Most of the afferent fibres mediating reflexes are myelinated and their conduction velocity is grater than 15 m/s. The cell body of primary sensory neurons are located in the nodose ganglion and transmit mostly to the caudal part of nucleus tractus solitarius (NTS), but others connections can be observed with the medial reticular formation, the dorsal motor nucleus, area postrema and nucleus cunneatus. NTS can modulate blood pressure, swallowing and hart rate. In particular, there are short pathways connecting the NTS with cardiomotor neurons in the dorsal motor nucleus able to slow down the heart rate (**?**).

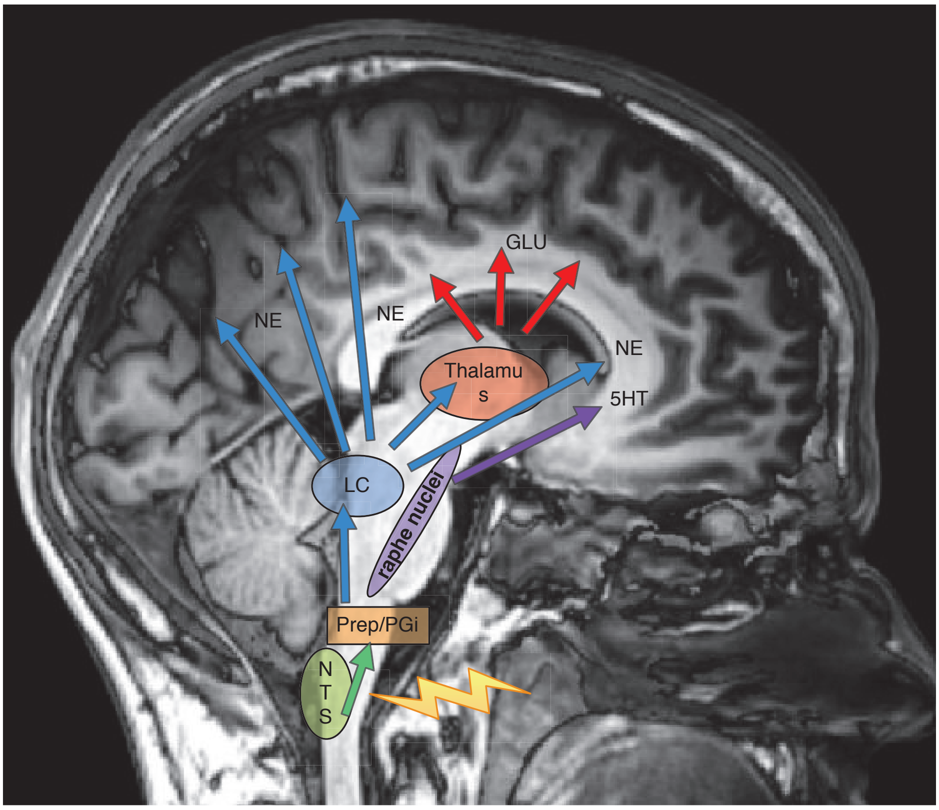

Figure 4.3: **Vagal Projection throughout the brain**. The image represents the principal vagal pathway activated by the stimulation. Vagal inputs arrive to locus coeruleus(LC) via the nucleus paragigantocellularis (PGi) and the nucleus prepositus hypoglossi (Prep). From LC, Noprepinephrine (NE) neurons projects to the thalamus, cortex and dorsal raphe nucleus (DRN). From the DRN glutamatergic and serotonergic fibers projectes to the cerebral cortex (**?**). Figure inspired by Fornai et al. (**?**)

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4.2.2 History of the VNS

In 1938, Bailey and Bremer (?) discovered that the effect of VNS in the cat cerebral cortex caused electroencephalogram changes. In 1951, (?) showed evoked responses in the ventroposterior complex of the intralaminar regions of the thalamus after cervical vagus nerve stimulation. In 1985, Zabara (?) reported inhibition of the neural process inducing an alteration of the brain activity and a reduction of seizures in dogs. His work was duplicated in monkeys and rats. After that, human pilot studies for patients benefits in various clinical conditions began (?). In 1997 the Food and Drug Administration (FDA) approved the surgical implantation of a vagus nerve stimulator for the treatment of seizures in drug-refractory epilepsy. Clinical trials were performed either as randomized trials with different intensity or cross-over trials with the stimulator on and off (?). Then it was approved for treatment-resistant depression and weight loss in 2015. In Europe, two tVNS devices have been approved for use in epilepsy in 2010 and pain in 2012 and another (?) for headache. In the US, it has not been specifically approved but it is legal and its effects are investigated for many conditions such as: Atrial fibrillation, depression, dementia, diabetes, memory, headache, pain tinnitus, Schizophrenia and stroke.

4.2.3 VNS implant device

The implant device is composed by a pulse generator programmable by telemetry connected with a bipolar flexible wire. The most common model is the commercially available Cyberonics pulse generator (?). The pulse generator is placed by the neurosurgeon, during general anaesthesia, under the skin, in a subcutaneous pocket, in the upper left side of the chest, centred at mid-neck level and on the anterior border of the sternocleidomastoid muscle. The lead terminates in a double coil electrode which is positioned on the cervical portion of the left vagus nerve (??). Programmable parameters consist in: current charge (corresponding to the intensity (mA) of the electrical stimulus), the pulse width (duration in microseconds of the electrical pulse), the pulse frequency (Hz), and the on/off duty cycle (on and off time window length). Initial settings need to be settled taking into account not only efficacy (optimal regulation for controlling seizure) but also tolerably, which improves over time.

Adverse consequences are related to the stimulation of body parts innervated by the vagus nerve, but since 80% of fibres are "afferent", the pulses are propagated from the electrodes attached on the nerve toward the brain. The implantation on the left side is more reliable to prevent/minimize cardiac complications, notably the cardiac dysrhythmia such as bradycardia or asystole. In fact, the right vagus nerve supplies the sinoatrial node, while the left one innervates the atrioventricular node and has proportionally less number of cardiac efferent fibers (?). Right VNS stimulation is under investigation as a treatment for heart failure (?). In parallel with surgically implanted VNS other non-invasive vagus nerve stimulation techniques were developed to provide possible alternatives able to produce similar effects without the surgical potential risks and high financial costs. Notably, the transcutaneus VNS (t-VNS) targets cymba conchae, a depression of the external ear, in a brunch of afferent projections. Several studies investigated neural activations during tVNS in healthy subjects population using fMRI. Main findings reported similar activation patterns as patterns found following surgical implant (??).

4.2.4 Neurochemical consequences of VNS

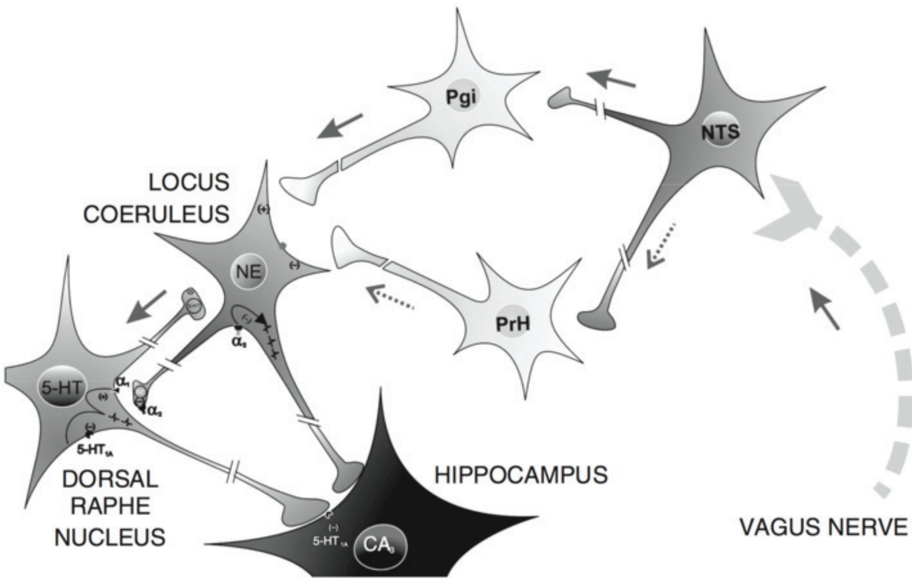

Figure 4.4: **Pathways of Activation of the Locus Cperuleus** This figure shows in detail the pathways involving the nucleus paragigantocellularis (PGi) and the nucleus prepositus hypoglossi (Prep). Credits: http://brainstimulationclinic.squarespace.com/vagus.

When the vagus is peripherally stimulated, the nucleus of the solitary NTS plays the major role. Vagal inputs are relayed from the nucleus tractus solitarius to the locus coeruleus via a disynaptic pathway 4.4, a paragigantocellularus (PGi) and the nucleus prepositus hypoglossi (Prep). The stimulation tends to facilitate the excitatory (PGi) pathways rather than the inhibitory one (Prep) on neurons in the LC. LC in turn projects with norepineprine (NE) neurons to the dorsal raphe nucleus (DRN), thalamus and the cerebral cortex. The thalamus projects by glutamatergic fibers to the cerebral cortex and the dorso raphe nucleus (DRN) projects also to the cerebral cortex by serotonergic (5-HT) fibers. There is evidence that the locus coeruleus (LC) is a major area of downstream of NTS since suppression of epileptic seizure induced by VNS is lost in part when this area is inactive (**?**). Therefore the principal pathway through which the vagus nerve stimulation may influence the cerebral cortex is thought to be across the locus coeruleus (LC).

4.2.5 VNS in epilepsy

The most recognized and reviewed application of the vagus nerve stimulation is at present the treatment of refractory epilepsy. Since the effect of vagus nerve stimulation in humans is mainly reported in this literature, here I will review the most interesting findings elucidating its mechanisms of action and cerebral activations.

Nearly one third of epileptic patients are refractory to anti-epileptic agents (**?**). Among them, some have focally resectable lesions and others don't. In this latter case, they are considered suitable for VNS implantation. The first human implant of a vagus nerve stimulator device was reported in literature by Penry et al. in 1988. He conducted a study over 4 patients, resulting in a complete seizure control in two of them, in a reduction of 40% of seizure frequency in one and no change in the last one, with 6 to 10 months follow up (**?**). Two prospective pilot studies on 14 patients with a follow up of 14 and 35 months, reported a reduction in seizure frequency of about 47%. In a larger study, 198 patients were treated with high or low intensity VNS, showing that patient receiving high stimulation had an average reduction of 28% in total seizure frequency compared to 15% in low stimulation group (**?**). Long-term outcome was documented over a cohort of 28 patients implanted for 5 years. At 12 months seizure frequency decreased from baseline by 28% and arrived at 72% at follow up, which ranged from 5 to 7 years (?). Englot et al. (?) performed the first meta-analysis analysing data from 3321 patients coming from 74 different studies. Seizure frequency was reduced by 50% after more than 1 year of therapy. At the follow-up 50% reduction was reported in approximately 50% of the patients.

Possible mechanisms of action

Although vagus nerve stimulation represents a valid option for the treatment of medically refractory epilepsy in adults and children, the underlying mechanism of action remains elusive. The establishment of the exact mechanism for VNS efficacy in patients with intractable epilepsy is of fundamental importance, since it would allow not only further developments of this technique, but also the identification of newer fields of applications. Main hypothesis underlying its mechanism of action includes:

- **Norepinephrine mediated mechanism**: Previous studies have shown that VNS may upregulate endogenus noradrenergic activity, increasing brain concentration of norephinephrine by activating the locus coeruleus (LC). Indeed it is already known from animal studies that increasing brain noradrenaline levels following traumatic brain injury (TBI) can promote functional recovery. Amphetamine and norepinephrine re-uptake inhibitors have been demonstrated to improve functions in humans after stroke, depth perception after visual cortex ablation, tactile placing after motor cortex injury. Moreover, intravenous noradrenaline infusion facilitate motor recovery following sensory-motor cortex injury.(?). By contrary lesion to the LC diminished the rate and extent of functional recovery rate following ablation (?). Several different mechanisms can account for this effect, among them: the reduction of ischemic injury, induction of long-trem potetntiation (LTP) attenuation of inflammation, and reduction in seizure activity.

- **Modulation of depolarization activity**: in the acute post-injury period further structural damages (secondary damags) can be triggered by non-convulsive seizure. Two further depolarization phenomena induced secondary injuries, the peri-infarct depolarization and the glial calcium wave. Peri-infarct depolarization is a pathological seizure activity, happening at the interface of the lesion. This activity can contributes to the so called cortical spreading depression, which is a global depolarization spreading across the cortex, and causing a supplemental damage. Glial calcium wave reduction is also considered to be the substrate of neuroprotective effect of anti-epileptic drugs.

- **Decreasing glutamate-mediated exocitotoxicity**: VNS seems to exert a neuroprotective effect reducing the post-traumatic hyper-excitability, which is a major cause of secondary neuronal injuries. The loss of cortical neurons, the modification in their circuitry and the loss of inhibitory gamma aminobutyric acid (GABA), leading to hyper-excitability of pyramidal cells, together induce in the weeks following TBI predisposition to seizures and exacerbation of injury. When neurons are damaged extracellular glutamate increases, and binding with NMDA receptors increases calcium influx. The intracellular calcium can initiate apoptosis cascades in the neurons. VNS can lower extracellular glutamate level. Thus VNS appears to protect GABAergic neurons from destruction and it reduces the incidence of seizure activity.

- **Synaptic plasticity and recruitment of endogenous Neural Stem Cells** Some evidences show as in addition to the other neuroprotective effects, VNS may potentiate synaptic plasticity and recruit neural stem cells. In fact, rat studies showed that the facilitation of long term potentiation LTP induced by VNS in the hippocampus and cortex in rats, was likely mediated by norephineprine (**?**).

- **VNS attenuates cortical edema and blood brain barrier breakdown**

- **Ghrelin-Mediated actions of VNS**

- **Ghrelin-Mediated actions of VNS**

- **Synaptic plasticity and recruitment of endogenous Neural Stal Cells**

- **Decreasing intracranial pressure**

4.2.6 VNS-induced cerebral activations

As already mentioned, vagal afferents traverse the solitary tract and form synapses mainly in the nuclei of the dorsal medullary complex. Among these nuclei, the nucleus of solitary tract (NTS) receives the greatest number of vagal afferent synapses. Through the parabrachial nucleus, its projections involve several structures such as the hypothalamus, the thalamus, the amygdala, the anterior insula, the infralimbic cortex, the lateral prefrontal cortex and other cortical regions. Through the amygdala, the NTS gains access to the limbic system (amygdala-hippocampus-entorhinal cortex). In addition, the NTS projects to the LC, providing widespread noradrenergic innervation to the entier cortex and to the raphe nuclei providing serotonergic innervation of the brain **?**. Neuroimaging studies of VNS have been conducted in both patients and healthy humans, using either implanted or transcutaneus stimulations. Many groups have used different imaging techniques to better understand the pathways activated by the stimulation and to describe VNS effects on the brain. Since these investigations were conducted using different methodologies (PET, SPECT, fMRI) and different protocols (changing stimulation parameters, including acute and chronic effects) the literature appears inconsistent and sometimes in contradiction.

The most robust findings seem to be the involvement of the thalamus, which plays a key role in processing somatic sensations and regulating cortical activity. A well documented effect of VNS is the increase of induced cerebral blood flow (metabolic rate) in bilateral thalami during both immediate effect and prolonged studies. Prolonged effects of VNS administration may affect thalamo-cortical regulation of brain circuitry and indirectly the modulation of EEG oscillatory brain activity. It was also observed that the left VNS implant cause an additional metabolic increase in the right inferior postcentral gyrus **?**. The thalamus is formed by numerous thalamic nuclei containing the talamocortical relay neurons, which in principle drive the entire cortex and all subcortical structures. Among the multiple hypothesis about the VNS mechanisms underlying improvement in seizure suppression is that thalami are regions generating active prevention of seizure onset. All studies using PET and SPECT reported changes in the thalamic activity after VNS in epilepsy. By contrast, studies investigating VNS activation using fMRI reported weak thalamic involvement. This inconsistency could be due to different time resolutions, and limits relative to fMRI to assess subcortical structures. Cerebellar activation are consistent with vagus nerve projection anatomy. fMRI studies found changes mainly in the orbitofrontal cortex, anterior temporalpoles, insula, and the hypothalamus (?).

A single case study, by Fan et al., involving a 4-year-old patient suffering from a myoclonic astatic epilepsy (MAE) revealed significant changes in the EEG patterns after 6 months of stimulation, resulting in increased beta-gamma spectrum rhythm. Brain diffusion-tensor imaging at 10 months showed increased functional anisotropy (generated by axons limitating molecular motion to preferential directions) in the right fimbiae fornix at the level of cerebral peduncle (?). Fractional anisotropy measures the microstructural organization and direcionality, it is thus a direct indicator of myelination and axonal diameter. In general, fractional anisotropy increases with ageing and myelination in the cortex, while diffusivity decreases (?).

In a study designed to evaluate cortical activations during VNS, Narayanan and colleagues (?) tested 5 epileptic patients using fMRI. Parameters were settled at 30Hz of frequency, 0.5-2mA of intensity and a cycle of 30s ON followed by 30s OFF. They detected activation bilaterally on the thalamus (predominantly left), insular cortices bilaterally, ipsilateral basal ganglia and postcentral gyri, right superior temporal gyrus, and inferomedial occipital gyri (on left more than on right). The most robust result was the activation of the thalami and insular cortices.

Recently, it has been assessed that tVNS could trigger the activation of classical pathways of central vagal projections as well. In particular, a fMRI study done in 12 healthy adults undergoing 7 min of mild auricular tVNS could significantly affect the central projections of vagus nerve. In this study, the regions activated by tVNS were: the nucleus of solitary tract (NTS) projecting to the spinal trigeminal nucleus, parabrachial area, locus coeruleus, dorsal raphe nucleus, periaqueductal gray, thalamus, amygdala, insula, nucleus acumbens, bed nucleus of the stria terminalis, and hypothalamus. On the contrary a deactivation was found in the hypothalamus and hippocampus ?. Anterior thalamic activation is consistent with fMRI and PET studies performed in patients having a chronic VNS implant. The anterior thalamic activation was mainly observed in patients who responded to the VNS (?).

Kraus et al. (?) conducted a fMRI study over an healthy population using tVNS. They found an affect in the BOLD signal, which decreased in limbic brain areas, including the amygdala, hippocampus, parahippocampal gyrus, and middle and superior temporal gyrus and increased in the insula, precentral gyrus, and thalamus. These results were paired with psychometric data, which revealed an improvement in well-being after tVNS. The sham stimulation did not show similar fMRI or psychometirc effects. In a follow-up study anterior and posterior wall tVNS effects were investigated separately, drawing the conclusion that the anterior wall evoked the deactivation of limbic brain areas at the parahippocampal gyrus and the posterior cingulate cortex, as well as other regions such as the thalamus, locus coeruleus and solitary tract (?).

Fang et al. (?) studies patients suffering from depression and after 1-month of VNS stimulation they found that tVNS can significantly modulate the default mode network in patients suffering from mild or major depressive disorder. In particular, they found decreased functional connectivity between DMN and anterior insula and parahippocampus and increased connectivity between the DMN and precuneus and orbital prefrontal cortex. The increases of connectivity was associated with depression scale reduction.

Another important VNS-induced effect is to trigger neural plasticity. Different studies (see section VNS and rehabilitative therapies) used this hypothesis to test VNS efficacy for a wide types of applications. The rationale for these studies come from the evidence that chronic VNS induces long-lasting increases of brain derived neurotrophic factor (BDNF) in the hippocampus. This BDNF expression increase may serve to promote and maintain new neuronal connections and therefore is a substrate of neuronal plasticity. This effect was also observed in the cerebral cortex of rats ?. In particular, the chronic VNS promotes acute effect maintenance, such as trophism and new cell proliferation rather than increases cell proliferation indefinitely ?. Therefore, VNS can affect neuronal plasticity by altering the synaptic activity. In fact, it is likely to cause increased transsynaptic neurotransmission in the nucleus of the tractus solitarius (?) and to produce changes in receptor density in sites receiving projections from the latter (?).

4.2.7 VNS and EEG

VNS and EEG in early animal model

In animal models, the effect of vagus nerve stimulation in the background EEG activity is highly dependent on the different stimulation parameters. In fact, both synchronization and desynchronization were previously observed (?). Since seizures are characterized by highly synchronized EEG activity, synchronization and desynchronization are associated with increased resistance and susceptibility to seizures, respectively (?).

Bailey and Bremmer (1938) first discovered that electrical stimulation of the vagus nerve in cats caused desynchronization (?). Krhal et al. had demonstrated that lesions in the LC suppressed the VNS anti-epileptic effect, proving its importance, and the desynchronization and the arousal-promoting affect as well (?). Magnes et al. directly stimulating the nerve in "encephale isole" cats observed that low frequency electrical stimulation (1-16 Hz) in the region of the nucleus of solitary tract, resulted in widespread EEG synchronization. By contrary, vagus nerve stimulation at higher frequency (>30Hz) produced EEG-desynchronization (?). Thompson et al., demonstrated respectively EEG gamma-spectrum (>40 Hz) in direct cortical and thalamic recordings during high frequency VNS at 1.5mA ?. All these results can be synthesized by observing that desynchronization seems to result from high-frequency stimulation of fibres with a low level of myelination, instead synchronization is more associated to low-frequency stimulation of highly myelinated fibres (?).

VNS and EEG in epileptic patients

Also for epileptic patients it was hypothesized that the widespread cortical desynchronization caused by VNS could be a major mechanism of action causing the anti-epileptic effect. In fact, hypersynchrony is a well known pathological pattern in epileptic patients (??). Several studies reported VNS-induced increase of thalamic activity, which is interestingly related to the reticular activating system, and cortical desynchronization suggesting a possible involvement of the thalamus in anti-epileptic effect ?.

Marrosu et al. investigated EEG power spectrum changes in 11 epileptic patients 1 month and 1 year after VNS implantation. They found a decreased synchronization of theta frequency and an increase in gamma power spectrum (20-50Hz) and synchronization. However, this gamma modulation can be seizure-independent and related to improved attentional performances (?). Another study on Lennox-Gastaut syndrome (LGS) patients, treated with VNS, reported improved alertness in 76.7% of patients. Improved alertness seemed to be consistent with another behavioural study in 10 epileptic patients conducted by Rizzo et al. (?) who reported major effects of vagus nerve stimulation on daytime alertness and a reduction of nocturnal rapid eye movements sleep. This can be interpreted as a possible effect of the VNS on structures involved in sleep-wake cycles. A recent study to evaluate quality of life (QOL) after VNS was conducted on the VNS Therapy Patient Outcome Registry, containing data from 5000 patients (including 3000 with $>$ 12 months follow-up). Behavioural improvements were reported also in alertness (48-50% of patients), post-ictal state, cluster seizure, mood change, verbal communication, school/professional achievements and memory.

In order to evaluate the effect of the VNS on the EEG signals, functional connectivity indexes were frequently used as an investigation tool. Fraschini et al., showed that the mean phase locking values (PLI) (a measure of synchrony between signals) computed 5 years after VNS surgery on responsive epileptic patients, were significantly lower only over the gamma (30-48 Hz) frequency band (?), indicating a global signal desynchronization. Fraschini et al. further investigated the architecture of functional brain networks in VNS-responding patients. Following the hypothesis that VNS would change the network topology in responders, they used minimum spanning tree metrics to try to demonstrate that in responders significant alterations occur in the inter-ictal configurations, which tended to go back to more efficient and integrated patterns. They found a re-organization of theta networks in responders patients, which showed an increased diameter and eccentricity. Loss of integration in theta band was already described in patients prone to occurrence in seizures ?.

Bartolomei et al., evaluated 5 patients suffering from drug resistant epilepsy. Activity during the VNS ON and VNS OFF periods was considered. The main findings showed that in comparison to OFF periods, the ON periods had higher values of functional connectivity in 4 out of 5 patients. Interestingly, the patient showing decreased connections was the only responder, suggesting a possible mechanism related to the therapy success (?). De Vos et al. used a measure of symmetry among omologus channels (pdBSI, paired Brain Simmetry Index) to find predictive interictal EEG features for seizure reduction. This study showed that non-responders had higher pdBSI in theta and alpha band, once again suggesting that seizure-reduction is associated to increase asymmetry in signal coupling (?) .

4.2.8 VNS and rehabilitation therapies

Recently,it has been proposed that coupling the vagus nerve stimulation (VNS) with rehabilitative techniques could promote adaptive circuit changes in the brain, incrementing the training-dependent plasticity and enhancing recovery. The major goals of rehabilitative techniques are to treat pathological neural activity and restore lost functions. Traditional rehabilitation often does not provide significant improvements, leaving the patient in a chronic disability.

Animal models of chronic tinnitus, ischemic stroke, intracerebral hemorrhage and traumatic brain injury benefit from being specifically paired with VNS burst. As already highlighted, the electrical stimulation of the vagus nerve drives the activity in the noradrenergic locus coeruleus and cholinergic basal forebrain and the subsequent release of neuromodulator throughout the cortex. If this effect is reduced, the VNS effects is also blocked. As already mentioned, these neuromodulators systems play an important role in the expression of cortical plasticity (?).

Experiments in rats take advantage from these considerations and showed how pairing left cervical VNS with tones can actually induce a reorganization of the tonotopic map, increasing the proportion of neurons responding to a particular frequency in the primary auditory cortex (?). To study plasticity within the motor cortex, Porter et al. paired left cervical VNS with successful trials in a lever press task. They found an increase of the area corresponding to the body part (upper-limb or paw) used in the task and no change for the other (control) representations (?).

VNS was tested with success in patients with severe chronic tinnitus. They received left cervical VNS paired this time with a range of tones, excluding the tinnitus frequency. The therapy lasted for 2.5h per 20 days. Four patients out of 10 demonstrated a clinical significant improvement, and the benefit persisted for 2 months. This treatment is potentially applicable in various cases of maladaptive plasticity triggering sensory disorders, for example the phantom limb pain and chronic pain. In fact, it is known that the pain intensity correlates with the level of over-representation.

Preclinical studies done by Pruitt et al. extended previous animal studies demonstrating that VNS can improve the recovery of motor function, if coupled with successful trials (VNS occurred within 45ms). Since VNS did not reduce the size of the lesion, it was hypothesized that motor recovery wasn not due to a neuroprotective mechanism (?).

Chapter 5

Restoring consciousness with vagus nerve stimulation

5.1 Introduction

Understanding the mechanisms of action and pathophysiology underlying alterations of the conscious state following severe head traumas is a major issue in current clinical research. Although our knowledge on disorders of consciousness has progressed (see Chapter 4), at present a few studies aim at developing techniques to accelerate recovery and optimize the chances of healing for patients with unfavourable prognosis. Some attempts have already been done, as already discussed, for example physical therapy techniques, including passive range of motion therapies (?), pharmacological interventions (?) and electrical stimulation (?). Unfortunately, only a few interventions show to be reproducible and effective.

Notably, Schiff et al. demonstrated a significant late function recovery after DBS on MCS patients targeting the anterior intralaminar thalamic nuclei (?). While there have been some encouraging results, evidences are insufficient and other studies of validation are required. Other techniques were recently tested as potential treatments for consciousness disorders. For instance, short duration transcranial direct current stimulation (tDCS) applied for 20 minutes on dorsolateral prefrontal cortex on coma patients have been demonstrated to improve CRS-R scores, mainly in MCS patients (?). Pharmacological interventions have also been tested as treatment aiming at modulating key neurotransmitter systems mediating arousal and attention. The GABA agonist Zolpidem was shown to trigger an immediate, though temporary, transition from VS to MCS, inducing a re-emergence of command following, visual pursuit and gesture, albeit in some few responsive patients. VS and MCS patients, treated for 4 weeks with amantadine, which acts on monoamines such as noradrenaline and serotonin, showed a faster rate of behavioural improvement (?).

Recent results show that direct stimulation of the vagus nerve (VNS) activates thalamo-cortical neural pathways important in promoting arousal and conscious state. The modulation of vagal activity also appears to affect the cognitive and attentional systems (?) and enhance mood and cognition (?). Further, the stimulation of the vagus nerve at the neck level is known to activate numerous brain regions, among them the locus coeruleus, regulating arousal and known to promote and increase the release of norepinephrine (?).

It has been demonstrated that vagus nerve stimulation could enhance neural plasticity responsible for supporting the recovery of functions after brain damages. Different preclinical models of traumatic brain injuries (TBI) exist to study vagus nerve stimulation effect in rats. Using a fluid percussion model of TBI, Smith et al. (?) demonstrated that the VNS stimulation initiated 2h post injury enhanced the cognitive and motor recovery rate. In particular, rats treated with VNS (?) showed both an accelerated and a more complete recovery compared to lesioned untreated rats. The surviving rate also increased. Stimulation parameters were settled as follows: 30s of pulses duration, 0.5mA of current intensity, at 20Hz biphasic pulses. Behavioral tests inlcuded beam walk, skilled forelimb reaching, and Morris water maze test. The same results were achieved one year later, initiating the VNS 24h later the induction of a fluid percussion lesion. According to Smith et al. these results may be mediated by VNS activation of the locus coeruleus (LC), leading to the release of norepinephrine in the nueraxis ?.

Moreover, animal models have shown that VNS may play a neuroprotective role promoting neurogenesis in the hippocampus (?), promoting the generation of neurotrophic factors (brain-derived neurotrophic factor, BDNF) (?) and it also appears to have a positive effect on systemic inflammation responsible for heart failure and brain death. Various studies tried to investigate the molecular mechanisms underlying the therapeutic action of the VNS in both treatment-resistant epilepsy and depression. Follesa et al. tested in the rat brain whether VNS increases the expression of brain-derived neurotrophic factor (BDNF), fibroblast growth factor, nerve growth factor and norepinephrine concentration. They found that acute nerve stimulation (3h treatment, 2 days after surgery) increased the expression of BDNF and fibroblast growth factor, in the hippocampus and cerebral cortex and increased the concentration of norepinephrine in prefrontal cortex. These results shows how VNS can trigger molecular changes in the rat brain involving neurotransmitter and growth factors regulation, but only chronic stimulation modulates the firing rates of monoaminergic neurons. (?). Other animals studies have found that VNS modulates noradrenergic neurotransmission (?) which is important for arousal regulation and wakefulness (?) by activating the locus coeruleus.

We hypothesize that through its action on the thalamo-cortical system, the VNS could increase the probability of awakening in patients with an unfavourable prognosis and accelerate this same awakening in those who have received a more favourable prognosis.

5.2 Abstract

In the following study we investigated the mechanisms responsible for the return of consciousness induced by vagus nerve stimulation (VNS). We reported a single case study of a patient who's been living in a vegetative state for 15 years who benefited from the implantation of a vagal nerve stimulator. The patient was followed for 9 months (including pre and post-VNS periods), during this period we could observed the transition from vegetative to minimally conscious state, as assessed by CRS-R clinical score, mainly signalling that the patient improved his ability to follow external stimulations. These results were supported by several neuroimaging evidence such as measure of cortical and sub-cortical activity and the evaluation of functional connectivity, which enabled us to record increased communication between brain regions previ-

ously identified as a "hot zone" for consciousness. We believe that these novel findings may have far-reaching implications not only for the clinical care of patients with disorders of consciousness but also for current theories of consciousness. Our article was submitted and accepted by ***Current Biology*** in August 2017. The following section contains the full submitted material.

5.3 Publication

CellPress

Current Biology
Magazine

challenge to our understanding of this process. Development of a robust *in vitro* spermatogenesis system for mammals would provide useful insights. So far, numerous approaches using the entire tissue or separated single cells from the testis in 2D or 3D cell culture systems have been explored, which is encouraging. Additionally, a robust *in vitro* spermatogenesis system might help define the causes of infertility in humans.

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Restoring consciousness with vagus nerve stimulation

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Patients lying in a vegetative state present severe impairments of consciousness [1] caused by lesions in the cortex, the brainstem, the thalamus and the white matter [2]. There is agreement that this condition may involve disconnections in long-range cortico–cortical and thalamo-cortical pathways [3]. Hence, in the vegetative state cortical activity is 'deafferented' from subcortical modulation and/or principally disrupted between fronto-parietal regions. Some patients in a vegetative state recover while others persistently remain in such a state. The neural signature of spontaneous recovery is linked to increased thalamo-cortical activity and improved fronto-parietal functional connectivity [3]. The likelihood of consciousness recovery depends on the extent of brain damage and patients' etiology, but after one year of unresponsive behavior, chances become low [1]. There is thus a need to explore novel ways of repairing lost consciousness. Here we report beneficial effects of vagus nerve stimulation on consciousness level of a single patient in a vegetative state, including improved behavioral responsiveness and enhanced brain connectivity patterns.

Consistent with an important role of the thalamic-cortical axis for awareness, one study has demonstrated an increased behavioral responsiveness after deep thalamic stimulation limited to the stimulation period [4]. Here we propose to activate the thalamo-cortical network based on vagus nerve stimulation. The vagus nerve carries somatic and visceral efferents and afferents distributed throughout the central nervous system, either monosynaptically or via the nucleus of the solitary tract (NTS) [5]. The vagus directly modulates activity in the brainstem and via the NTS it reaches the dorsal raphe nuclei, the thalamus, the amygdala, and the hippocampus [5]. In humans, vagus nerve stimulation increases metabolism in the forebrain, thalamus and reticular formation [6]. It also enhances neuronal firing in the locus coeruleus which leads to massive release of norepinephrine in the thalamus and hippocampus, a noradrenergic pathway important for arousal, alertness and the fight-or-flight response [7].

Following the hypothesis that vagus nerve stimulation functionally reorganizes the thalamo-cortical network, we tested its effects on the cortical activity of a patient lying in a vegetative state for 15 years following traumatic brain injury. Behavioral, electroencephalographic (EEG) and 18F-FDG PET recordings were performed before and after surgical implantation of a vagus nerve stimulator. Stimulation was gradually increased to a maximum intensity of 1.5 mA, and its effects were monitored over six months post-implantation. After one month of stimulation, when intensity reached 1 mA, clinical examination revealed reproducible and consistent improvements in general arousal, sustained attention, body motility and visual pursuit. Scores on the Coma Recovery Scale-Revised (CRS-R) test improved, mostly in the visual domain, as stimulation increased, from a score of 5 at baseline (last exam) to 10 at highest intensities (1.00–1.25 mA), indicating a transition from a vegetative to minimally conscious state.

Scalp EEG data comparing the patient's resting state pre- and post-stimulation revealed a significant increase in theta band (4–7 Hz) power (one-sample t-test, $p < 0.0001$ false discovery rate corrected, Figure S1A,B in Supplemental Information, published with this article online), a brain signal found to reliably distinguish minimally conscious patients from vegetative ones [8]. Data driven blind source separation analysis identified eight sources responsible for the stimulation-induced power increase, distributed over the occipito-parietal, inferior temporal and fronto-central regions in addition to a

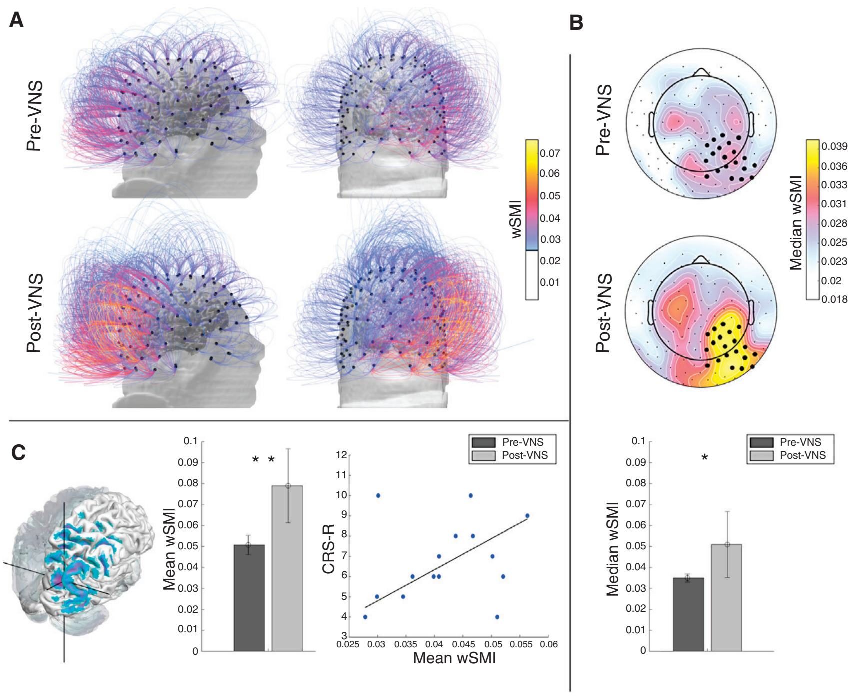

Figure 1. Information sharing increases after vagus nerve stimulation over centroposterior regions.

(A) Sagittal (left) and coronal (right) views of weighted symbolic mutual information (wSMI) shared by all channels pre- and post-vagus nerve stimulation (VNS) (top and bottom, respectively). For visual clarity, only links with wSMI higher than 0.025 are shown. (B) Topographies of the median wSMI that each EEG channel shares with all the other channels pre- and post-VNS (top and bottom, respectively). The bar graph represents the median wSMI over right centroposterior electrodes (darker dots) which significantly increases post-VNS (permutation test over sessions: Wilcoxon test, $p = 0.0266$). (C) Localization of the most VNS-reactive theta source showing significant increase of information sharing post-VNS. Sources' localization is presented over the patient's cortical surface (probability map, sLoreta current source density: light blue scale) combined with his FDG-PET metabolism (gray scale) as measured three months post-VNS. The source was localized in the inferior parietal lobule. The bar graph represents the mean wSMI shared with all other selected sources pre- and post-VNS (dark gray and light gray, respectively) (permutation test over sessions: Wilcoxon test, $p < 0.01$ Bonferroni corrected). CRS-R clinical score increased as a function of information sharing over a cortical posterior theta network (Robust regression, $p = 0.0015$).

deeper source most likely localized in the insula (Figure S1C). These areas belong to the default mode network whose activity appears to reflect the degree of consciousness in non-communicative brain-damaged patients [1]. The highest stimulation intensity induced an increase in theta power over the right inferior parietal and parieto-temporal-occipital border (Figure S1C), a region labelled as a hot-zone for conscious awareness [9]. We calculated weighted symbolic mutual information (wSMI), a measure of cortical connectivity and information sharing known as a sensitive index of consciousness. This measure captures activity in different frequency bands and robustly discriminates the vegetative from minimally conscious state within theta frequency signals (4–10 Hz) [10]. By using a parameter of $\tau = 32$ ms we found that the median wSMI across channel pairs was significantly higher after vagus nerve stimulation over a parietal region within a cluster including the right centro-temporo-occipital electrodes (Figure 1A,B).

The wSMI procedure was also applied on sources previously identified by blind source separation as reactive to vagus nerve stimulation. The purpose here was to isolate regions that most contributed to information sharing, thus suppressing sources with interfering noise activity. Cortical areas showing significant increase in mutual information after stimulation included the inferior parietal, precuneus, posterior cingulate and pre-motor/motor regions (Figure 1C and Figure S1E). The highest mean wSMI value was found in the inferior parietal cortex/intraparietal sulcus (Figure 1C). This global increase in mean wSMI over theta sources was significantly correlated to the CRS-R scores of clinical improvement (Robust regression, t (-0.0128;3.9436), dfe = 14, $p = 0.0015$, Figure 1C). These results demonstrate that vagus nerve stimulation enhances information sharing within a centro-posterior network. This is consistent with the observation reported on a large population of brain injured patients showing that activity within centro-parietal regions constitute the marker of a conscious state [10]. Importantly, they demonstrate the critical role of the parietal cortex as a central hub for broadcasting neural signals among posterior and central sites in order to strengthen consciousness.

Finally, 18F-FDG PET results corroborated EEG findings by showing extensive increases of activity in occipito-parieto-frontal and basal ganglia regions as early as three months after implantation of the stimulator. Vagus nerve stimulation enhanced the metabolic signal in the thalamus, a target of the vagus nerve (Figure S1D).

These findings show that stimulation of the vagus nerve promoted the spread of cortical signals and caused an increase of metabolic activity leading to behavioral improvement as measured with the CRS-R scale and as reported by clinicians and family members. Thus, potentiating vagus nerve inputs to the brain helps to restore consciousness even after many years of being in a vegetative state, thus challenging the belief that disorders of consciousness persisting after 12 months are irreversible [1]. The direct connection between the NTS where the vagus nerve originates and the thalamus may be at the origin of the significant increase in theta signal recorded at the cortical level. In particular, the parietal cortex appears to be a major player in guiding the expansion of neural activity across brain areas. The enhanced neural activity might also be mediated by neurotransmission changes given that vagus nerve projections target key regions important for the liberation of norepinephrine and serotonin [7].

5.4 Supplemental Information

Supplemental Information: Restoring large scale brain activity and consciousness with vagus nerve stimulation

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Supplemental Figures and Tables

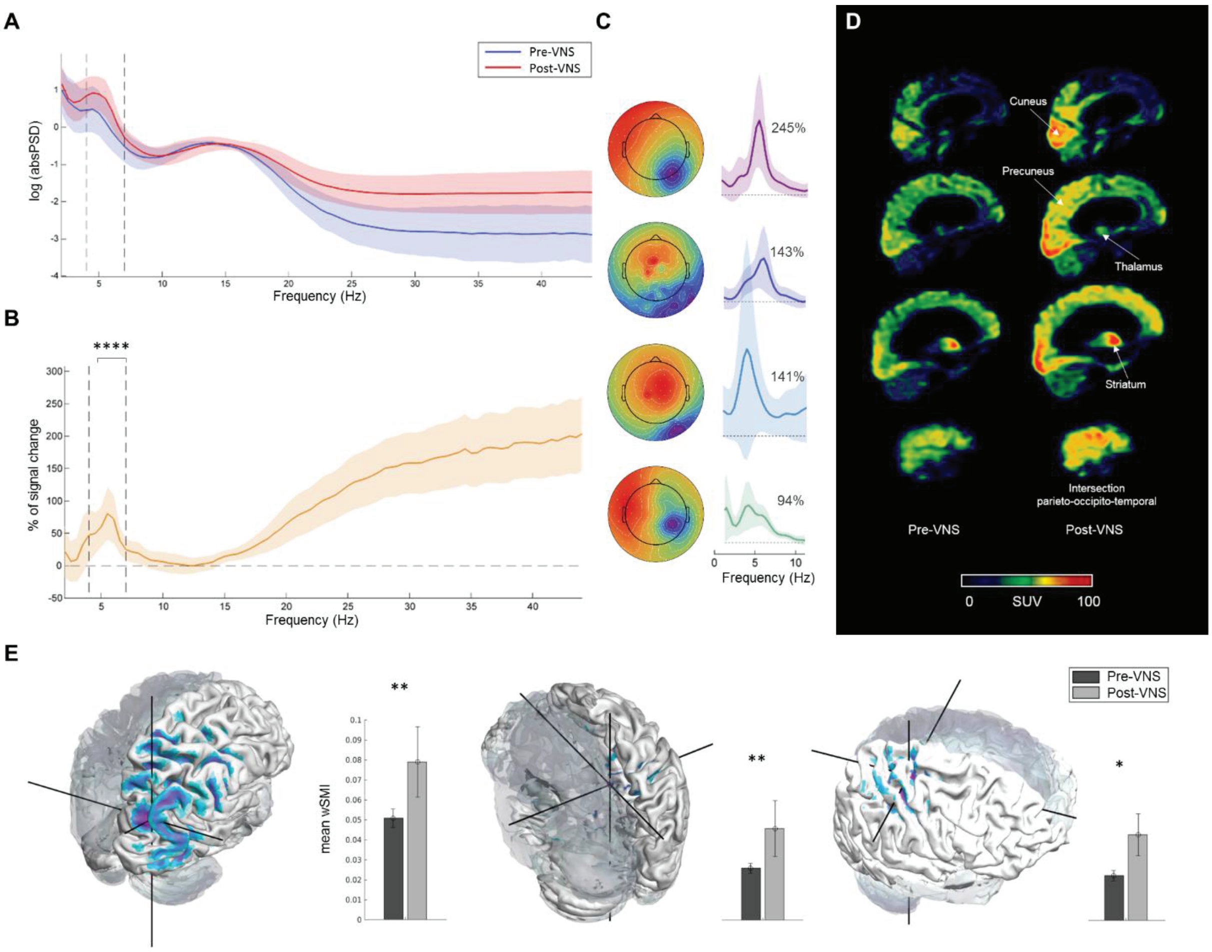

Figure S1. EEG signals and Pet-scan metabolic activity after VNS.

(**A**) Theta power increases after VNS over centroposterior regions. EEG power spectra at the scalp level pre-VNS (blue) and post-VNS (red). A significant absolute power increase was found in the theta (4-7Hz) frequency band. High beta (20-31Hz) and gamma (>32Hz) power increase was also observed, although their scalp distribution suggests the influence of muscle artifacts, reflecting the patient improved spontaneous facial motility post-VNS. **(B)** Percent of signal change in the EEG power spectrum. Signals post-VNS show a significant increase in theta band power (4–7Hz) (one-sample Student t-test, $p<0.0001$ corrected by false discovery rate). **(C)** Selection of four independent components (ICs) ranked by percent of signal change over theta peak (5.5Hz) as revealed by blind source separation analysis (one-sample Student t-test corrected by false discovery rate). From top to bottom: inferior parietal lobule, precuneus/posterior cingulate cortex, occipital cortex and pre-motor/motor area. **(D)** Fluorodeoxyglucose FDG-PET acquired during baseline (pre-VNS) and 3 months post-VNS. Metabolism increased in the right parieto-occipito-temporal cortex, frontal regions, thalamus and striatum. **(E)** Localization of VNS-reactive theta sources showing significant increase of information sharing post-VNS. Sources' localization is presented over the patient's cortical surface (probability map, sLoreta current source density: light blue scale) combined with his FDG-PET metabolism (gray scale) as measured three months post-VNS. VNS-reactive sources were localized in the inferior parietal lobule, in the precuneus/the posterior cingulate cortex and in the premotor/motor area (from left to right, respectively). Bar graph represent the mean wSMI shared with all other selected sources pre- (dark gray) and post- (light gray) VNS (permutation test over sessions: Wilcoxon test, $p<0.01$ Bonferroni corrected).

Table S1. CRS-R and wSMI scores as a function of time and VNS intensity. Summary of CRS-R clinical scores and the wSMI indexes computed over electrodes and VNS-reactive independent components, presented in temporal order. The dataset consists of three baselines recorded on the pre-VNS period (baseline 1, 2 and 3, light gray) and 13 sessions recorded on the post-VNS period. B=baseline, S=stimulation, wSMI=weighted symbolic mutual information, IC=independent components, red line=groups test/re-test sessions, d=day, w=week, m=month, - indicates missing CRS-R data because of clinician unavailability.

Timing	Recording	VNS Intensity (mA)	CRS-R score	wSMI	wSMI - IC
-2m	B 1	Off	6	0.0296	0.0362
-1m	B 2	Off	4	0.0299	0.0279
Time 0	B 3	Off	5	0.0330	0.0299
1d	S 1	0.25	7	0.0401	0.0437
1w	S 2	0.25	4	0.0367	0.0510
1w +1d	S 3	0.5	6	0.0312	0.0408
2w	S 4	0.5	6	0.0367	0.0521
2w + 1d	S 5	0.75	5	0.0287	0.0345
3w	S 6	0.75	7	0.0321	0.0408
3w + 1d	S 7	1	7	0.0475	0.0502
4w	S 8	1	10	0.0320	0.0464
4w + 1d	S 9	1.25	10	0.0253	0.0301
5w	S 10	1.25	9	0.0466	0.0563
5w + 1d	S 11	1.5	8	0.0502	0.0468
1m + 1w	S 12	1.5	-	0.0365	0.0461
1m + 2w	S 13	1.5	-	0.0383	0.0271
3m	S 14	1.5	6	0.0370	0.0399
3m + 2w	S 15	1.5	-	0.0396	0.0516
4m	S 16	1.5	-	0.0478	0.0462
6m	S 17	1.5	8	0.0479	0.0437

Supplemental Experimental Procedures

Participant and Protocol

Participant clinical history

A 35 years old male single patient lying in a vegetative state (VS) since 15 years was included in this study. The patient suffered from severe traumatic brain injury (TBI) following a road traffic accident. On admission to the hospital he presented diffuse brain swelling, subarachnoid and ventricular hemorrhage, bi-frontal and left temporal lobe contusions, basal skull fracture involving the right and left temporal bones and the left orbit. The initial intra-cranial pressure was elevated (40 mmHg). No signs of anoxia were reported. Four weeks' post-injury, the patient was diagnosed as in vegetative state (VS) by a multidisciplinary team since he fulfilled all the criteria according to international guidelines [S1].

A cerebral MRI (turbo spin echo T2-weighted images [TSE-T2], gradient echo T2 [T2*] images, fluid-attenuated inversion recovery T2 images [FLAIR] and 3D T1) performed in September 2015 showed traumatic lesions in fronto-basal and temporo-basal regions associated to an enlargement of the ventricular system predominantly on the left side and with brain-stem atrophy in the mesencephalon. The coma recovery scale revised (CRS-R) [S2] performed repeatedly during the months preceding inclusion was stable with a score ranging between 4 and 6 (07/09/2015: 6/23 [0-1-2-1-0-2]; 06/01/2016: 6/23 [0-1-2-1-0-2]; 27/02/2016: 4/23 [0-0-1-1-0-2]. Standard neurophysiological assessments including somesthetic, auditory and visual evoked potentials, mismatch negativity (MMN) and response to own name were tested regularly during the 15 years of vegetative state. Brainstem auditory responses were preserved on short auditory evoked potentials. Somatosensory evoked responses to median nerve stimulation (SEP) and middle latency auditory evoked responses (ML-AEP) showed a bilateral abolition of somatosensory and auditory cortical responses. Event-related potentials to auditory stimuli were also abolished: no N100-P200 complex, no mismatch negativity and no P300 response to own name were observed.

Experimental design

Following the clinical screening, the patient was formally included in the study to receive the Vagus Nerve Stimulation (VNS) treatment. Prior the experimental test, informed consent was obtained from the patient's legal representative (parents) and the whole protocol and procedure was approved by the Ethical Committee CPP Sud-Est IV and promoted by the Hospices Civils de Lyon. The study protocol was registered in Clinical Trials (ClinicalTrials.gov identifier: NCT02591069).

Prior the beginning of the study, physical and rehabilitation clinicians (JL and LT) evaluated whether the patient's clinical state met the inclusion and exclusion criteria. Inclusion criteria were: 1. age between 18 and 60 years-old; 2. vegetative or minimally conscious state following anoxia, traumatic or vascular brain injury confirmed by brain imagery and lasting for more than 6 months after the insult; 3. respiratory autonomy; medical stability. Exclusion criteria were: intubation state; tracheotomy, pregnancy; vagotomy; previous history of conscious disorder; contra-indications for VNS such as severe sleep apnea, psychosis, arrhythmia, a unique vagus nerve.

The neurosurgeons (MG, PB) implanted the vagus nerve stimulator device (VNS, Cyberonics, Houston, TX), coupled with a pulse generator programmable by telemetry, in the upper left side of the patient's chest. A double-coil electrode was wrapped around the vagus nerve at the neck level. The device was switched on one month after implantation at an initial current of 0,25mA with a pulse frequency of 30Hz and a pulse duration of 500ms. The protocol followed the standard stimulation cycles, used for epilepsy treatment, consisting in 30 seconds of stimulation interleaved by 5 minute of rest. During the first month and a half, the current amplitude was increased of 0,25mA every week until the maximal current of 1,5mA was reached. The stimulation parameters were kept unvaried throughout the following months.

Data acquisition

Behavioral assessment

The Coma Recovery Scale-Revised CRS-R [S2], was used to assess the clinical state of the patient along the whole protocol. During baseline and VNS sessions CRS-R evaluation was performed by two expert clinicians (JL, LT).

PET data acquisition

All PET data were acquired with Siemens Biograph mCT-64 PET/CT tomograph at CERMEP Neuroimagery Center (Centre d'étude et de recherche multimodale et pluridisciplinaire d'imagerie du vivant, Groupement Hospitalier Est, Lyon, FRANCE). The radioligand used was the [^{18}F]-Fluorodeoxyglucose (FDG). A bolus of

[^{18}F]-FDG was injected through an intravenous catheter 40 min before acquisition (injected dose, scan 1: 154 MBq; scan 2: 146 MBq). Measures for tissues and head support attenuation were performed with a 1 min low dose CT scan (<0.3 mSV) acquired before emission data acquisition. PET data were acquired in List-Mode during 20 min and attenuation corrected image was reconstructed using OSEM-3D iterative method (12 iterations, 21 subsets, 4mm Gaussian filter) incorporating PSF and time of flight [200 x 200 voxels in plane and 109 slices (2.03 x 2.03 x 2.03 mm)]. A first PET scan was performed during the first month post-implantation while the stimulator was off and a second at the end of 3 post-VNS. Each exam was monitored the clinician (JL) and by the clinical and pharmacological staff of the Cermep Neuroimaging center.

EEG data

EEG recordings were collected during 20 experimental sessions. Recordings consist of 1h of resting state acquisitions obtained while the patient was comfortably installed on his bed, with the head slightly raised ans resting on the pillow. EEG signals were recorded simultaneously with VNS and ECG signals. VNS signal was recorded at the neck in proximity of the vagus nerve stimulator. Three baseline sessions were recorded before the beginning of the VNS stimulation, one before and two after the surgery. Further, we recorded EEG data 1 day and 6 days after each current intensity augmentation, thus obtaining 12 sessions during 1 month. The other sessions were recorded at 2, 3, 4, 5 and 6 months after the beginning of the stimulation.

EEG preprocessing

Electroencephalographic (EEG) signals were recorded using the Brain Product™ actiCHamp system with 128 active electrodes (actiCAP 128Ch Standard-2) mounted in an elastic cap at 10-5 system standard locations [S3]. All electrodes impedances were kept below 50 kOhms. EEG data were recorded at a sampling rate of 5kHz with an online reference at the Fz electrode, then all data post-processing was done offline using the Matlab^{TM} software. First, the EEG signal was band pass filtered using a zero-phase Chebyshev type II filters (Low pass – cutting frequency: 46Hz, transition band width: 2 Hz, attenuation: 80 dB; order: 36, sections: 18 |High pass – cutting frequency: 0.5Hz, transition band width: 0.5 Hz, attenuation: 80 dB; order: 7, sections: 4) and re-referenced to common average. For every session, the first and last 20s of data were discarded in order to avoid any edge effect. Data recorded during the 30s of VNS actual stimulation were identified using the VNS signal recorded with a bipolar electrode place on the neck and the discarded. Data were divided in epochs of 2s, with 50% of overlap. Brain artifacts (eye blinks and movement, ballistocardiac noise, muscular artifact, etc.) were detected and rejected using an automated recursive procedure of epoch rejection: a covariance matrix was calculated for each epoch. Then, for each epoch, the Frobenius norm of the difference between the epoch covariance and the mean-covariance was calculated. Any epoch with a Frobenius norm superior to the mean norm + 3.5 times the norm standard deviation was discarded. Then, for each session, an independent component analysis (ICA)/blind source separation (BSS) algorithm was used to isolate and remove remaining interferential non-brain sources [S4].

Data driven sources estimation

We applied group independent component analysis (gICA) to obtain a direct estimation of the neural sources reproducible across all recording sessions. A procedure using the Second Order Blind Identification [S5] algorithm was privileged among other methods due to its robustness with respect to inter-session variability and its ability to separate sources with non-proportional power-spectra without deleterious prior dimension reduction [S6]. To this aim, 1001 lagged-covariance matrices with time delays from 0 to 1/5s were computed on each epoch to be then averaged across epochs and across sessions. The resulting diagonalization set was then joint-diagonalized using the UWEDGE algorithm [S7] leading to the identification of 128 independent components (ICs). Finally, the ICs were ordered by decreasing order of explained-variance and only the first 20 sources were evaluated as being reliable neural sources with a stable dipolar topography (a stable dipolar topography being mandatory for accurate source localization). The other ICs were discarded.

Model driven sources localization

A forward lead field model was generated using the symmetric boundary element method (symmetric BEM) with the OpenMEEG Software [S8] and the Brainstorm platform [S9]. The patient's anatomy, segmented from T1-weighted magnetic resonance images was considered. Then, only preserved cortical regions, identified from the measured [^{18}F]-FDG metabolism, were retained. Finally, for each source identified after Blind Source

Separation (BSS), the columns of the estimated mixing matrix were used as the inputs of the inverse problem solved by the Standardized Low Resolution Electromagnetic Tomography (sLORETA) method [S10].

Frequency Analysis

Scalp analysis

Absolute power spectra density (PSD) were computed using the Welch method (2s Hamming window, 50% overlap, FFT length 2^{14} samples). For each VNS ON session, the percent of absolute PSD change at each sensor were computed with respect to the mean PSD computed in the three baselines. Then the median percent of signal change was calculated across all electrodes. One-sample Student *t-test* corrected by false discovery rate [S11] was employed to identify frequencies showing significant changes during VNS.

Sources analysis

Absolute power spectra density (PSD) were computed at the source level using the Welch method (2s Hamming window, 50% overlap, FFT length 2^{14} samples). For each VNS ON session, the percent of absolute PSD change at each IC were computed with respect to the mean PSD computed in the three baselines. One-sample Student *t-test* corrected by false discovery rate over all 128 ICs was used to assess which sources showed a significant difference in absolute PSD compared to baseline in theta band. Among the twenty most reliable and reproducible preselected neural sources, eight have shown a significant power increase in the theta band after VNS. These eight sources have been then selected for further analyses.

Weighted Symbolic Mutual Information (wSMI)

Scalp data

We computed over preprocessed data a weighted symbolic mutual information index (wSMI), previously described by King at al. [S12] as a promising measure to discriminate between different states of consciousness. This index is derived from the permutation entropy analysis and is used to detect non-linear coupling between pair of electrodes. This method consists in reducing signals to a limited set of discrete symbols, determining the symbols' probability density and counting their mutual occurrence over two time-series. Symbols are defined by considering groups of sub-vectors, including a certain number of points (k = 3) sampled with a particular temporal interval, which determines the frequency band for which the measure become sensible. Every sub-vector corresponds to a particular symbol, assigned according to different reciprocal patterns that the three points can assume. For k = 3, 6 possible patterns exist. The parametrization τ = 32ms and k = 3, which lead to wSMI values particularly sensitive to patterns occurring in the θ band, was specifically selected following King et al. study [S12] showing that the combination of these values is the most sensitive to discriminate between different states of consciousness. To compute the wSMI, the EEG was first filtered with a low-pass Chebyshev type II filter with a cutting frequency at 10 Hz to avoid any aliasing during the symbolic transformation. Then, the scalp signal was spatially filtered using a Laplacian Transform [S13] in order to reduce redundancy in the data, as recommended in King et al. [S12] protocol. An estimate of the coupling between each pair of electrodes, after symbolic transformation can be obtained with the following formula:

$$wSMI(x,y) = \sum_{x} \sum_{y} w(x,y)\, p(x,y) \log_2 \frac{p(x,y)}{p(x)p(y)}$$

Where x and y are all symbols pairs found simultaneously in signals X and Y respectively; *p(x,y)* is the joint probability of symbol co-occurrence of x in X and y in Y; *p(x)* and *p(y)* are the marginal probabilities of those symbol in their time series. In order to avoid trivial conjunction of symbols due to common sources a weight *w* was applied. In this way, conjunctions due to volume conduction or polarity change were set to zero. To reduce the dimensionality and avoid to compare each electrode pair, statistic was performed over a comprehensive variable: the median wSMI that each EEG channel shares with all other channels. We wanted to test whether the wSMI pre-VNS was lower compared to the wSMI post-VNS. Since previous literature reported that median wSMI increases mostly over centroposterior region, we test the average median wSMI over electrodes within this region of interest. A cluster of electrodes was selected by taking all parietal locations in the patient preserved right hemisphere, then a non-parametric permutation test (10 000 permutations) was ran to assess the increment of median wSMI post VNS.

Sources data

wSMI was also computed on the sources space as an index of information sharing between the sources previously identified as the generators of the VNS induced θ power increase observed on the scalp. wSMI was computed between all sources pairs and the magnitude of information sharing for each IC was estimated as the mean wSMI that the IC shares with all other IC. Sources were tested using non-parametric permutation test (10 000 permutations) to assess the increase of mean wSMI post-VNS and then corrected by applying a Bonferroni correction.

Finally, in order to test if wSMI values correlate with the observed patient's behavioral improvement, a robust regression (*robustfit* function Matlab™ software, 'bisquare' weighting function) was computed between the CRS-R scores and the mean wSMI across sources.

Supplemental Results

On the day following the surgery of the vagus nerve stimulator implantation a clear wide opening of the eyes was observed for several hours. This sudden improvement could be related to an overstimulation of the nerve during surgery. This behavior was again observed when the stimulator was switched *on* one month after implantation. Each time stimulation intensity was increased, recurring episodes of cough were observed together with facial flushing and wide opening of the eyes. These manifestations lasted a couple of minutes. Globally, the patient was significantly more alert after the stimulation was turned *on*. Several behaviors expressing awareness have been detected by clinicians and family member starting from an intensity of 0.5 mA and which have been replicated when the intensity reached 1 mA. These included oriented eyes movements (mainly the right eye because of a ptosis of the left eye) on the speaker's direction, wide eyes opening when the experimenter approached the patient's face, eye movements pursuit following a mirror, spontaneous head movements towards the speaker while moving around the patient's bed, slow left-right head movements on verbal command. Furthermore, meaningful affective behaviors have been also elicited in response to emotional stimuli. For instance, a smile on the left cheek and tears were observed while listening to his preferred music. During the VNS period, an episode of bronchial stasis was observed that justified a short stay at the hospital. It should be noted however that the patient already had regular bronchial stasis before his inclusion in the protocol.

Supplemental Discussion

Experimental procedure: pitfalls and advantages

One of the limit of our experimental design is the lack of a placebo or an off condition. When we started the protocol we anticipated this difficulty but since this is a study involving one single patient we were forced to also consider other arguments that were against. First, it is unethical to switch a stimulator in a vegetative state patient that is getting clinically better. This is hard to accept by family members and even by clinicians. Second, as shown by our results the beneficial effect of VNS on patient's brain activity across sessions seems cumulative. This makes difficult to define a wash out period. To reduce criticisms on the lack of an off condition, during participants' selection phase, we deliberately choose to conduct this first experiment in a patient having received a pessimistic outcome on his chances to recover consciousness. Fifteen years of VS where no response occurred to any kind of stimulation or no improvements following clinical rehabilitation or pharmacological intervention we do believe constitute compelling arguments in favor of a causal effect of VNS as this is the only procedure that improved the patient's state. One may still argue that surgical intervention per se might have had a beneficial effect. As reported in the main text and in the methods (see above) there was a delay of one month between the surgical implantation and the time when the stimulator was switched on. During this off period no significant changes were observed. Yet, after stimulation, one month was a sufficient delay to induce changes and to observe patient's behavior recovering. In the light of these evidence we strongly believe that VNS is at the origin of patient's improvements. Following our single case study where we demonstrate the beneficial effects of VNS, larger populations of VS and MCS can be tested using for instance a multiple baseline across subject design where the issue of an off stimulation condition can be addressed with patients' stimulation scheduled at different time intervals. This type of study takes years to be completed. Therefore, a single case investigation remains an important step to demonstrate the feasibility and the benefits, along the possible side effects, of VNS.

Technical advantages of blind source separation (BSS)

Previous studies performed in patients with severe to mild disorders of consciousness have examined scalp activity in neural sources to search for neural markers of conscious states [S14]. Model driven source separation has been used to dissociate signals from closed locations *selected a priori*. Model driven source separation method present for different raisons several shortcomings [S15]. First, modelling damaged brains using a forward model is challenging and prone to errors. Second, the sensitivity to noise of model driven inverse solutions are often incompatible with noisy EEG recorded in clinical situations. Third, if some distributed inverse solution are good at localizing one active source [S10], they can poorly dissociate three simultaneously active sources [S16–S19].

Because we were conscious of these difficulties, we choose a rather different approach based on blind and data driven procedure. To dissociate the neural sources mixed in scalp measurements we used blind source separation (BSS / also known as ICA – independent component analysis). Because of the "blind" nature of the BSS approach, no knowledge of volume conduction (volume inhomogeneity and anisotropy) or assumptions about source waveform is needed [S20]. Further, BSS can recover systematically distributed neural sources from "resting" EEG [S21] and can recover multiple independent components that are spatially closed despite unavoidable inter-session variabilities [S6,S22]. Therefore, this procedure allows to identify for any type of patient the most reliable sources components that are contributing to the recorded scalp EEG, regardless of distortions found in the lesioned brain.

In the wSMI analysis the purpose of BSS procedure was to select the neural components reactive to VNS in order to reduce spurious connectivity due to interfering sources and obtain a more reliable index of patient's consciousness level. The original wSMI analysis of King et al [S12], reproduced in this paper, needed a spatial transformation (Spatial Derivative of scalp potentials) before the estimation of functional connectivity. The rationale for the method presented in the second part of the paper is that the use of a spatial transform derived from the patient's measurements using BSS will suppress most of the noisy components that can interfere with the wSMI estimates while preserving potentially critical tangential sources that could be suppressed by the current source density (CSD) transform [S20]. With the original spatial transform, a correlation between the measured functional connectivity and the observed variation of the clinical score (Robust regression: t (1.0716;1.9863), dfe = 14, p=0.0669) was already measured. But as predicted, with the BSS derived spatial transformation the observed linear relationship between wSMI and the CRS score was far better (Robust regression: t (-0.0128;3.9436), dfe = 14, p = 0.0015). Second concerning the estimation of the localization of the identified components, a forward head model was built, considering the patient's anatomy and metabolism. Then the localization of the cortical generators of each identified independent components were estimated using Standardized Low Resolution Electromagnetic Tomography. This strategy was privileged due to its good performances to localize point-test sources [S10,S22]. However, the accuracy of the localization estimates is indeed sensitive to errors in the head modeling of the patient despite the good quality of the spatial patterns estimated via BSS. That's why the estimated localizations of the components within the right occipito-parietal lobules are reported using an appropriate precision (most probable lobule/structure). Nevertheless, the results reported on the extracted signals of the independent component are not concerned by these issues.

Effect of VNS: cumulative against instantaneous?

An interesting issue is the important delay (one month) needed to observe stable improvements following VNS. Our results show that brain activity and regional connectivity progressively improve as VNS stimulation increases. This suggests a cumulative and time lag beneficial effect of VNS rather than being an instantaneous one. Several studies have applied other techniques as potential treatments for consciousness disorders. For instance, short duration transcranial direct current stimulation (tDCS) applied for 20 minutes on dorsolateral prefrontal cortex of coma patients has been shown to improve CRS-R scores, mainly in MCS patients [S23]. A similar observation has been made in a single patient using deep brain stimulation (DBS). It must be noted, however, that tDCS and DBS are techniques that directly act upon cortical and subcortical activity, respectively. In contrast, VNS is a peripheral stimulation although the vagus nerve has direct projections to brain stem regions and via the thalamus to the cortex. Hence, because of such differences, VNS effects might be indirect, therefore appearing late in time.

Pharmacological interventions have also been tested as treatment aiming at modulating key neurotransmitter systems mediating arousal and attention. The GABA agonist Zolpidem was shown to trigger an immediate, though temporary, transition from VS to MCS, inducing a re-emergence of command following, visual pursuit and gesture, albeit in some few responsive patients [S24]. VS and MCS patients, treated for 4 weeks with amantadine, which acts on monoamines such as noradrenaline and serotonin, showed a faster rate of behavioral improvement [S24].

In line with this, it was found that in rat models of coma, both acute and chronic VNS, stimulates the same neurotransmitters (serotonin and noradrenaline) as well as growth factors in the hippocampus and cerebral cortex, but only chronic stimulation modulates the firing rates of monoaminergic neurons [S25–S27]. In parallel, behavioral effects in this animal model were observed after eight days post-injury [S28]. In the present study, we have shown that VNS beneficial effects appear after one month of stimulation. This suggests that the improvements we found may be linked to the upregulation of serotonergic and noradrenergic neurons. Furthermore, this timing is also consistent with the one observed in epileptic patients. Indeed, it has been found that VNS reduces epileptic seizures after several weeks or months, probably reflecting an increase of cerebral blood flow (CBF) notably in the parietal cortex and thalamus [S29].

Author Contributions

AS proposed the concept study, the working hypotheses and supervised the experimental protocol; MC, GL and AS designed the EEG protocol and MC and GL performed the exams and the analyses. AL performed the Pet-scan exams and analyses. JL, LT, NAO performed the clinical exams and JL supervised the clinical protocol; MG and PB performed surgery and implanted the stimulator. MD and GD provided inputs during the study. AS and MC wrote the paper and GL the EEG method section. All authors discussed the data and provided feedbacks and suggestions.

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5.5 Unpublished figures

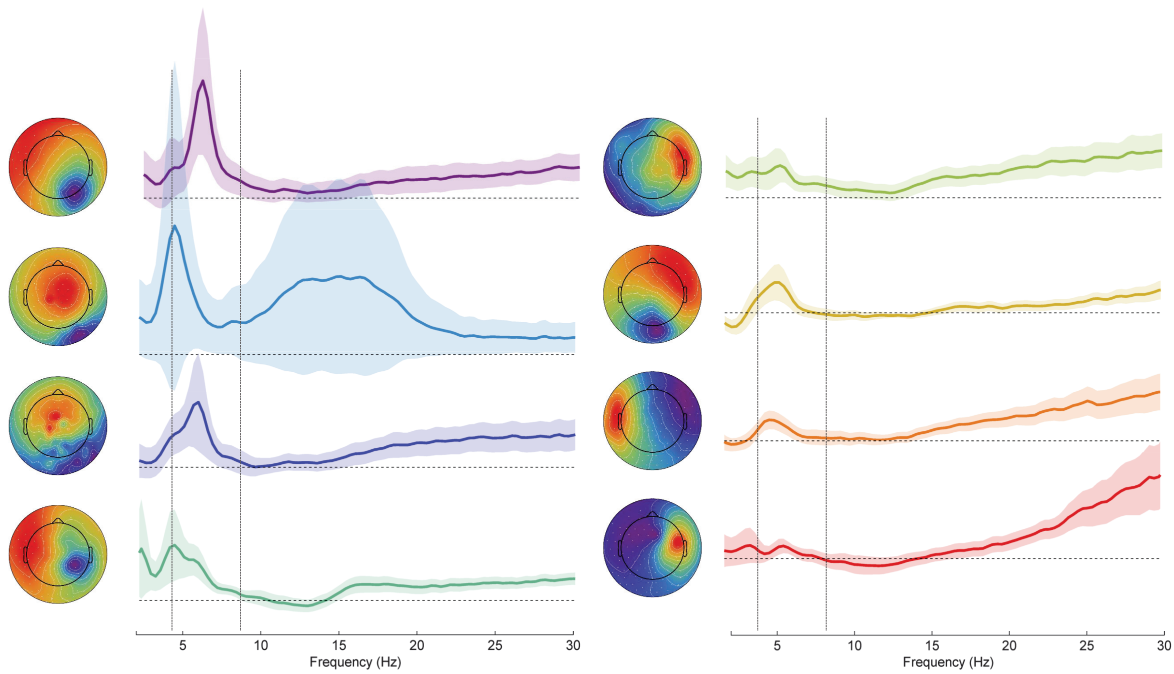

Figure 5.1: **VNS-recative theta sources.** Eight independent components (ICs) ranked by percent of signal change over theta peak (5.5Hz) as revealed by blind source separation analysis (one-sample *Student t-test* corrected by false discovery rate). From top to bottom: inferior parietal lobule, precuneus/posterior cingulate cortex, occipital cortex and pre-motor/motor area, extrastriate cortex, Brodmann area 18/19, occipito-parietal sulcus, basal ganglia/left insula, superior frontal cortex, Brodmann area 6/8.

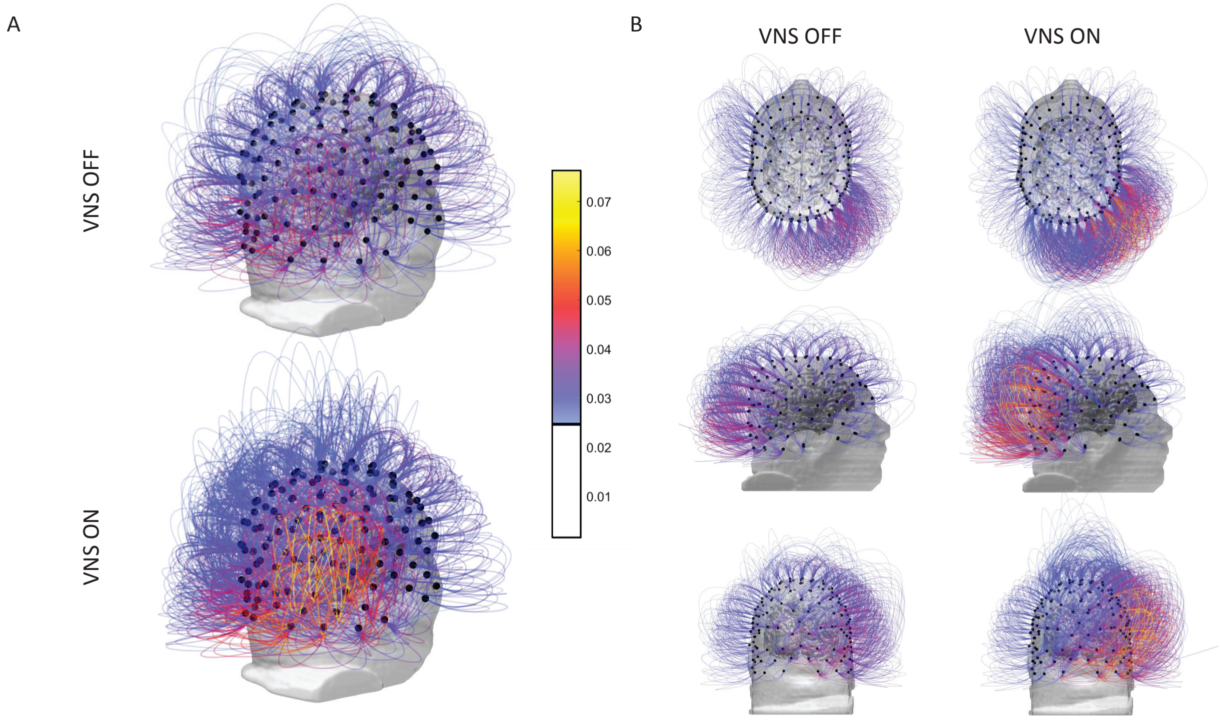

Figure 5.2: **weigthed Symbolic Mutual Information (wSMI) at the scalp level pre and post-VNS.**(A) Lateral view of the wSMI connections across all electrodes for pre-VNS (OFF) and post-VNS (ON).(B) Transversal, sagittal and coronal views of wSMI pre-VNS (OFF) and post-VNS (ON).

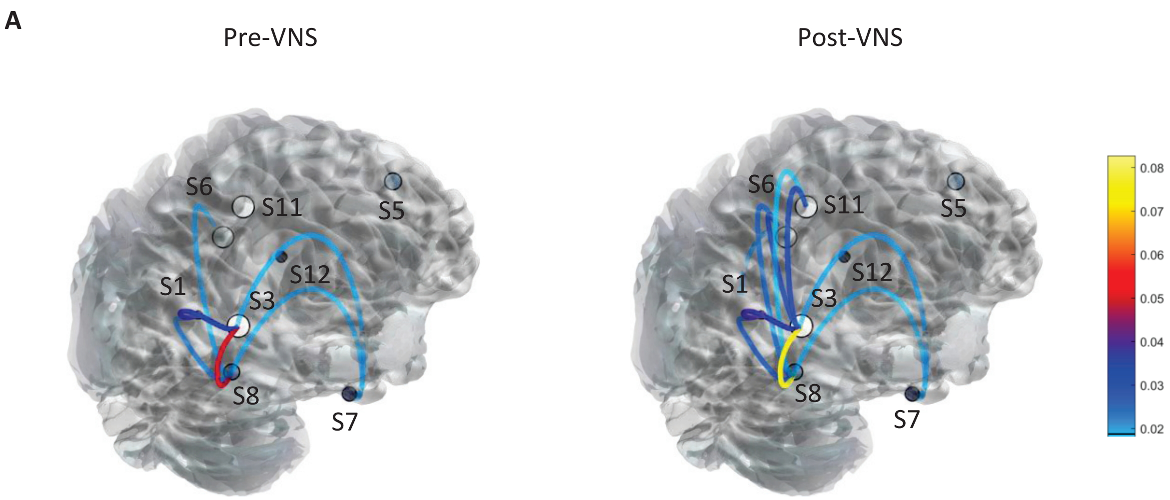

Figure 5.3: **weigthed Symbolic Mutual Information (wSMI) at the sources level pre and post-VNS.**

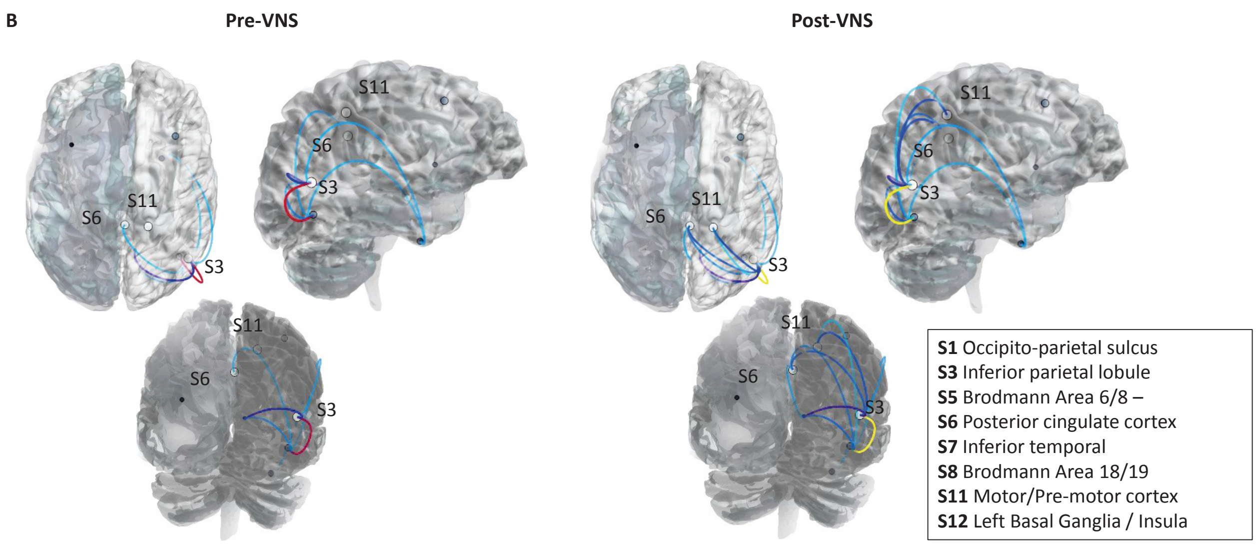

Figure 5.4: **weigthed Symbolic Mutual Information (wSMI) over VNS-reactive theta sources.** Functional connectivity measured as information sharing among previously selected VNS reactive sources over theta power. Panel (A) shows wSMI connectivity pre-VSN (left) and post-VNS (right). Panel (B) represents the same pattern, showing traversal, sagittal and coronal views respectively. Only the 3 sources which changed their mean wSMI across all other sources are labelled (S3,S6,S11).

Chapter 6

Discussion

Taken together, the studies presented in my PhD thesis show several important results. First, we suggested a neural substrate underlying motor awareness, highlighting the specific role of the parietal cortex, and in particular the precuneus, in bringing the sensorimotor conflict into awareness. Similar results have been found in previous works conducted on both patients reporting specific parietal lesions (?) and patients who were cortically stimulated (?). In our opinion, the precuneus activity can reflect the process of discrepancy during the comparison between initial intentions and actual feedbacks. Therefore, we think that this activity is linked to the process of emergence of a motor awareness following a mismatch detection. The fact that this activity does not directly encode visuomotor features is in line with the view that it reflects endogenous elaboration of information and conceptual rather than perceptual functions (?). The precuneus activation is known to be linked to the elaboration of conscious representation of information, mental images and spontaneous thoughts for constructing problem solving strategies and planning (?). In parallel, we found that this mechanism is still quite immature between 7 and 11 year old children. The shift of task-specific areas into different cortical regions was accompanied by a shift in the behavioural performances, showing that children used different strategies in constructing their motor awareness judgements. Here, we propose here that neuroimaging and behavioural results reflect a different balance in the mechanism of comparison between expected and actual sensory feedback. In particular, the lack of a stable forward model in children would force the balance of these two elements to be in favour of sensory information, which in turn would bias and guide the construction of motor awareness. Thus, the comparator process taking into account mainly visual cues, (?) will not assure optimal performance, in term of executive reproducibility and formation of subjective threshold of motor awareness.

In the second study, we characterized the connectivity patterns underlying the shift between the vegetative state and the minimally conscious state, induced by the vagus nerve stimulation (VNS). We found that information sharing, reflecting activity mainly in theta band, increased over parietal regions, where the precuneus/posterior cingulate cortex were central regions orchestrating the spread of activity over other cortical and subcortical areas. Our findings are in line with previous EEG studies, showing various information sharing patterns characterizing different states of consciousness (?). In fact, increased wSMI over centroposterior region was already described as a sign of augmented consciousness. Further evidence supporting the role of the precuneus as an active region in conscious processing includes functional imaging studies showing that the deactivation of this area and the posteromedial cortical area during slow-wave sleep, REM sleep, hypnotic state

Taken together, these two studies provide preliminary evidence that the highly cortical and subcortical interconnected area whose the precuneus belong may be a "hot zone" region for sustaining self-consciousneess and conscious experiences. In fact, it is at the interface between these two different (albeit intimately dependent) neural correlates of consciousness.

6.1 Limitations

The previous studies have of course some limitations. In my first study, I try to disentangle the encoding of visuomotor features determining objective performance from subjective motor awareness. Some criticism may arise, in fact causal decoding models are not always conclusive, since they identify only some features being only a potential causes of the response (?). Further, single subject analysis would have been more suitable in a paradigm including subjective perception thresholds. However, the large number of trials required to obtain the statistical robustness prevented us from proceeding in this way. We then compared adult and children populations, having a consistently different numbers of trials due to less reproducible behaviour in children.

Frequency analysis are usually performed in the literature of subjective awareness (**?**). Previous findings showed how transient activity is mostly related to objective performance and subjective awareness is instead characterized by distributed slow cortical potentials. However, we cannot conclude on this point.

In the second study, we conducted a single case experiment including a patient laying vegetative state for 15 years. Main concerns can arise by considering that the change could be triggered by the operation and intensive care. In order to avoid these critics a crossover paradigm with On and Off alternated and blind period would have been suitable. However, as already discussed in the supplemental part of the paper, it appears unethical to switch off a stimulator in a vegetative state patient that is getting clinically better. Following this single case study, helpful in demonstrating the beneficial effects of VNS, a larger populations of VS and MCS can be tested using for instance a multiple baseline across subjects' design. However, this type of study takes years to be completed. Therefore, a single case investigation is an important step to demonstrate the feasibility and the benefits along the possible side effects of VNS.

Further, investigation techniques were also questionable, in the sense that CRS-R scores are not optimal to classify patients who lie to the limit of categorical classification, such as MCS+ and MCS-. For this reason, it would have been interesting to include our patients data (EEG recordings and CRS-R scores) in a larger database to compare his initial metrics with a larger population (**?**).

Although alpha rhythm has been described in some studies as a predictive signal of consciousness, it is also true that theta band outperform alpha band when considering wSMI analysis, as found by King et al. (**???**). Another important reason of why we predominantly found theta has probably a relation with VNS action. The vagus nerve regulates the parasympathetic system and theta has been reported as a signal linked to the processing of primary emotional body responses such as fear and fear learning (?), physical pain and pain perception or even empathy (?). We can then speculate that in our patient VNS has promoted the return to consciousness primarily via body changes within the autonomic system. Theta signals may then represent a first step of brain responses meaning attention to the body before gaining full attention to the external world.

6.2 Conclusion and perspectives

There are two levels of implication reflecting the two main axis developed in this PhD thesis. From a fundamental point of view, we tried to characterize the shift between two conscious pathological states, VS to MCS, using recently validated tools in the research domain. For the first time, we reported the potential of a new neuromodulatory approach in the field of disorder of consciousness, showing that significant signs of consciousness can be triggered by the reactivation of the vagal pathway. However, this point must be discussed carefully and validated over a larger cohort of TBI patients. Although we reported important behavioral and physiological changes, it is not clear weather all patients can benefit from this treatment or how they should be selected following clinical criteria and consequently in which extent and proportion this treatment can improve their conditions.

A second level involves the conscious access to motor awareness. Previous study hypothesized that this function was subserved by a frontoparietal connectivity network, reflecting respectively the executive and polymodal higher-order perceptual structures (?). However, our findings clearly point at parietal regions as showing a specific role in bringing the sensorimotor conflict into awareness. These findings are important to elucidate brain mechanisms underlying conscious processing. The way our brain integrates and filters motor relevant information can tell us how the brain build awareness. Therefore, this simple but powerful task can help to pinpoint higher-order motor mechanisms, and gives a key of investigation to shed light on developmental questions and possibly on core difficulties in developmental disorder of coordination and other related pathology.

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