Probing and modulating brain circuits by deep brain stimulation:

Functional connectivity between the sensory nuclei of thalamus and the periaqueductal grey in pain modulation;

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford

Trinity Term 2015

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Abstract

Backgrounds. The periaqueductal grey(PAG) plays an important role in the descending pain modulation systems, which has been known to inhibit incoming nociceptive signals at the level of the spinal dorsal horn. But pain mechanism involves the brain and the spinal cord to maintain homeostasis, so theoretically pain modulation mechanism should involve both direction: ascending and descending pathways to the brain and spinal cord dorsal horn. However, literature addressing this question is limited. The sensory thalamus is the most important brain area receiving sensory signals. Moreover, with the fact that evidence indicates the PAG and the thalamus have functional connections, therefore we wanted to test if there is a functional connection between the PAG and the sensory thalamus related to pain modulation.

Aims. To investigate the functional connectivity between the periaqueductal grey and the sensory nuclei of the thalamus in pain modulation

Methods. Three experiments have been conducted. The first experiment is to investigate the effect of PAG DBS on the sensory thalamus, and vice versa, in chronic neuropathic pain patients. The second experiment is to estimate the change of spectral coherence between the sensory thalamus and the PAG during various pain states, when chronic neuropathic pain patients were being peripheral stimulated by ice to evoke pain. The third experiment is to test whether the functional connectivity between the two areas would be affected by general anaesthetics propofol.

Results. PAG DBS inhibited the sensory thalamus with decreasing thalamic delta, theta, alpha and beta power, and sensory thalamus DBS excited the PAG with increasing PAG delta and theta power. The PAG and the sensory thalamus interact reciprocally at short latency, which may be related to pain modulation. The sensory thalamus and the periaqueductal grey also have high gamma coherence which would be inhibited by pain. This high-gamma coherence was increased during propofol induction period which is compatible with the hypothesis that propofol induces loss of consciousness via its GABAergic activity.

Conclusions. This thesis suggests that the sensory thalamus and the periaqueductal grey have a reciprocal connection. Functionally this connectivity might be related to pain modulation. Also this functional connectivity might represent the fact that the transmission and processing of pain signals is always monitored and modified by central pain modulation systems so that organisms can respond properly to the incoming signals.

Acknowledgements

I wish to thank Professor Tipu Aziz and Mr Alexander Green for providing me with an exciting opportunity to explore the neuroscience underlying deep brain stimulation for neuropathic pain. They are very helpful and supportive.

I thank Professor John Stein for his patient help to revise my research paper, and his very helpful constructive criticism during my transfer viva and final viva.

I thank Drs Shouyan Wang and Alek Pogosyan for devising software and teaching me how to analyse local field potentials.

I thank Professor Peter Brown for his suggestion and guidance during my DPhil transfer viva and his help for my paper revision.

I thank Professor Dipankar Nandi for his teaching, corrections and suggestions for my thesis.

I thank the patients who participated in the studies. I hope this work can leads to clinical improvement for them and for everyone.

I thank my friends and colleagues, Drs Ben Seymour, Holly Roy, Nor Faizal Ahmad Bahuri, Huiling Tan, Sandra Boccard and Alexander Weiss, for helping me a lot through the DPhil process.

I thank my parents and my brother for their love and support. I also want to thank my wife Cher and my children Candace and James. They perfect my life.

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Abbreviations

ACC: anterior cingular cortex

BOLD: blood-oxygenation-level-dependent

CNS: central nervous system

CT: x-ray computed tomography

DBS: deep brain stimulation

dlPAG: dorsolateral PAG

DLPT: dorsolateral pontine tegmentum

DTI: diffusion tensor imaging

DTI: diffusion tensor imaging

EEG: electroencephalogram

EQ-5D: Euroqol five-dimensional assessment tool

FFT: fast Fourier transforms

fMRI: functional MRI

FMS: fibromyalgia syndrome

IBS: irritable bowel syndrome

IC: internal capsule

IPG: implantable pulse generator

LFPs: local field potentials

lPAG: lateral PAG

MCP: midcommissural point

MD: medial dorsal thalamic nucleus

MOR: μ-opioid receptor

MPQ: McGill pain questionnaire

MRI: magnetic resonance imaging

MSC: magnitude squared coherence

MUA: multi-unit activity

NCCP: non-cardiac chest pain

NRM: nucleus raphe magnus

NTS: nucleus tractus solitarius

PAG: periaqueductal grey

PD: parabrachial area

PET: positron emission tomography

PFC: prefrontal cortex

PVG: periventricular grey

rCBF: reginal cerebral blood flow

RVM: rostral ventromedial medulla

SF-36: short form 36

SI: primary somatosensory cortex

SII: secondary somatosensory cortex

SPECT: single-photon emission computed tomography

SRD: subnucleus reticularis dorsalis

STFT: short-time fast Fourier transforms

SUA: single unit activity

VAS: visual analogue scale

vlPAG: ventrolateral PAG

Vmpo: posterior part of the ventromedial nucleus of thalamus

VPL: ventral posterior lateralis

VPM: ventral posterior medialis

Related publications, presentations and awards

PUBLICATIONS

Wu Dali, Wang S, Stein J. F., et al 2014 Reciprocal interaction between the human thalamus and periaqueductal grey may be important for pain perception. *Experimental Brain Research*, 232: 527-534

Wu Dali, Bahuri N.F.A., Wang S, et al 2015 Increased alpha synchronisation is prevalent in various subcortical brain regions under propofol-induced unconsciousness. (preparation)

CONFERENCE PAPERS

Wu Dali. Stimulation of the Human Periaqueductal Gray Modulates Activity in the Sensory Thalamus and Vice Versa. Annual Meeting of the Congress of Neurological Surgeons, Chicago, USA, Oct 2012.

Wu Dali. General anaesthetic propofol induces alpha synchronisation in various human cortical and subcortical regions. 30th International Congress of Clinical Neurophysiology, Berlin, Germany, Mar 2014.

AWARDS

The Stereotactic and Functional Neurosurgery Resident Award, Annual Meeting of the Congress of Neurological Surgeons, Chicago, USA, Oct 2012

Chapter 1: Introduction- How do we perceive pain

1.1 The puzzle of pain-the variable link between pain and injury

Because the link between injury and pain seems to be obvious, it is generally believed that pain is permanently the outcome of physical injury and that the pain strength we sense is related to the severity of injury. Generally speaking, this association between injury and pain remains valid; however, there are many occurrences in which the relationship does not hold up. For instance, some people are born without the ability to feel pain even when they are seriously injured, and many of us have injuries such as cuts and bruises without feeling any pain until many minutes or hours later. In contrast, there are severe pains that are not associated with any known tissue damage or that persist for years after an injury has apparently healed. Clearly, the connection between pain and injury is extremely variable: pain may happen without injury, and injury without pain.

People who are born unable to feel pain (congenital analgesia) offer convincing evidence on the value of pain (Sternbach 1968). Sternbach reported that many of these people bore extensive bruises, burns, and lacerations when they were children, regularly bit deep into the tongue while eating food, and struggled to learn how to avoid causing severe wounds on themselves. The inability to feel pain after an appendix rupture, which is usually go with severe abdominal pain, led to near-death in one case. Another person walked on a leg with a fractured bone until it broke completely. Most people who are insensitive to pain learn, with difficulty, to avoid damaging themselves severely. However, they survive because they have language to symbolise potential danger and to communicate. Animals, who have no such verbal communication, would have died. It is very clear that pain plays an important role in survival.

During the Second World War, Beecher (Beecher 1959) observed the behaviour of soldiers that were wounded severely and found that most of them denied having pain. Melzack and colleagues (Melzack et al. 1982) also found that 37% of the people who arrived at the accident and emergency unit with various injuries reported that they did not feel any pain until many minutes or hours after the injury. These situational analgesia highlights the variable link between injury and pain.

1.2 The psychology of pain

Psychological studies have revealed that pain is not just a function of the quantity of bodily injury alone. Rather, the quantity and quality of pain we perceive are also determined by our earlier experiences and how well we recall them, by our capability to appreciate the source of the pain and to grasp its consequences. Even the culture in which we have been brought up plays an essential role in how we feel and respond to pain. Whether we perceive pain or not, then, cannot be defined simply in terms of particularly types of stimuli. Rather, it is an extremely personal experience, which depends on cultural background, factors that are unique to each individual, and the meaning of the situation.

Many factors can influence quality and intensity of humans’ pain perception, such as their unique past history, the meaning they give to the pain-producing situation and their state of mind at the moment. As the result, it is not stimulation of nociceptive receptors that makes the start of the pain process. Noxious stimuli produce signals entering a dynamic nervous system that is already influenced by experience, culture, anticipation, anxiety and so forth. These brain processes actively select, abstract and synthesize information from the total sensory input.

1.3 The physiology of pain: organisation of central pain pathways

Just as aforementioned, pain is vital for our existence, and the connection of pain to noxious stimuli is multifaceted (Wall 1979).

Noxious stimulation induces a series of physiological changes in the organism. When harmful stimuli are applied, in order to minimise the risk of injury, the stimuli will excite a range of affective responses and motivational changes. Animals that are suffering from noxious stimuli will also have behavioural responses to pain, usually starting with a rapid withdrawal from the site of stimulation. In addition, other responses to pain may also occur, including motor behaviour, such as fight or flight, and autonomic activity responses such as changes in respiration, heart rate and blood pressure, and muscle tone. The purpose of those wide ranges of modulations is to prepare the body for emergency and escape. Moreover, the nature, location, and intensity of the pain will also be registered and the environmental context in which the pain occurred will be stored for future reference.

If noxious stimulation causes injuries, the pain sensitivity around the wounds will increase. There are several changes peripherally and centrally to cause the phenomenon, including local changes in the small diameter nociceptive sensory neurons and changes in the spinal cord and brain's sensory processing. Besides, the increase of pain sensitivity will also induce a motivational change to encourage rest to enhance wound healing. Descending pain pathways from the brain, at all times, influence and control pain processing at spinal levels, which registers both the environmental and bodily status (Bester et al. 2000; Bester et al. 2001; A. D. Craig 2002; Hunt and Mantyh 2001), which maintains a balance between pain sensitivity and survival after injury. However, in some cases chronic pain would develop. When the pain remains after recovery from injury, or results from direct injury to the nervous system, the normal controls from the brain to modulate pain sensitivity will be undermined, whose mechanism is still not fully understood. This multifaceted response to harmful stimuli over time suggests that nociceptive information is processed at different levels of the brain and spinal cord to deliver an integrated response.

It has been argued that pain has affective and discriminative components. Moreover, at least in humans, these two components have been shown that they can be dissociable by hypnosis or following cortical lesions such as anterior cingulotomy (Hofbauer et al. 2001; Price 2000; Rainville et al. 1999; Rainville 2002). The discriminative dimension describes the location and defines the feeling of the pain-stabbing, burning, and so on. The affective dimension, on the other hand, is responsible for an 'unpleasantness' value to the pain. It is thought that the primary sensory cortex is to analyse, at least, the first levels of sensory discrimination — that is, the 'what' and 'where' of the painful stimulation. The limbic system, including the limbic cortex and subcortical structures such as the amygdala, has something to do with the affective component of pain, which is attributed to the incoming nociceptive information based on the past history of the animal and the current behavioural context. Affective component of pain causes behavioural reactions that include a cohesive autonomic response through activation of brainstem and spinal centres that control sympathetic and parasympathetic outflow to the body.

Memory also plays an important role in the mechanism of pain perception. Many chronic pain states carry with them a memory of past peripheral injury, for example, with painful events that occurred before amputation of a limb (Katz and Melzack 1990). Pain memories can also be recovered in the absence of on-going pain by stimulation of particular areas of the thalamus, and retain both a discriminative and affective dimension (Lenz et al. 1995). Pain memories thus may be triggered by peripheral stimulation but do not seem to rely purely on activity within ascending pathways from the spinal cord and may require cortical involvement.

Nociceptive information leaves the spinal cord by a number of distinct pathways and terminates within other areas of the spinal cord and discrete areas of the brainstem and the thalamus, and nociceptive information must gain access to the areas of the spinal cord and brain that are concerned with patterning the fully elaborated and integrated pain response.

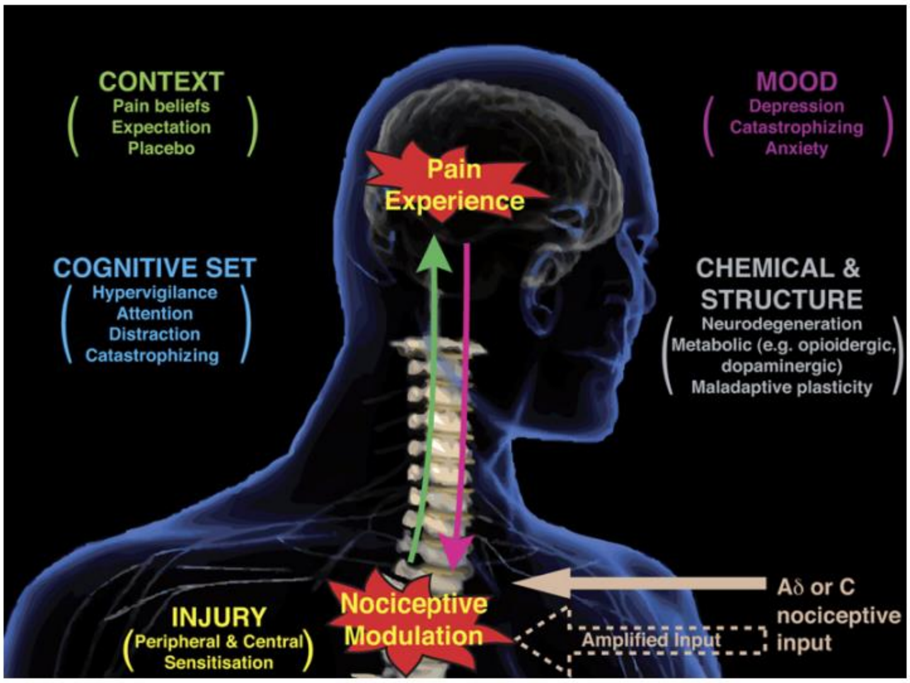


Fig 1.1 The connection of nociceptive input to pain perception is multifaceted.

1.4 The ascending pain pathways

1.4.1 Primary afferent termination within the spinal cord

Somatosensory information reaches the spinal cord and areas of the brainstem through primary afferent sensory fibres (Hunt and Rossi 1985; Hunt and Mantyh 2001; Nagy and Hunt 1983; Snider and McMahon 1998). Throughout the dorsal horn, particularly laminae I–II and V–VII, nociceptive information is relayed to spinal cord neurons. Neurons within laminae I and II and V and VI receive nociceptive information through unmyelinated C fibres and finely myelinated Aδ sensory afferents. C fibres form the major part of the sensory input to the cord. Nociceptive information from the skin is distributed between laminae I, II, and V, while visceral input terminates largely within laminae I and V, avoiding lamina II (Cervero 1994; Cervero and Laird 1999; Hunt and Rossi 1985; Lawson 2002; Sharkey et al. 1987; Snider and McMahon 1998). However, it should be noted that both lamina I neurons and lamina V neurons — the origins of two of the major ascending pathways — potentially receive all types of nociceptive input.

1.4.2 The projection from the superficial dorsal horn

The lamina I pathway has been repeatedly described in numerous studies and in a variety of animals including humans, where it is thought to have reached the highest degree of differentiation. Several sites of termination of the pathway have been described which hint at the function of this pathway in both homeostatic regulation and in supplying information to areas of the brain concerned with discrimination, affect and autonomic regulation.

It has been argued, especially by Craig and his colleagues (A. D. Craig 2002), that the lamina I pathway plays an important role in homeostasis. They also argued that the lamina I pathway is an ‘afferent sympathetic’ pathway, which directs both interoceptive and extereoceptive information to areas of the CNS concerned with autonomic regulation and affective expression. Other evidence also supports the view that the lamina I pathway is crucial for the regulation of spinal cord excitability, and therefore pain behaviour, through the activation of descending inhibitory and excitatory pathways from the brainstem (Bester et al. 2001; Suzuki et al. 2002).

The nociceptive projection pathway originating from the superficial dorsal horn (the ‘lamina I pathway’) arises from neurons mainly in lamina I (Bester et al. 1995; Bester et al. 2000; Wall 1979) but with a small contribution from laminae III and IV neurons (Ding et al. 1995; Naim et al. 1997; Todd et al. 2000; Todd 2002). Via the anterior white commissure, the axons of the tract cells cross over to the other side of the spinal cord, and to the spinal cord anterolateral corner. Decussation typically happens 1-2 spinal nerve segments above the entry point.

1.4.3 Spinal cord and medulla

Within the spinal cord, it is demonstrated that, in thoracic cord segments, ascending fibres terminate around the sympathetic preganglionic motor neurons of the intermediolateral column (A. D. Craig 1996, 2000, 2002). After that, they continue on to terminate in association with discrete areas of the caudal brainstem. It is found that heavy termination is in areas related with cardiovascular and visceral regulation, particularly the nucleus tractus solitarius (NTS) (Gamboa-Esteves et al. 2001) and ventro lateral medulla (Bourgeais et al. 2001; Lima et al. 2002). Painful stimulation produces responses in the respiratory and cardiovascular systems and afferents from both of these systems terminate within the NTS (Janig and Habler 2000).

Other areas of termination include the dorsal reticular nucleus, also known as the subnucleus reticularis dorsalis (SRD), and possibly the lateral reticular nucleus and adjacent reticular formation which has motor functions related to the cerebellum. The SRD receives bilateral input from deep and superficial layers of the spinal cord which is largely nociceptive and forms part of a ‘pronociceptive’ pathway that both projects to the medial thalamus and projects back upon the spinal cord modulating nociceptive transmission (Lima and Almeida 2002; Monconduit et al. 2002). This is one of many brain areas that both receive nociceptive input from the spinal cord and project back upon the dorsal horn to regulate the flow of pain-related information.

Such neural loops connecting the brain and spinal cord appear to be crucial to the ways in which pain sensitivity is regulated in the behaving animal. SRD neurons have extremely large receptive fields, in some cases covering the whole body. The dorsal part of the nucleus receives largely ipsilateral nociceptive input from laminae I and X, and destruction of the nucleus depresses nociceptive responses to acute and inflammatory nociception.

1.4.4 Pons and mesencephalon

Further rostrally, axons from the lamina I pathway terminate heavily within the contralateral parabrachial area (PB) (Bernard et al. 1995; Feil and Herbert 1995) and PAG (Villanueva and Bernard 1999). The PB area probably receives the densest set of terminal projections originating in spinal lamina I. PB is an area that integrates information from the NTS and visceral and somatic information from the body through the spinal cord. Neurons respond almost exclusively to nociceptive stimulation and their receptive fields can be extremely large, including the whole of the body area (Bernard and Besson 1990; Bernard et al. 1994; Bester et al. 1995; Matsumoto et al. 1996) as well as responding to visceral stimulation, such as following colorectal stimulation (Bernard et al. 1992). PB neurons are therefore exquisitely sensitive to intensity of the noxious stimulus rather than location or nature of the stimulus.

Limbic system, such as the ventromedial hypothalamus (Bester et al. 1997) and the central nucleus of the amygdala (Bernard et al. 1993), receives heavy projections from PB, which provides the nociceptive input to areas of the brain classically associated with affect (Bernard and Besson 1990; Bester et al. 1995; M. Davis and

Shi 1999; Shi and Davis 1999). The ventromedial hypothalamus has been associated with rage and aggression and projects upon the PAG (Keay and Bandler 2002). The amygdala regulates autonomic function and is necessary for fear conditioning (Adamec and Starkadamec 1983; Roeling et al. 1994). Together these areas may be important in regulating pain-related emotional responses, as well as in modulating longer-term functions such as motivational and metabolic states that follow injury. Importantly, these structures give rise to powerful descending projections that modulate nociceptive processing at the level of the spinal cord.

The primary brainstem route for modulating and coordinating pain behaviours is through the PAG of the midbrain (Basbaum and Fields 1984; Urban and Gebhart 1999). As would be expected, apart from receiving direct nociceptive inputs from lamina I neurons and neurons of the lateral spinal nucleus, the PAG also receives substantial inputs from PB and the ventromedial hypothalamus.

1.4.5 Diencephalon

It remains controversial about the organization of nociceptive inputs to the thalamus and to the cerebral cortex (A. D. Craig and Blomqvist 2002; Gauriau and Bernard 2004a, 2004b; E. G. Jones 2002; Treede 2002; W. D. Willis et al. 2002). Classically, it had been maintained that some specific parts of the medial and lateral thalamus receive nociceptive signals. The medial pathway, projected from deeper spinal laminae, terminated within nuclei of the intralaminar and medial thalamic nuclei that projected upon areas of limbic cortex, which is linked to affect and motivation. On the other hand, the lateral thalamic nuclei, received nociceptive information from the superficial layers of the spinal dorsal horn, projected to primary somatosensory cortex, which is related to discriminative characteristics of pain processing (Melzack and Casey 1968). However, recent data has blurred this simple mediolateral distinction. Anterograde tracing studies following small injections of tracer into lamina I of the spinal cord indicated a projection from lamina I and from the lateral spinal nucleus to the adjacent medial dorsal thalamic nucleus (MD) that projects upon the orbital and medial parts of the frontal cortex and to the cingulate cortex. That is, it is now argued that there is a projection from lamina I to both medial and lateral thalamic territories, and this lamina I pathway has access to both somatosensory cortex and limbic cortex (A. D. Craig 2002; Gauriau and Bernard 2002, 2004a, 2004b; W. D. Willis, Jr. and Westlund 2001).

In primates, it has been argued that Vmpo (A. D. Craig et al. 1994; A. D. Craig et al. 2000), a posterior part of the ventromedial nucleus of thalamus, is to receive input only from lamina I nociceptive and temperature-sensitive neurons and to project to insula cortex (A. D. Craig 2002; A. D. Craig and Blomqvist 2002). It has also been claimed that the projection area of Vmpo within the insula cortex represents a primary sensory representation for pain, temperature, itch, and other feelings from the body. Moreover, in humans, when Vmpo is stimulated, it can evoke pain and temperature sensation, and when Vmpo or its cortical field is lesioned, these sensations can be reduced specifically (A. D. Craig 2002).

1.4.6 Deep projections V–VII

Deeper-lying neurons, particularly lamina V and the adjacent laminae IV and VI–VII neurons of the spinal cord, also receive substantial nociceptive input and give rise to a major ascending pathway to the brain. Compared to lamina I neurons however, they generally have a wide dynamic range of response and are rarely nociceptive specific, have larger receptive field sizes, and respond to a variety of noxious and nonnoxious stimuli, as well as showing viscero-somatic convergence (Besson and Chaouch 1987; Hoffman et al. 1981; Le Bars et al. 1986; Maixner et al. 1989; Treede 2002; W. D. Willis and Coggeshall 1991). This has led to the suggestion that they are well fitted to providing information about the intensity but not the location of the stimulus.

1.4.7 Summary

The detailed anatomy of the ascending pain pathways is still not fully understood, but many studies have identified projections that terminate in a large number of different areas of the brain associated with autonomic, motor, discriminative, affective, cognitive, and motivational aspects of pain behaviour.

Deep and superficial pathways from the dorsal horn terminate in discrete areas of the brainstem. The superficial lamina I pathway, which reaches its highest level of differentiation in primates, seems to have a particular role in delivering nociceptive information to areas of the brain concerned with discrimination, affect, cognition, and motivation and from which extensive descending pathways emerge to modulate activity in many other areas of the brain and spinal cord. Deeper lying dorsal horn neurons can support nociceptive behaviour but the resetting of nociceptive sensitivity following skin or peripheral nerve damage critically requires the lamina I pathway and the engagement of descending pathways that regulate spinal sensitivity. How pain is perceived and located is still largely unknown but is assumed to require cortical processing of nociceptive information that could be provided through deep and superficial spinal pathways and from polysynaptic pathways within the brainstem.

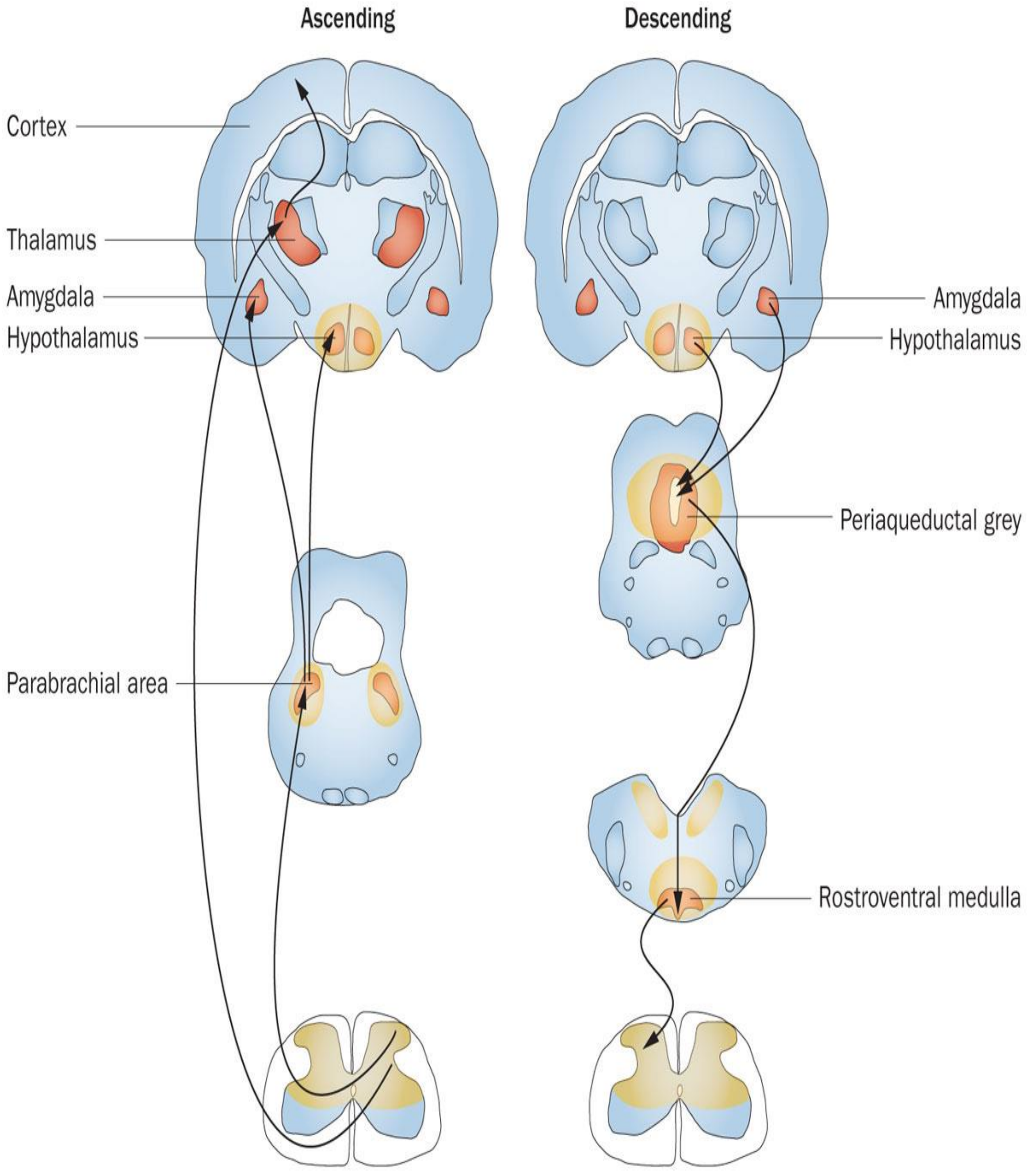


Fig 1.2 Ascending and descending pain pathways

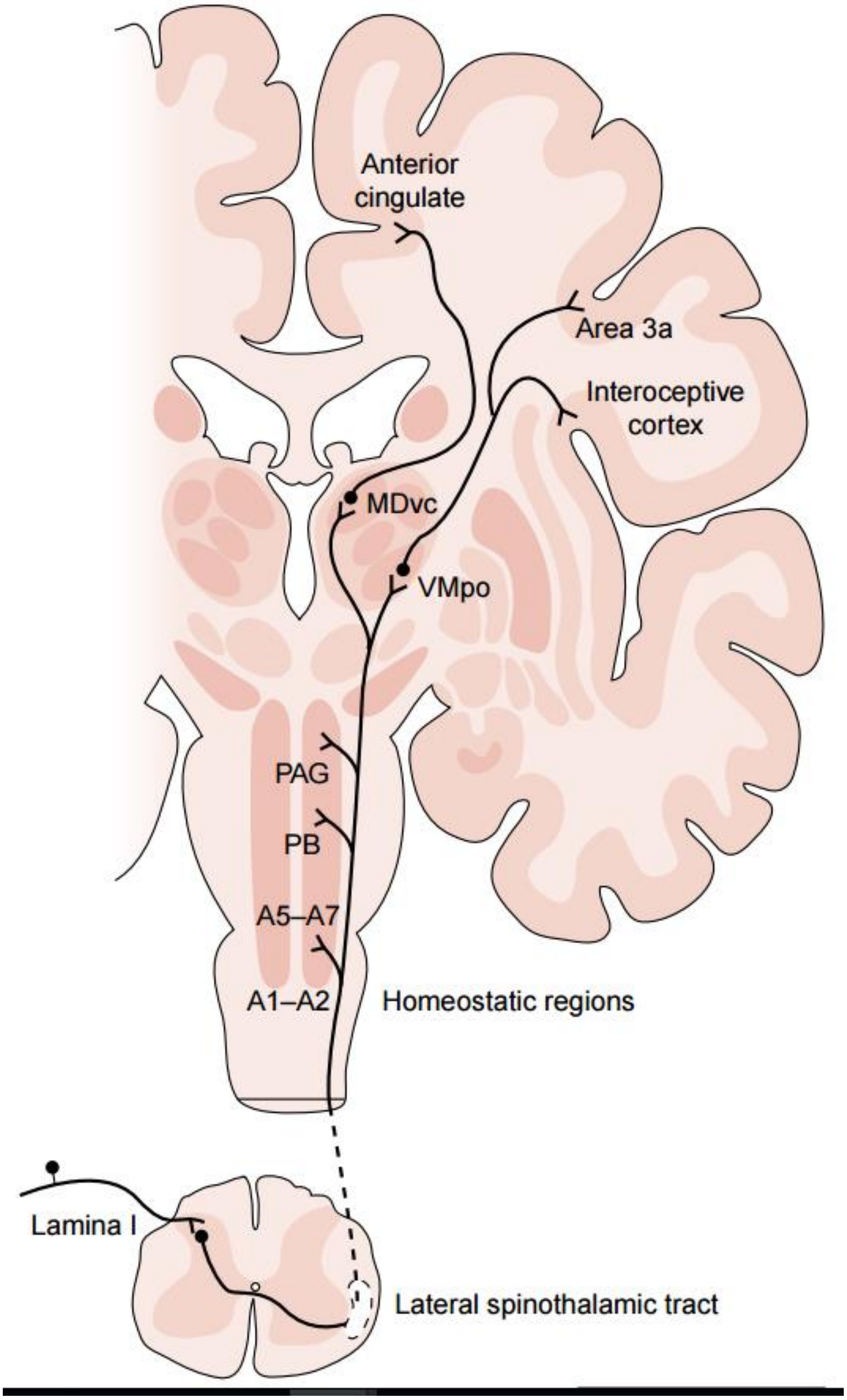


Fig 1.3 Lamina 1 pathways

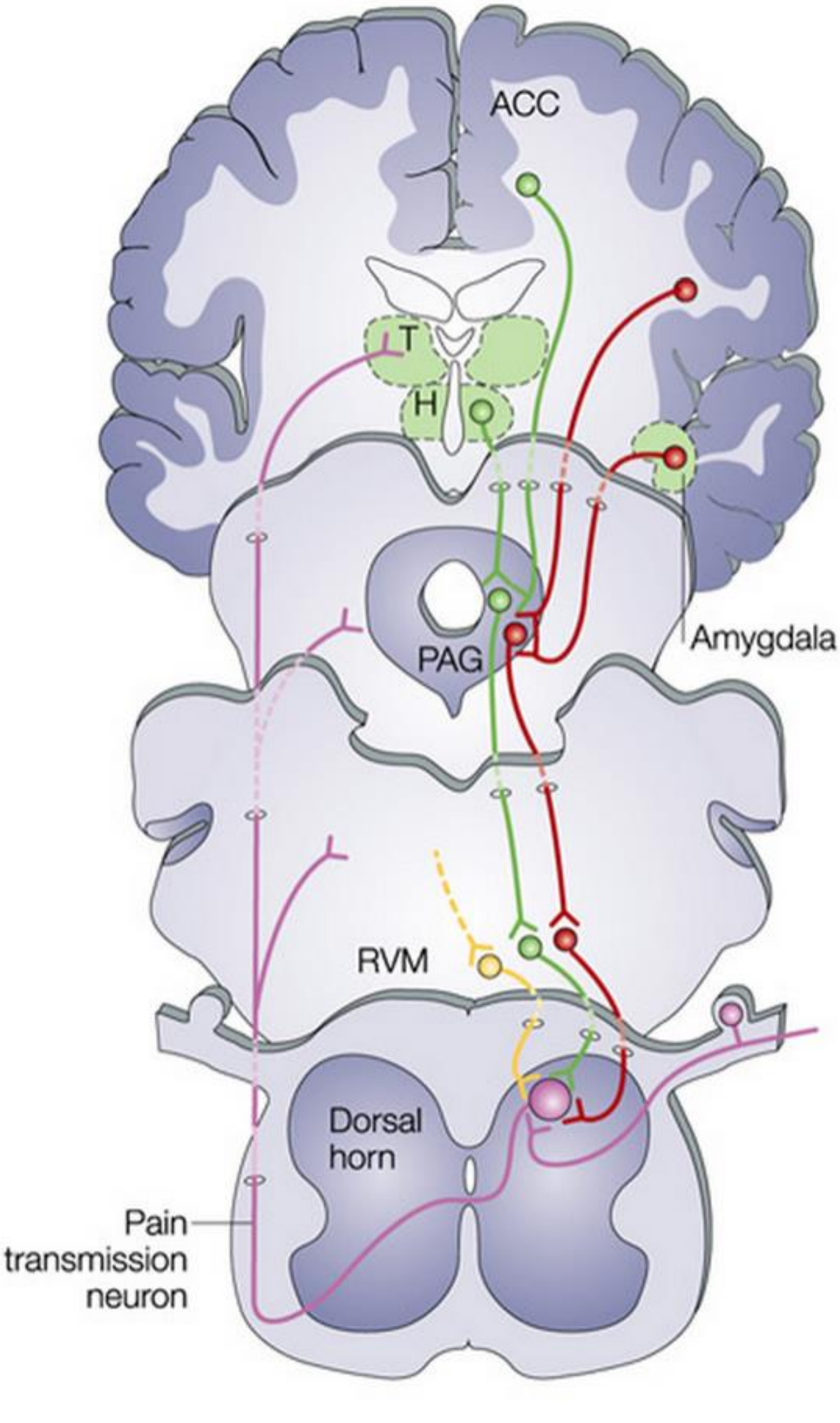


Fig 1.4 Descending pain inhibition and enhancement mechanisms.

1.5 Descending control of pain processing

1.5.1 Perspective

There was a report by Reynolds (Reynolds 1969) that generated widespread interest in descending control of spinal pain processing. He demonstrated that during, and for some time after, electrical stimulation in the rat midbrain PAG, abdominal surgery in the absence of an anaesthetic could be carried out. The stimulation-produced effects were subsequently confirmed and extended to humans (Hosobuchi et al. 1977; Richardson and Akil 1977a), which established the relationship between stimulation-produced antinociception in animals and stimulation-produced analgesia in humans. Further study revealed that opioids given directly into the same area of the PAG produced antinociception. Opioid receptors and endogenous opioid peptides were subsequently shown to be present in these and other areas in the brainstem that have since been implicated in nociception or modulation of nociception.

1.5.2 Anatomical organization of descending pain modulation

Early studies rapidly established the central importance of the ventrolateral PAG (vlPAG) in descending pain modulation. Selective activation of neurons in the vlPAG by excitatory amino acids or opioid analgesics such as morphine injected into the vlPAG was shown to inhibit spinal nociceptive reflexes (T. S. Jensen and Yaksh 1984; Yaksh and Rudy 1978) and spinal nociceptive transmission (S. L. Jones and Gebhart 1988). It was soon realised, however, that descending effects from the vlPAG were indirect and acted through relays in the brainstem region known as the rostral ventromedial medulla (RVM). This region includes a large number of serotonin-containing neurons in the nucleus raphe magnus (NRM) (Steinbusch 1981) that project to the spinal cord dorsal horn (Bowker et al. 1982).

The contribution of spinally projecting RVM serotonin neurons to the antinociception produced by activation of neurons in the vlPAG was demonstrated by numerous studies (Aimone et al. 1987; Fang and Proudfit 1996; T. S. Jensen and Yaksh 1984; Yaksh 1979; Yaksh and Tyce 1979). It was soon established that direct activation of spinally projecting neurons in the RVM produced antinociception, using the same agents that were antinociceptive when applied in the vlPAG (electrical stimulation, glutamate, or morphine).

1.5.3. Descending inhibition

Spinal nociceptive transmission can be inhibited by stimulation in the sensory cortex, ventrobasal thalamus, hypothalamus, and widespread areas in the midbrain, pons, and medulla (Gebhart 1986; Donna L. Hammond 1986; S. L. Jones 1992). Descending influences from these sites contribute to inhibition of spinal nociceptive transmission indirectly by multi-neuronal relays in the brainstem, where they modulate the excitability of neurons that send projections to all levels of the spinal cord. Descending inhibition from the PAG or RVM is mediated by both serotonergic and noradrenergic receptors in the spinal cord. In addition, it was shown that electrical stimulation in RVM increased the spinal release of both noradrenaline and serotonin (D. L. Hammond et al. 1985).

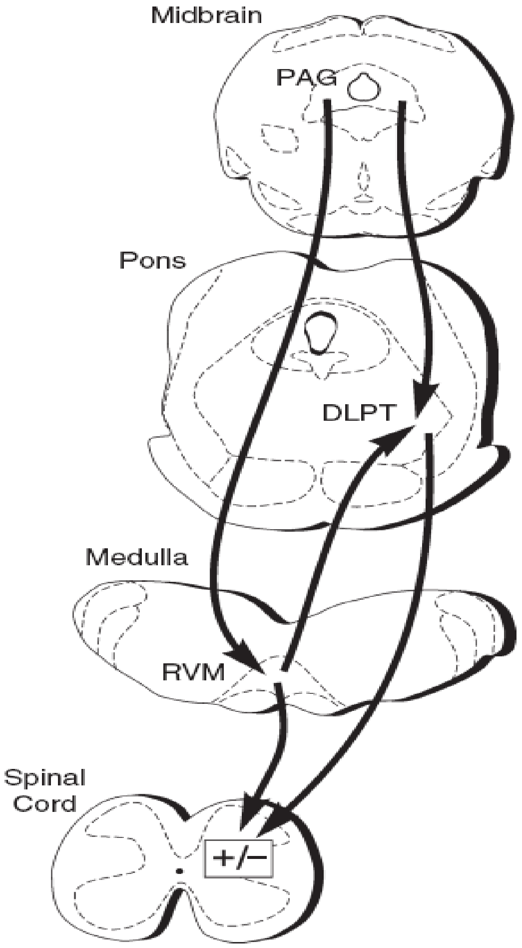
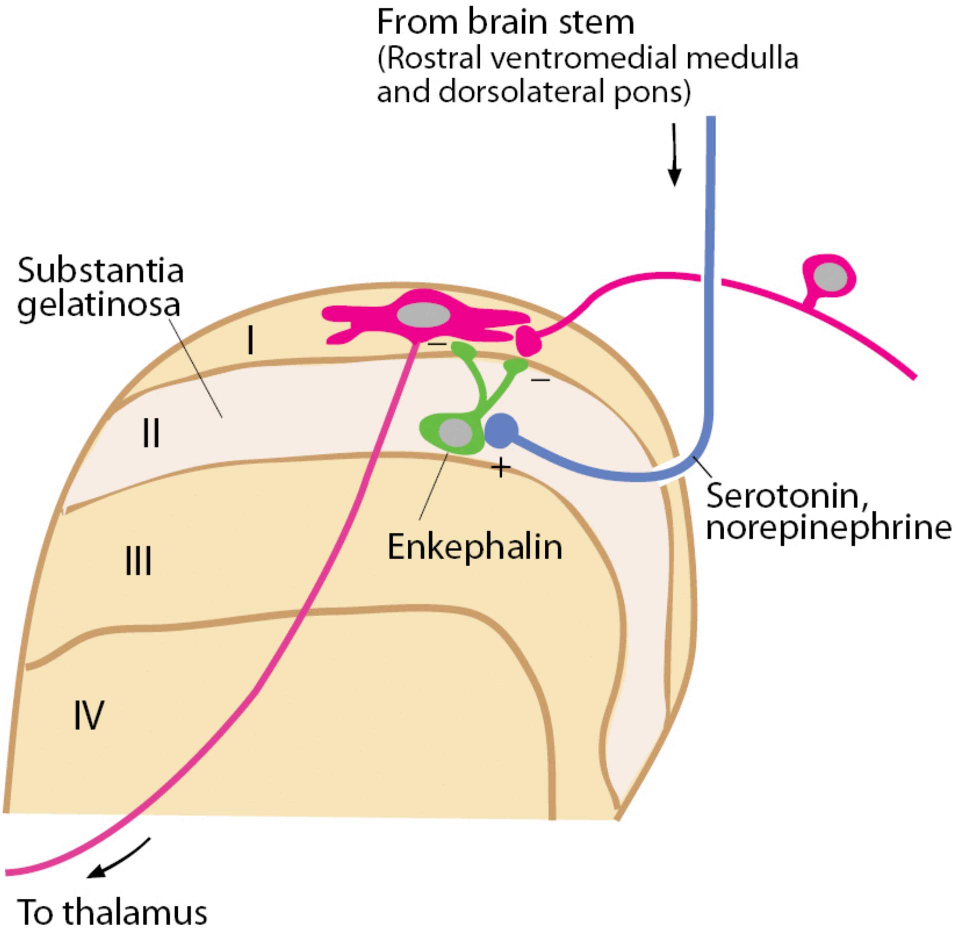


Fig 1.5

Summary of brainstem pain modulation pathways that reflects the current state of understanding. Neurons in the vlPAG modulate the activity of neurons in both the RVM and the DLPT that constitute parallel descending pathways to the spinal cord dorsal horn. These descending pathways can both inhibit and facilitate nociceptive processing in the dorsal horn, and the relative activity of these opposing actions controls the output of second order nociceptive neurons that project to more rostral brain sites that relay nociceptive information to somatosensory cortical and other brain regions where pain is perceived and interpreted. Abbreviations: DLPT, dorsolateral pontine tegmentum; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; +/-, excitatory/inhibitory.

The existence of an endogenous pain modulation system implies a physiological function that can be engaged or activated by appropriate stimuli. The stimuli that activate this modulatory system have been studied and several classes of such stimuli identified. Certainly, opioids given systemically for control of pain produce receptor-mediated actions in the PAG and RVM, and thus functionally activate the endogenous pain inhibitory system. Although opioids like morphine generally inhibit neurons, they appear to indirectly activate descending inhibitory neurons by inhibition of local GABAergic interneurons that disinhibit (activate) the descending neurons. For example, in the PAG it has been shown that morphine inhibits a GABAergic interneuron that disinhibits descending excitatory influences on neurons in the RVM (Moreau and Fields 1986).

Natural stimuli that have also been shown to engage the endogenous pain control system include stress (stress-induced analgesia), fear and anxiety, intense exercise (e.g. running a marathon), and sexual activity (Bodnar 1986; Watkins and Maier 2000). Some forms of acupuncture may also access this system (Chen et al. 1998). Disease states also certainly engage the endogenous modulatory system. For example, cardiopulmonary input (Randich and Maixner 1984, 1986; Randich and Gebhart 1992) and experimentally-induced illness (Watkins and Maier 2000) both alter nociceptive response thresholds by accessing the modulatory system. Experimentally increasing blood pressure in normotensive individuals increases response thresholds to experimentally applied noxious stimuli (Maixner and Randich 1984), whereas illness behaviour typically leads to increased sensitivity to stimuli, or hyperalgesia (Watkins and Maier 2000).



Opioids in the pain-modulation circuit in the spinal cord dorsal horn.

The prototypical μ-opioid receptor (MOR) agonist is morphine. It is through both pre- and postsynaptic mechanisms at multiple CNS sites that MOR agonists produce analgesia. Through actions on primary afferents (Stein et al. 2003) and nociceptive relay neurons in the dorsal horn (Glaum et al. 1994; Grudt and Williams 1994), MOR agonists can directly inhibit pain transmission at spinal levels. The MOR can be found in the insular cortex, amygdala, hypothalamus, PAG, DLPT, RVM and spinal cord dorsal horn, which are known supraspinal components of the pain-modulation circuit. Behavioural responses to noxious stimulation can be inhibited when MOR agonists are microinjected into each of these sites.

1.5.4 Summary

Since the seminal observation by Reynolds (Reynolds 1969), more and more evidence have been found to help us understand the circuitry and function of brainstem neurons that modulate nociception. There is now compelling evidence that neurons in the vlPAG control the activity of neurons in both the RVM and the DLPT that constitute a parallel descending pain modulation pathway to the spinal cord dorsal horn. Nociceptive processing in the dorsal horn can be both inhibited and facilitated by these descending pathways, and it is the relative activity of these opposing actions that controls the output of second order nociceptive neurons that project to more rostral brain sites that relay nociceptive signals to somatosensory cortical and other brain regions where pain is perceived and interpreted. The presence of persistent or chronic pain can significantly alter the function of descending control systems, which appears to be at least partially accountable for the origination and maintenance of these pain states.

1.6 Neurosurgical treatment of chronic neuropathic pain

Neurosurgeons have taken advantage of the aforementioned knowledge and translated it to clinical use, using DBS to treat chronic neuropathic pain patients. First I shall briefly summary what neuropathic pain is and then introduce the deep brain stimulation treatment for pain.

1.6.1 Neuropathic pain

Neuropathic pain has been redefined, by International Association for the Study of Pain, as “pain caused by a lesion or disease of the somatosensory system” (Troels S. Jensen et al. 2011b). Regarding nociceptive pain (eg tissue damage), it is the otherwise normal somatosensory system that handles sources of peripheral noxious stimulation (eg inflammatory mediators). On the contrary, the lesion or disease that leads to an abnormal and dysfunctional somatosensory system is the main cause of neuropathic pain is (Woolf 2004). According to the definition, neuropathic pain includes various clinical conditions that can be characterised in terms of anatomy (eg, peripheral vs central) and aetiology (eg, traumatic, infectious, degenerative, toxic and metabolic).

Neuropathic pain symptoms can be categorised into two groups: positive and negative. The positive symptoms contain both spontaneous (stimulus-independent) and evoked (stimulus-dependent) pain and other symptoms including tingling (ie, paresthesias) (Gilron et al. 2006). The negative signs and symptoms in the involved nerve territory include weakness, numbness, and loss of deep tendon reflexes. Neuropathic pain can be continuous or intermittent and may be described with various descriptors of pain quality. Allodynia (pain in response to a normally nonpainful stimulus, such as contact of clothing on skin), and hyperalgesia (increased pain in response to a normally painful stimulus) are the pain evoked by stimulus. It can often be observed that these sensory abnormalities extend beyond dermatomal or nerve territory distributions, which leads to the wrong diagnosis of a functional or psychosomatic disorder.

1.6.2 Deep brain stimulation for pain

Disorders or injuries in the somatosensory systems can produce neuropathic pain (T. S. Jensen et al. 2011a), and patients with this type of pain usually suffer from more severe and prolonged pain symptoms than patients with other types of chronic pain (Torrance et al. 2006). For decades neurosurgeons around the world have made great attempts trying to find better treatment options for medicine-refractory pain patients and have tested numerous pain-related components of the human nervous system. They have lesioned, injected analgesics or anaesthetics, or electrically stimulated those pain-related structures to see if these procedures could relieve pain. Those pain-related structures include the peripheral nervous system, the spinal cord, midbrain and thalamus. So far there have been many neurosurgical treatments for chronic neuropathic pain patients, one of which is DBS (Boccard et al. 2013) .

The history of taking advantage of invasive stereotactic brain stimulation with electricity as an investigation tool can be traced back to the beginning of the twentieth century, when Horsley and Clarke were still working at UCL. Scientists used this technique to activate the central nervous system and by observation of the physiologic responses to the stimuli they were able to determine the function of the regions stimulated (Tan and Black 2002). However, using electrical stimulation in the central nervous system to modulate the functions of brain circuits to obtain medical effects is a relatively new addition to the options for intractable pain. Because of the procedure’s comparatively low risk for side effect, this treatment intervention has been studied and refined enthusiastically. It can be reasonably predicted that DBS has the potential to positively affect all of the previously untreatable pain in the future.

There have been many reports, from 1950s, about electrical stimulation of certain human brain areas that could relieve pain, even though some of the brain regions were not intended to be stimulated for pain at the beginning, but at least those reports demonstrated the possibility of cerebral electrical stimulation as a pain treatment. Heath noticed that, in a patient with terminal cancer, stimulating the septal area not only reduced her depression and stress of her terminal ill by producing dysphoria and euphoria, but also relieved her pain (Heath 1954). In addition to Heath, there were also some other scientists who found similar outcomes of septal stimulation. In 1956, Pool et al. demonstrated that the septal area in the area of the anterior third ventricle could be a target of stimulation to relieve pain (Plotkin 1982). Later Gol also described some pain patients treated with septal electrical stimulation, although his result was not very significantly positive (Gol 1967).

In 1969, Reynolds described that the rats could bear surgical procedures on them without evidence of pain when the periaqueductal grey was electrically stimulated, which produced deep analgesia (Reynolds 1969). In 1971, Mayer et al. also demonstrated a similar phenomenon of periaqueductal grey stimulation in the rat. Both of these studies encouraged abundant animal studies to examine the underlying mechanism of pain modulation by exogenous brain stimulation (Mayer et al. 1971). Based on these studies, in order to find suitable sites for chronic stimulation to treat pain, Akil and Richardson initiated a human study to investigate the outcome of acute periventricular (PVG) and periaqueductal (PAG) stimulation. They reported that analgesia effect could be attained if the stimulation was on the region along the third ventricle wall, which extends from a centimetre above the intercommissural line caudally into the area of the raphae nuclei, (Richardson and Akil 1977b). Moreover, they selected a location in the PVG for chronic investigations because stimulation at this area produced minimal side effects, compared with stimulating the sites farther caudal in the brainstem (Richardson and Akil 1977b, 1977a). So far it has been shown that stimulation in the PVG and PAG area, where is believed to be the endogenous opiate system, can relieve chronic pain.

The thalamus has also been thoroughly studied as an area that has the potential to be an effective target for pain treatment, especially the sensory nuclei of the thalamus. The sensory nuclei of the thalamus are thought to be sensory relay centres, including the ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclei, which receive sensory signals from the main touch and proprioception sensory tracts projecting from the dorsal columns. (Ervin et al. 1966). Mazars et al., in 1960, demonstrated that VPL stimulation relieved pain (Mazars et al. 1960). In 1966, Ervin et al. also stated that thalamic stimulation relieved a patient’s pain. In addition to human studies, animal experiments also showed similar results. There were experiments in the cat showing that electrical stimulation of the sensory thalamus induced analgesia (Richardson 1970). In the early 1970s, Hosobuchi et al., further started using chronic stimulation of the internal capsule (IC) and VPM, originally, to relieve the symptoms of anaesthesia dolorosa, which followed section of the fifth nerve to treat trigeminal neuralgia (Hosobuch et al. 1973). Stimulating these nuclei produces paraesthesia in the pain area, and then patients lose the chronic burning paresthetic and aching quality of the pain.

In addition to the aforementioned brain regions, an area more lateral than the PAG, the lateral upper brain stem (the nuclear mass of Kolliker–Fuse) was also revealed the ability to reduce pain when stimulated. In 1992 Young et al. reported that, in three of his patients, analgesia was produced by chronic stimulation of this area (Young et al. 1992).

In terms of physiology, pain relief produced by PAG stimulation and sensory thalamus stimulation may be due to different mechanisms. Originally, scientists thought it was the direct effects on pain transmission produced by stimulation of these areas that caused pain relief, but when later Akil and Liebeskind found that naloxone, a specific opiate antagonist, could inhibit the pain relief effect induced by PVG stimulation, it began to be thought that the underlying mechanism of modulation of pain was more complicated (Akil et al. 1976). It has been noted that, in the rodent, researchers found cell bodies for the central opiate pathways in various areas of the hypothalamus: the infundibular, arcuate and periventricular nucleus of the hypothalamus. Anteriorly the cell bodies project axons through the septal area, and then, superiorly and posteriorly, through the septal area and medial to the thalamus in the periventricular grey to the raphe nuclei in the ventral PAG and inferior to the locus coeruleus, where this tract terminates (Watson et al. 1977). Pilcher et al (Pilcher et al. 1988) has also identified this beta-endorphin system in the human brain. Richardson and his colleagues revealed, in patients, that analgesia could be produced by stimulating anywhere along this system (Richardson 1982).

The noradrenergic fibres from the locus ceruleus and the secondary serotonergic fibres from the raphae nuclei then, through the spinal cord’s dorsolateral funiculus, descend to impinge on the spinal cord dorsal horn (Basbaum et al. 1976; Basbaum

1992). It has been suggested that an intermediate opioid interneuron is existed and is activated by these descending tracts that then stops pain at the first synapse in the spinal dorsal horn (Basbaum et al. 1976). As a result, the primary effect of PVG and PAG stimulation is thought to excite the same pathway that is activated by systemic opioids of the mu type (Akil and Liebeskind 1975; Rhodes and Liebeskind 1978), which activates secondarily the descending inhibitory tracts originating from the raphe nuclei and locus coeruleus and possibly the parabrachial and Kolliker–Fuse nucleus, to stop incoming signals from somatic origin at the first synapse for pain impulses in the dorsal horn (Young et al. 1992).

There are some controversies raised about whether analgesia produced by activating these tracts is via opiate pathways. Akil et al. (Akil et al. 1978b; Akil et al. 1978a) have revealed that endogenous opiates are released into the ventricular fluid when this system is activated, and naloxone blocked analgesia in animals (Akil et al. 1976) and reduced analgesia in humans (Hosobuchi et al. 1977; Richardson and Akil 1977a). Fessler et al. questioned Akil's reports (Fessler et al. 1984) about the fact that Akil and his colleagues used contrast material to visualise the cerebral ventricles during operation to help place electrodes, but Akil and his colleagues argued that, in their studies, they took the baseline levels of opiate in the ventricle and the post-stimulation opiate levels long after contrast material administration (Akil et al. 1978b; Akil et al. 1978a). Moreover, Yaksh et al. have demonstrated that direct opiate activation of the PVG/PAG has shown activation of serotonergic and noradrenergic mechanisms in the spinal cord, and the effect of pain relief by stimulation is reduced by chemically blocking of these monoamine mechanisms (Yaksh 1979, 1985, 1987). Later Young et al. in the human patient also found that

PVG stimulation induced methionin enkephlelin and beta-endorphin release (Young and Chambi 1987; Young et al. 1993). Thus, it is believed that PAG and PVG stimulation excites opiate fibres that extend from the hypothalamus (PVG) to the raphe nucleus and locus coeruleus, where they terminate and stimulate noradrenergic and serotonergic fibres that descend through the dorsolateral funiculus, which terminate in the spinal dorsal horn to inhibit pain at the first synapse, possibly through an opiate interneuron. In the parabrachial nuclei area, the Kolliker–Fuse nucleus has been defined as owning noradrenergic cell bodies projecting inferiorly to the spinal cord. Therefore, stimulating the zone might directly activate the descending noradrenergic inhibitor fibres to the dorsal horn and produces pain relief. As a result, stimulating regions like this would cut one synapse in the chain of events, since directly it excites the descending noradrenergic fibres and consequently is more effective in patients that have a faulty opiate mechanism, high opiates tolerance or habituation. It has been reported that stimulation results in slight adverse side effects and, in three of six patients, was effective to relieve pain (Young et al. 1992).

It remains unclear, at least in detail, about the inhibitory mechanism of stimulating the internal capsule and the VPL and VPM of the thalamus. It is known that stimulation of the spinal cord’s nucleus Cuniatus and Gracilis can relieve pain, through which the internal capsule and the VPL and VPM of the thalamus are extensions of the dorsal columns extending upward. Experiments in the cat show that the effects of pain relief by stimulating the spinal dorsal column are not obliterated completely by sectioning of the dorsal column below the level of activation, which indicates the existence of an additional more central inhibitory pathway (Richardson

1970). Furthermore, sensory thalamus single-cell recordings demonstrate an on/off mechanism triggered by the dorsal column stimulation, which would suggest a multifaceted inhibitory mechanism of communication between the position, touch and proprioception sense fibres in the dorsal columns and the pain transmission system in the dorsal horn at the origin of the ventrolateral spino-thalamic and spino-reticular tracts spinal cord, as well as in the synapses in the cortex of the brain, thalamus and the brainstem (Richards 1973). In 1960, Mazars et al. (Mazars et al. 1960) reported that stimulating the somatosensory pathways in the spinal cord produced relief of pain and Ervin et al. (Ervin et al. 1966) treated one patient suffering from pain when stimulating the sensory nuclei of the thalamus.

Taking advantage of chronic thalamic stimulation was reported by Hosobuchi et al. for trigeminal rhizotomy-induced anaesthesia dolorosa (Hosobuch et al. 1973). However, they reported that it is needed to use intermittent stimulation continuously over a long period of time, by a programmed type of stimulation, to obtain good pain relief, for the stimulation would become less effective over time. The inhibitory mechanism of stimulating the sensory nuclei of the thalamus, internal capsule and dorsal columns that produces paraesthesia in the patient’s pain area is according to the “gate theory of pain transmission.”, Melzack and Wall’s novel pain researches (Melzack and Wall 1965) presenting that pain inhibition could be produced by activating proprioception and touch fibres. The effect of stimulation of dorsal column and VPM/VPL/IC is multifaceted. The mechanism of the inhibition seems to be driven at each synapse of the pathway of pain through the dorsal horn, brainstem, and thalamus, not only depend on mechanisms of inhibition involving the entry mechanism in the spinal cord as in the opiate system (Richardson 1970). Hence activation of the VPM, VPL, and IC is more effective for relieving deafferentation pain, as well as pain produced in the nervous system because it is able to inhibit pain neurons at multiple levels, if these structures are intact.

As aforementioned, the PAG has been known to play an important role in modulation of pain perception (Bandler and Shipley 1994; Basbaum and Fields 1984; Budai et al. 1998; Heinricher et al. 2009; Millan 2002), and DBS of the region has been successfully carried out all over the world and has effectively relieved many chronic medicine-refractory pain patients, even though this treatment is still not a proven therapy in the US because of its lack of a randomized controlled trial (Bittar et al. 2005; Boccard SG 2012; Levy et al. 2010).

In addition to its descending projections, the PAG also provides efferents to the forebrain, which target the thalamus and hypothalamus. Thus whether the PAG modulates pain via its descending projections alone is still debated. In monkeys neuroanatomical tracing studies have shown that the PAG has direct ascending projections to the nucleus reticularis thalami, the nucleus medialis dorsalis, the intralaminar thalamic nuclei and the midline thalamic nuclei, through the periventricular bundle (Benarroch 2012; Krout and Loewy 2000a; Mantyh 1983b). In humans, diffusion tensor imaging (DTI) studies have also demonstrated connections between the PAG and the medial dorsal nucleus of the thalamus (Hadjipavlou et al. 2006; Linnman et al. 2012; Sillery et al. 2005), even though current DTI methods do not allow for inference on the directionality of information flow within tracts and have an obvious limitation in time-resolution to detect synaptic activities. Moreover, Nandi and colleagues have shown that stimulation of the human periventricular grey affected the sensory thalamic local field potentials immediately, which was correlated with pain relief (Nandi et al. 2002; Nandi et al. 2003). Thus it is still possible that the PAG may exert its pain-modulation effect by its ascending projections in addition.

1.7 Connectivity of the PAG

1.7.1 Diffusion tensor tractography

Through probabilistic Diffusion tensor imaging (DTI) tractography, we are allowed to identify white matter tracts by diffusion weighted imaging. The principle diffusivity direction can be followed in a voxel to voxel manner, which helps us achieve the identification of white matter tracks. Even though anterograde and retrograde tract tracing in animals remains the gold standard, particularly in primates (Aggleton et al. 1980; Dujardin and Jurgens 2005; Mantyh 1983b, 1983a), these pathways in humans can also be confirmed with the help of DTI. Due to the limitations of current resolution, tractography is only suitable primarily for large fibre pathways. Therefore, pathways through regions of fibre crossing or complexity, or smaller pathways may not be identified. Furthermore, it should be noted that DTI also has other limitations: 1) inference on the directionality of information flow within tracts cannot be drawn by current DTI methods, and 2) the temporal resolution of DTI is not good enough to detect the changes of synaptic activities. Even though the PAG connections cannot be addressed directly, DTI has been used to help identify several cerebral circuits, including the superior, medial, and inferior cerebellar peduncles, the temporo-/parieto-/occipitopontine tract, the frontopontine tract, medial lemniscus, and the corticospinal tract (Stieltjes et al. 2001). Besides, there are several studies that have taken advantage of diffusion imaging tractography using the PAG as the starting point (Hadjipavlou et al. 2006; Owen et al. 2007; Owen et al. 2008; E. A. C. Pereira et al. 2010a; Sillery et al. 2005). The PAG has been found to have connections with the frontopolar and middle frontal gyri, the rostral ventral medulla, the dorsomedial, ventromedial and ventrolateral prefrontal cortex, the amygdala (through the thalamus and hypothalamus) and the thalamus (medial dorsal nucleus). Probabilistic DTI in pre-operative patients with PAG DBS for chronic pain also found similar results (Owen et al. 2007; Owen et al. 2008). Moreover, it has also been argued that when future technical has been advanced, one day probabilistic DTI can be used as a planning tool for operation. It is also noted that ventral PAG has connections with anterior cingulate cortex, nucleus accumbens, ventromedial prefrontal cortex and amygdala. While dorsal PAG connects with primary somatosensory cortex and ventral posterior thalamus.

DTI tractography studies have also been performed in healthy controls, and the seeds were reported lesion sites. It is noted that seeds in the sites for limbic leucotomy, subcaudate tractotomy and anterior capsulotomy all had projections to the PAG (Schoene-Bake et al. 2010).

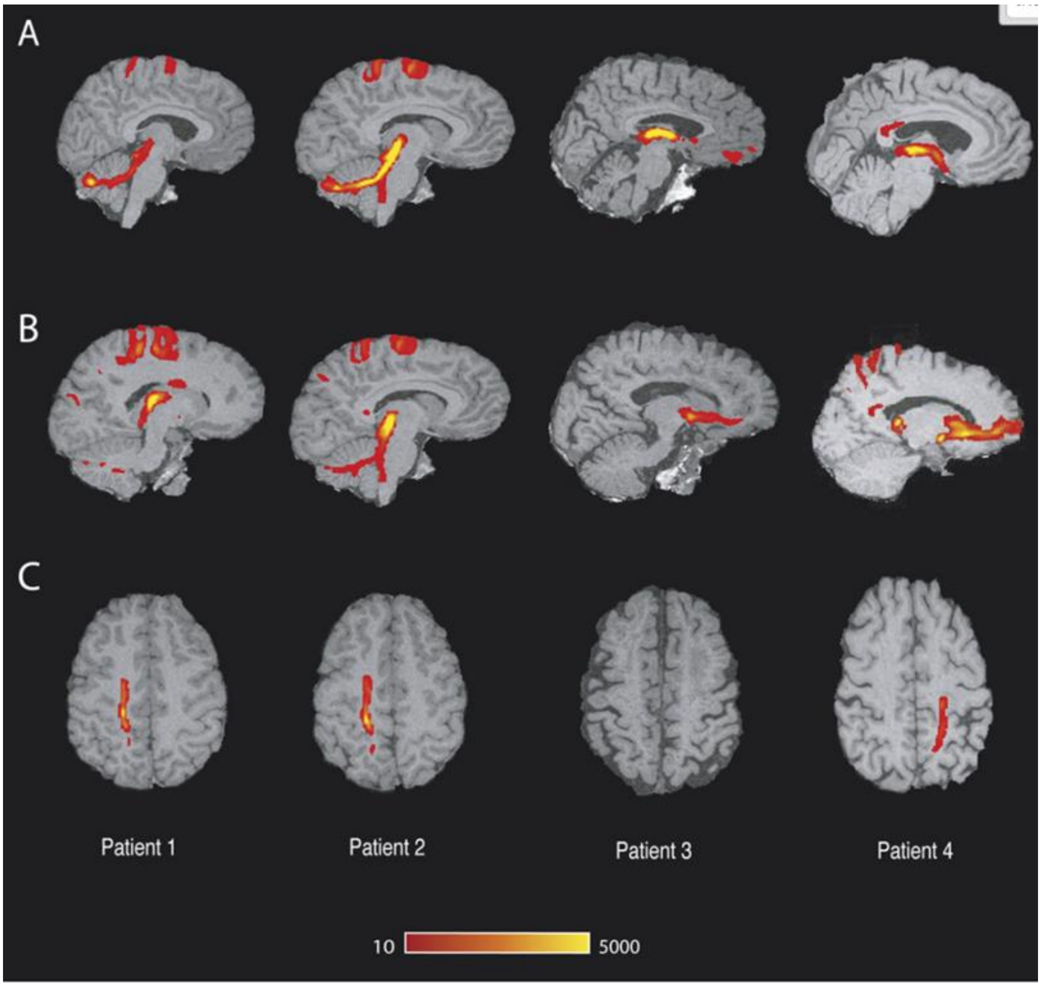


Fig 1.6 DTI tractography showing the PAG's connections (Owen, S. L. F., et al. (2008), 'Pre-operative dti and probabilisitic tractography in four patients with deep brain stimulation for chronic pain', *Journal of Clinical Neuroscience,* 15 (7), 801-05)

1.7.2 Functional connectivity

The correlations between spatially remote neurophysiological events can be defined as the functional connectivity between sites. In neuroimaging, a temporal correlation between regional fluctuations in cerebral blood flow or BOLD signal is usually used to mean functional connectivity .

Resting state connectivity of the PAG

Cerebral blood flow’s correlated slow fluctuations can be shown in separated brain regions, and this finding can be used for resting state functional connectivity MRI (Friston et al. 1993). BOLD signal (Blood-oxygenation-level-dependent) (Biswal et al. 1995) can also show this phenomenon. Even though functional connectivity does not measure anatomic connectivity or directionality directly, more and more evidence has suggested the fact that anatomy adequately constrained functional connectivity, which allows researchers to characterise the architecture of distinct brain systems (Van Dijk et al. 2010). Tomasi and colleagues conducted a study to analyse functional hubs in 979 healthy subjects (Tomasi and Volkow 2011), it was revealed that three subcortical networks involve the PAG, and the network hubs were in the the amygdala, the thalamus and the cerebellum. The PAG was also found, in an independent component analysis, to belong to a “salience network,” with the main nodes in orbital frontoinsular cortices and dorsal anterior cingulate (Seeley et al. 2007). Kong and colleagues conducted a study investigating PAG’s resting state connectivity in 100 healthy controls using a seed based approach, and demonstrated that the anterior insula, the hippocampus, the globus pallidus, the ventromedial medulla, the cerebellum, and the rostral and pregenual ACC have significant functional connectivity to the PAG. Negative functional connectivity was seen to the lateral orbital prefrontal cortex, the posterior insula, the middle occipital gyrus and the post-central gyrus. Furthermore, females had a higher functional connectivity to the midcingulate cortex, and males to the right prefrontal cortex, left medial orbital prefrontal cortex, the left uncus and the right insula (Kong et al. 2010).

There are also some clinical studies on the PAG's resting state connectivity: the PAG is connected functionally to the dorsal putamen in healthy subjects, but in obsessive–compulsive disorder patients the connectivity is significantly less so (Harrison et al. 2009). The basolateral amygdala connectivity to the PAG is lower than the centromedial amygdala connectivity to the PAG, but there is no significant differences between healthy controls and generalized anxiety disorder patients (Etkin et al. 2009). In fibromyalgia patients with severe spontaneous pain, the connectivity is compromised between the PAG and right executive attention network, including posterior parietal regions overlapping the superior parietal lobule and intraparietal sulcus, and dorsolateral prefrontal cortex (Napadow et al. 2010).

Even though fMRI has helped demonstrate many functional connectivity between brain areas, we should bear in mind the fact that fMRI's time resolution is not good enough to detect synaptic activity, therefore we should be very cautious when determine whether various brain areas are directly connected or not.

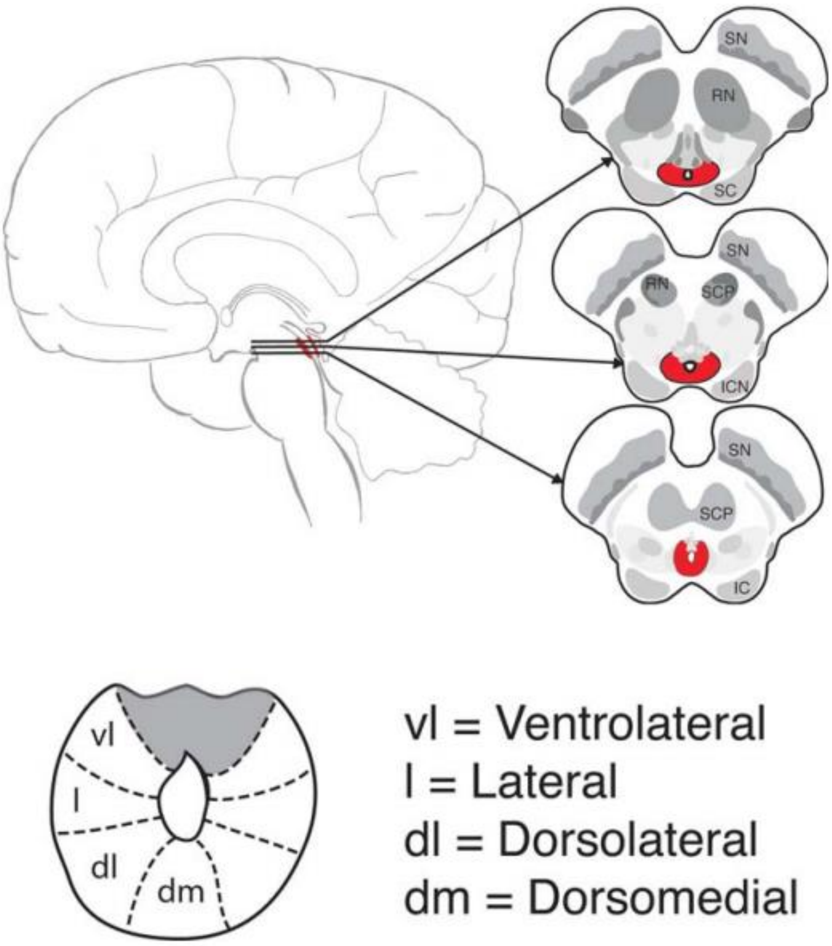


Fig 1.7 Position of the PAG within the human midbrain surrounding the cerebral aqueduct, and divisions of the PAG derived from animal models.

1.7.3 Functional organization of the PAG — two forms of analgesia

Analgesia evoked from the PAG is best viewed as a component of one or more complex adaptive emotional coping responses. The PAG is functionally organized into four longitudinal columns of neurons. Two distinct forms of analgesia can be produced from activation specifically of the ventrolateral PAG column or the dorsolateral/lateral PAG columns. Typically as a response to extreme, inescapable physical stress, including traumatic injury, a long-acting, opioid-mediated analgesia is a component of a vlPAG-mediated passive coping or conservation-withdrawal reaction which promotes recovery and healing. In contrast, a short-acting, non-opioid-mediated analgesia represents a component of a dlPAG- or lPAG-mediated active coping or defensive reaction to an escapable threat or stress, including acute pain.

Anatomical data indicate that each PAG column lies embedded within a distinct forebrain circuit that includes select medial and orbital PFC, hypothalamic and amygdaloid areas. These circuits provide a basis whereby psychological (as well as physical) stressors can influence neural activity in the PAG in a graded and distinct columnar manner.

Activation of the PAG is invariably accompanied by pronounced changes in autonomic function. In humans, an involvement of the PAG has been implicated in several ‘visceral’ pain states (e.g. migraine, NCCP, IBS).It is argued that in such cases, pain may arise as a consequence of abnormal activity in the PAG which generates pain as a result of ‘dysfunctional’ ischaemic changes or spasm in regional smooth muscle and/or alterations in descending antinociceptive control.

1.8 Cerebral signature of pain: the pain matrix revealed by imaging studies

1.8.1 Acute pain experience and its neural correlates

Numerous studies have been performed that used various imaging methods to perceive the brain areas which are active during acute painful stimulation. In most studies, the insular, secondary somatosensory (SII), and anterior cingulate (ACC) cortices (Brodman areas (BA) 24 and 32) were active, usually bilaterally (A. K. P. Jones et al. 1991; Tracey and Mantyh 2007). In many studies, additional areas of activation included primary somatosensory cortex (SI) contralateral to the stimulation, the thalamus, the prefrontal cortex (BA 10, 45, 46, 47), the supplementary motor area, the cerebellum, the basal ganglia (striatum), the peraqueductal grey, the amygdala, the hippocampus, and the posterior parietal cortex (BA 40).

1.8.2 The central representation of chronic pain

Di Piero *et al.* (Dipiero et al. 1991) used PET to determine pain-related activation patterns in patients with cancer pain and found, as the most prominent characteristic, decreased rCBF in the thalamus. In a subsequent study, patients who underwent cordotomy, with substantial pain relief, were tested and reversal of this activation pattern was observed. The reduced activation in the thalamus in states of chronic pain and its increase related to effective treatment was confirmed in several other studies of neuropathic pain conditions (Duncan et al. 1998; Hsieh et al. 1995; Iadarola et al. 1995).

Several studies have examined central activation patterns in fibromyalgia syndrome (FMS). Mountz *et al.* (Mountz et al. 1998) reviewed PET and SPECT studies in FMS and came to the conclusion that the most prominent finding in chronic patients is a reduced activation in the thalamus. In addition, FMS patients showed reduced blood flow in the region of the caudate nucleus. The authors related these reduced activations to hypersensitivity of spinal cord neurons. Using fMRI, Gracely *et al.* (Gracely et al. 2002) noticed that in fibromyalgia patients, a number of brain regions showed noticeably hyperreactive to painful stimulation, including SI cortex. These data are in accordance with previous EEG studies that examined laser-related evoked responses and found enhanced activity of the long-latency components in FMS patients (Gibson et al. 1994; Lorenz et al. 1996).

Silvermann *et al.* (Silverman et al. 1997) found that painful colorectal distention in irritable bowel syndrome patients did not activate the ACC as in healthy controls, but activation was found in the left prefrontal cortex (BA10), suggesting that chronic pain might activate other brain regions than does experimental pain. Grachev *et al.* (Grachev et al. 2000) used proton magnetic resonance spectroscopy to determine to what extent abnormal brain chemistry is present in the anterior cingulate, the thalamus, or the prefrontal cortex. They observed reduced levels of N-acetyl aspartate and glucose in the dorsolateral prefrontal cortex in patients with chronic low back pain.

Several studies have inspected the central correlates of headache. Weiller *et al.* (Weiller et al. 1995) found activation of the auditory and visual association cortices, the cingulate cortex, as well as an area in the dorsal midbrain that included the periaqueductal grey and dorsolateral pontine tegmentum, including the nucleus ceruleus, related to migraine attacks without aura. These activations seem to be specific for migraine headaches (May et al. 2000). In cluster headache, by contrast, the posterior hypothalamus may play a special role (May and Goadsby 2001).

The neural correlates of allodynia have been examined in patients with neuropathic pain and in normal subjects, using capsaicin-provoked allodynia. Whether related to capsaicin application or neuropathic pain, some data suggest that allodynia is processed in a different way in the cerebral cortex than is nociceptive pain. Witting *et al.* (Witting et al. 2001), using a capsaicin-evoked tactile allodynia, found an allodynia-specific activation in parietal association cortex (BA5/7). Lorenz *et al.*(Lorenz et al. *2002)*, comparing heat pain and thermal allodynia in a PET study, found allodynia-specific activations in the medial thalamus, putamen, and prefrontal cortex. Further, Peyron *et al.* (Peyron et al. 1998; Peyron et al. 2000) could not find activation of the ACC during allodynia in patients with central pain, even though the ACC is almost always activated in studies of nociceptive pain using PET or fMRI. Baron *et al.* (Baron et al. 1999), similarly, also did not detect activation of the ACC when investigating dynamic tactile allodynia during a capsaicin model in normal subjects and argued that Aβ-mediated pain has a unique cortical presentation. However, this explanation is not supported by other investigators; they rather argued that ultimately the same cortical structures are activated by pain arising from aberrant or normal processes. For instance, after capsaicin was injected in normal subjects, Iadarola*et al.* (Iadarola et al. 1998) revealed activation of the ACC during dynamic tactile allodynia. Similarly, Petrovic *et al.* (Petrovic et al. 1999) showed ACC activation related to dynamic tactile allodynia in a neuropathic pain patient.

1.8.3 Chronic pain and cortical reorganization

Animal models have revealed that spinal cord neurons can be sensitised by intensive and/or long-lasting state of pain (Woolf and Salter 2000). Also this abnormal pain state can cause a changed representation of the painful area in the cortex (Benoist et al. 1999) and the thalamus (Vos et al. 2000). In patients suffering from chronic pain, it can be observed that they are hyperreactive to tactile or noxious stimuli. These modifications in nociceptive sensitivity might be related to cortical changes, even though some data have already suggested that some of these changes in nociception are associated with peripheral as well as spinal and thalamic mechanisms.

Flor *et al.* (Flor et al. 1997) stated that, in patients with chronic pain, raised responses to painful and non-painful tactile stimulation could be noted by magnetencephalography. A significantly higher magnetic field, in the time window less than 100 msec, could be revealed by stimulation at the affected site of the back, but not at the finger, whereas, in the later time windows, both types of stimulation caused higher fields in patients compared to controls. When the patients had a longer history of low back pain, this hyperreactivity of the somatosensory system was noted more severe. When researchers localised the source of this early activity, they found it was originated from SI. Although there was no significant difference between patients and controls in terms of the localization of the fingers, in the patients it was noted that the localization of the back was more inferior and medial, suggesting an expansion and shift toward the cortical representation of the leg. These data suggest that chronic pain induced an extension of the cortical representation zone connected to nociceptive input, similar to the extension of cortical representations that have been reported to happen with other sorts of behaviourally relevant stimulation. Nociceptive input is very important to the creature, and it might be beneficial to augment the representation of this type of stimulation to prepare the creature for the satisfactory response. The quantity of increase of the back region was positively correlated with disease history indicating the fact that this pain-related cortical reorganisation develops over time.

This kind of cortical modification may be similar to what Katz and Melzack (Katz and Melzack 1990) have termed, in phantom limb pain patients, a ‘somatosensory pain memory’. While they referred mainly to explicit memories (i.e. the patients’recollection that the phantom pain was similar to previously experienced pains), somatosensory memories can also be implicit. Implicit pain memories are based on changes in the brain that are not open to conscious awareness but lead to behavioural and perceptual changes of which the patient is not aware, such as hyperalgesia and allodynia. It is therefore impossible for the patient to counteract these pain memories. In the absence of peripheral stimulation, pain perception can be produced by this sort of memory trace, because an increase of a representational zone is linked to higher acuity in the perception of tactile input.

In patients with complex regional pain syndrome, similar modifications in the cortical processing of sensory information were also noticed (Juottonen et al. 2002). These reorganisational processes are also modifiable by cognitive and affective processes. For example, Buchner *et al.* (Buchner et al. 1999)showed that attention modifies the somatotopic map in SI cortex. Flor (Flor 2002) reported that verbal reinforcement of increased or decreased pain ratings leads to a persistence of elevated pain ratings in chronic pain patients that are accompanied by an elevated electrocortical response originating in SII cortex.

1.9 The question the thesis would like to address: The gap in the literature

PAG DBS can relieve chronic neuropathic pain but we know that 1) the descending pain modulation pathway is contralateral to the pain transmission site, and 2) symptoms of neuropathic pain does not rely on peripheral nociceptive stimulation. As a result, it can be argued that the PAG might have the ability to modulate pain via its ascending connections with other brain regions, in addition to modulating the activity in the spinal dorsal horn. Furthermore, it is believed that sensory systems are simply mapping from stimuli to responses (Carpenter and Reddi 2012), ie sensory processing is for organism to have proper responses to maintain homeostasis, pain signals can be modified if needed. Sensory thalamus is the most important area to receive sensory information, therefore it is logical to think whether the activity in it could be modulated, and the PAG is one of the possible areas to play the role, for the PAG is in a vital position in the descending pain modulation system, and the descending pain pathways, from the brain, at all times influence and control pain processing, which registers both the environmental and bodily status. Moreover, because affective component of pain causes behavioural reactions that include a cohesive autonomic response through activation of brainstem and spinal centres that control sympathetic and parasympathetic outflow to the body, theoretically, endogenous pain modulation mechanisms should involve both directions (upwards to the brain and downwards to the spinal cord). Therefore, the study of functional connections between the PAG and the sensory thalamus is important if we want to understand pain mechanism better; however, we could only find little literature addressing this question.

1.10 The aim of the thesis

The aim of the thesis is to identify the functional connectivity between the sensory thalamus and the periaqueductal grey in pain modulation.

We estimated the functional connectivity between the sensory thalamus and the periaqueductal grey by 1) studying these two areas' interaction during DBS of either site, 2) calculating their spectral coherence change along with the patients' various pain states, and 3) investigating the effect of general anaesthetics on the functional connectivity between these two areas, to see if the pain-related functional connection can be affected by GABA-mediated activity.

I shall discuss the methodology of this thesis in chapter2, and place detailed experiment methods in the following result chapters

Chapter 2 Methodology

2.1 Deep brain stimulation as an investigation probe

The experiments presented in this thesis were all taking advantage of the deep brain stimulation's extraordinary versatility in terms of it therapeutic and research usages. Deep brain stimulation, which introduces electrodes and then delivers beneficial electrical stimulation in brain circuits with dysfunction, not only produces lessening of symptoms but also presents us the exceptional opportunity for investigating these circuits' function. As a result, deep brain stimulation offers us an entrance into brain circuits' workings and dynamics.

Implanting DBS electrodes is an invasive neurosurgical procedure. However, it is regularly done under local anaesthesia, and precise stimulation mapping can be performed because patients are fully awake. After surgeons have implanted the leads in the desired target below the brain convexity, they connect the leads to a programmable pulse generator (IPG), which is similar to contemporary cardiac pacemakers. Surgeons then implant IPG in a subcutaneous pocket below the collarbone. Once implanted, physicians can externally access DBS devices and adjust stimulation parameters to clinical effects. Physicians are able to fine-tune electrical stimulation to get best clinical effects and prevent unwanted side effects or off-target spread of current. Parameters that can be modified include the optimal contact selection (within the electrode array), whether to stimulate bipolarly or monopolarly, stimulation frequency, amplitude, and pulse width.

Why DBS is useful to neuroscientists is mainly because it can be used both as a investigation tool and as a modulator of neuronal activity (Kringelbach et al. 2010; Lozano et al. 2010). When doing physiological mapping to select the most suitable brain target for electrode placement, surgeons can record signals from single or populations of neurons, and the neurons can be at rest or in response to various cognitive or motor tasks (Androulidakis et al. 2008; K. D. Davis et al. 2005; Sheth et al. 2012). Abundant data have been generated by this, which is helping us better understand the circuitry and the anatomy of psychiatric and neurological conditions. Electrophysiological signatures of target neurons can be identified, in awake humans, by microelectrode recordings along brain trajectories, and it can also be revealed how target neurons are involved in regulating miscellaneous functions such as movement (Hutchison et al. 1998), pain (Hutchison et al. 1999), reward (Zaghloul et al. 2009), decision making (Sheth et al. 2012), and plasticity (K. D. Davis et al. 1998). DBS electrodes can be externalised, and researchers have the opportunity to record local field potentials from DBS macroelectrodes placed within deep structures in the brain. Many important findings have been made by this method, including characterisation of pathological activity such as beta oscillations in PD patients' subthalamic nucleus (STN) (C. Hammond et al. 2007; Jenkinson and Brown 2011; Kuehn et al. 2008; Kuehn et al. 2009; Kuhn et al. 2005), how movement planning and execution involve basal ganglia nuclei activity (Paradiso et al. 2004), and studies about modulation of autonomic functions and pain (Green et al. 2006b; Green et al. 2007; Green and Paterson 2008; Green et al. 2010; Erlick A. C. Pereira et al. 2010b; Erlick A. C. Pereira et al. 2010c; Sitsapesan et al. 2013; Sverrisdottir et al. 2014; Wu et al. 2014).

In terms of medical benefits, regardless of clinical indication, to stimulate neuronal targets so that the activity of pathology can be modified is the principle of DBS treatment, and by this means to affect up- and downstream, regional and local projections. Therefore, by its functional connectivity, relative prominence, and role within a circuit driving the target behavior or symptom, physicians define a theoretically effective target. A rich opportunity for discovery is provided by tracking the behavioural and molecular results of stimulation within these pathological circuits.

2.2 DBS of the sensory thalamus and the periaqueductal grey in pain patients

2.2.1 Patient selection: indication for DBS pain treatment

What kinds of pain patients are suitable for DBS? Patients who are suffering from chronic medicine-refractory neuropathic pain are indicated for the treatment. The specific aetiology of the chronic pain seems to be less important to DBS efficacy than its symptom history. However, the pain must have a definable organic origin with the patient refractory to or poorly tolerant of pharmacological treatments.

Abundant evidence has shown that chronic pain causes brain modification; therefore, chronic medicine-refractory pain can be seen as central pain, thus neuropathic. Hence one important thing to patient selection for DBS is to ensure that patients' chronic pain is neuropathic, not psychogenic. In order to achieve that we at least need a team of neurosurgeons, neuropsychologists and pain specialist to determine the characters of pain.

In order to select suitable chronic pain patients for DBS treatment, we need to do many evaluations, such as 1) comprehensive neuropsychological evaluation, to exclude psychoses, addiction and medically refractory psychiatric disorders and ensure minimal cognitive impairment, 2) quantitative pain assessment and health related quality of life: VAS (scale 0-10) to rate pain intensity, the McGill pain questionnaire (MPQ) for pain evaluation and additional qualitative information and quality of life assessment, short form 36 (SF-36) and the VAS part of the Euroqol five-dimensional assessment tool (EQ-5D) to assess patients' quality of life. As aforementioned, these assessments are for DBS patient selection, not for the study in this thesis.

2.2.2 DBS targets

Thalamic and midbrain DBS targets are contralateral to the painful side of the body. Sites for DBS can be divided anatomically first into somaesthetic regions of the ventrobasal thalamus (sensory thalamus), second into more medial midbrain regions surrounding the aqueduct of Sylvius (PAG).

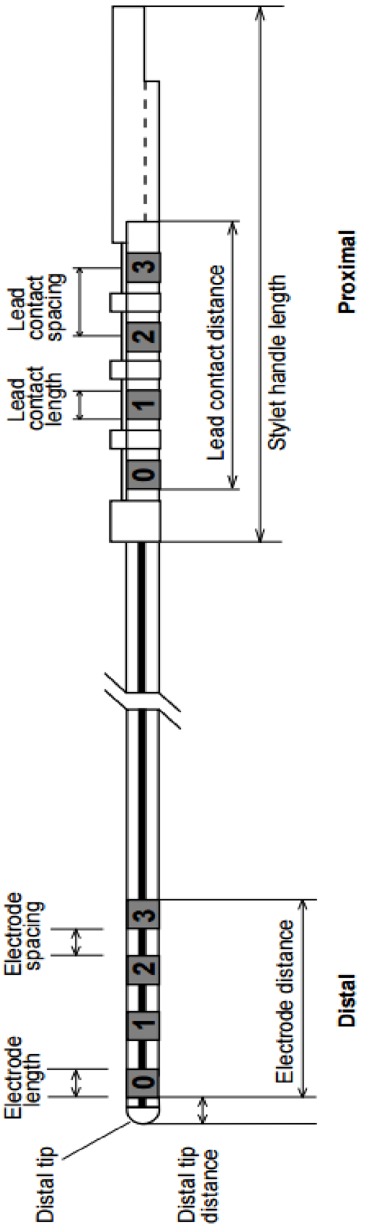
The surgeon's ultimate adjustment of intracerebral electrode position is directed by awake patient reports of somaesthetic localisation during intra-operative stimulation. Such subjective information may alter the final electrode position by up to several mm from pre-operative target co-ordinates. The targeting was performed on fused stereotactic magnetic resonance imaging/computed tomographic scans of 2 mm in thickness, using Radionics Image Fusion (Radionics, Burlington, MA) and StereoPlan (Radionics). The sensory thalamus targets (VPL/VPM) are described as follows; The VPM lies medial to VPL and is targeted for facial pain, whereas the more lateral VPL is targeted for arm and leg pain (consistent with the known homunculus of a horizontal man). As these targets cannot be distinguished visually, the thalamic targets are based on coordinates relative to the midcommissural point (MCP). For VPM this is 5-8mm posterior (y), 10-12mm lateral (x) and at the level of the AC-PC plane (z=0). For VPL, y and z are the same, but x= 12-14mm and just medial to the posterior limb of the internal capsule. Awake intraoperative testing confirms that the stimulation is somesthetically correct and the electrode can be re-sited if necessary. The PAG is targeted at the level of the superior colliculus but with the electrode tip in the ventrolateral PAG region, 3-5mm from the midline and just anterior to the aqueduct. The anterior and posterior commissures were identified on the axial images. The intended target for placing the deepest electrode contact was marked at the PAG at a level of less than 10 mm below the anterior commissure/posterior commissure line; between the dorsal part of the red nucleus and the superior colliculus in the anteroposterior plane; and approximately 5 mm lateral to the lateral boundary of the aqueduct and the third ventricle. All electrodes were localised on post-operative CT fused to pre-operative MRI to confirm location.

2.2.3 Surgical techniques

Informed written patient consent is obtained after detailed explanations of the surgical risks and potential benefits of the procedure and counselling for its duration of approximately two hours under moderate sedation and local anaesthesia with the head fixed and cranial stereotaxis applied. A week or more prior to surgery, patients have a T1 weighted MRI scan. For surgery, a Cosman-Roberts-Wells (CRW) base ring is applied to the patient's head under local anaesthesia. A stereotactic CT scan is the performed and the MRI scan is volumetrically fused to it using computerised image fusion and stereotactic planning programmes to eliminate spatial distortions that arise from magnetic field effects. The co-ordinates for the PAG and VPL/M and entry points are then calculated. A frontal trajectory avoiding the lateral ventricle is preferred in order to prevent 1) deflection of electrodes as they traverse the ependymal surface en route to the brain parenchyma and 2) bleeding in any of the number of vascular structures present in the ventricle. Moreover, evidence shows that avoiding the ventricles seems to reduce the need for multiple insertion through the brain to arrive the favourite target, and can greatly improves the accuracy of anatomical targeting during placement of the electrode for DBS (Zrinzo et al. 2009).

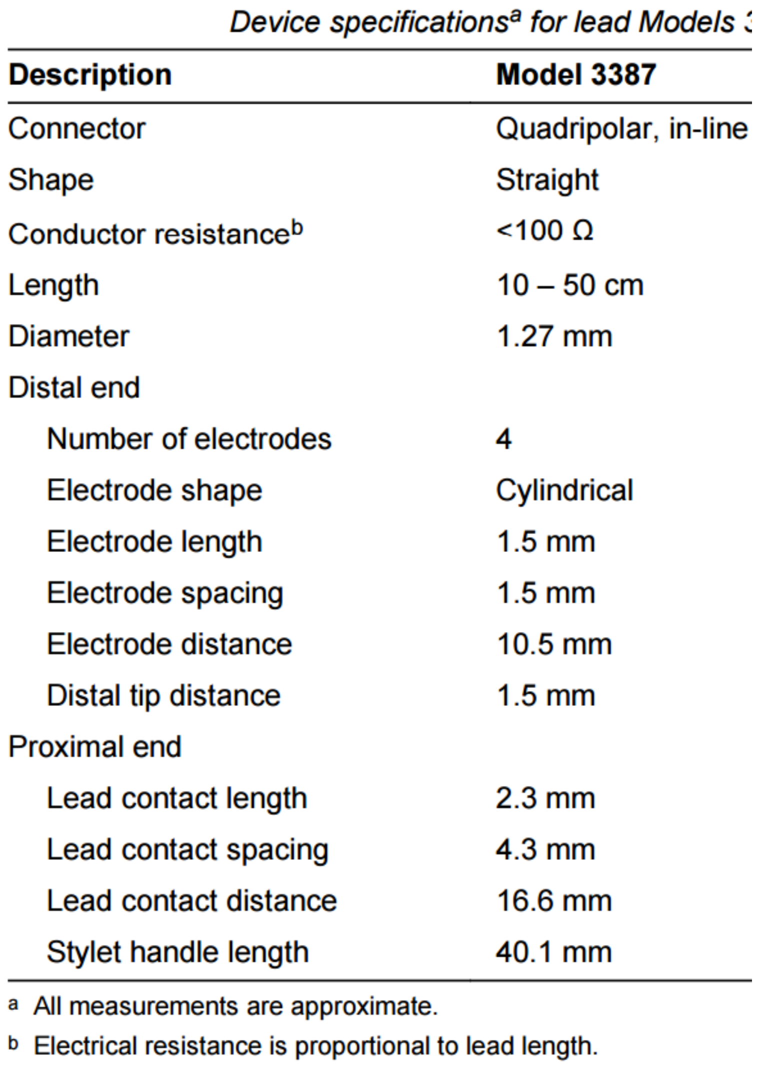
After a 3cm parasagittal scalp incision and separate 2.7mm twist drill craniostomy per electrode, both VP and PAG have been implanted with Medtronic model 3387 or St Jude Medical model 6143 quadripolar electrodes. Final electrode position is determined by intraoperative clinical assessment reliant upon subjective reporting by the conscious patient. Bipolar 5-50 Hz stimulation is performed initially, pulse width 100-450 µs, amplitude 0.1-3 V. Sensory thalamus stimulation aims to supplant painful sensation by pleasant paraethesia and PAG stimulation seeks to induce a sensation of warmth or analgesia in the painful area. Adjustment is primarily somatotopic so as to evoke appropriate topographic responses, but the assessor should be alert to pyramidal signs suggesting capsular involvement with thalamic DBS, and with PAG DBS for oscillopia and reports of visual disturbances caused by superior collicular involvement or facial paraesthesia arising from medial lemniscus stimulation. Each electrode is fixed to the skull by a miniplate and its lead externalised parietally via temporal extensions. Immediately after surgery, a further stereotactic CT is performed and co-registered as before to confirm electrode position.

After a week of post-operative clinical assessment, a decision is made whether to permanently implant the electrodes in a second operation under general anaesthesia. They are connected to an IPG implanted subcutaneously, usually infra-clavicularly or alternately intra-abdominally in subcutaneous fascia.



Electrode, lead contact, and stylet.

DBS electrodes: Medtronic model 3387



Device specifications[a] for lead Models 3

Description	Model 3387
Connector	Quadripolar, in-line
Shape	Straight
Conductor resistance[b]	<100 Ω
Length	10 – 50 cm
Diameter	1.27 mm
Distal end	
Number of electrodes	4
Electrode shape	Cylindrical
Electrode length	1.5 mm
Electrode spacing	1.5 mm
Electrode distance	10.5 mm
Distal tip distance	1.5 mm
Proximal end	
Lead contact length	2.3 mm
Lead contact spacing	4.3 mm
Lead contact distance	16.6 mm
Stylet handle length	40.1 mm

[a] All measurements are approximate.

[b] Electrical resistance is proportional to lead length.

2.2.4 Clinical Assessment for stage 2 operation (IPG implantation)

All electrodes are externalised for a week of trial stimulation. During this period, the patient records VAS scores at least twice a day and is kept blinded to DBS settings. Targets are trialled individually for 1-2 days using the stimulator parameters described to determine which settings of quadripolar electrode contact polarities confer maximum analgesia to the optimal somatic region. Monopolar stimulation is also trialled if bipolar settings fail to give pain relief. After this period, both electrodes are trialled together for 1-2 days. If the patient is satisfied with the degree of pain relief obtained, full implantation of the efficacious electrodes is performed and the DBS commenced at the optimised stimulation parameters. In general, surgeons do not decide between permanent implantation of PAG, sensory thalamus or dual site stimulation on any criteria other than demonstrable efficacy in each individual patient.

Patients ideally leave hospital the day after IPG implantation and their progress is followed by clinic appointments at one month, three months, six months and then six monthly thereafter. Initially they are given a pain diary to record their VAS and stimulator settings weekly for review at follow-up. In addition to being able to switch the DBS on and off at will, they are usually only given control over its voltage up to 6V.

2.3 Local field potentials recorded from DBS electrodes

Externalised DBS electrodes can be used to record local field potentials (LFPs) from DBS contacts placed within deep brain structures.

LFPs are a summation of synchronized dendritic, synaptic and membrane currents that are averaged over a volume of tissue extending a few millimeters from the tip of the recording electrode (Mitzdorf 1987). In the brain, they reflect the average electrical activity of thousands of neurons. The LFPs, produced by a group of cells, can also, in an indirect way, reflect the changes of membrane potential (the synaptic and action potentials), which the cells undergo coordinately (Hubbard et al. 1969). Thus studies using such potentials produce important data about the cells' average activity in the group, and is helpful for us to understand the physiological characteristics of any neural assembly (Hubbard et al. 1969). Most researchers agree that the activity of nearby neurons dominates the composition of LFPs (Bressler et al. 1993; Eckhorn et al. 1988; Roelfsema et al. 1997). Experiments on anaesthetized monkeys, involving comparing simultaneously recorded neuronal signals, including LFPs, single unit activity (SUA) and multi-unit activity (MUA), and functional magnetic resonance imaging (fMRI), from the visual cortex, have also demonstrated the functional relevance of LFPs. It was noticed that, among other signals such as MUA and SUA, LFPs showed the best correlation with BOLD fMRI signals (Logothetis et al. 2001). LFPs contains important integrative synaptic processes that cannot be captured by inspecting a few neurons' spiking activity alone, even though the signal is ambiguous and more difficult to interpret than spikes, for multiple neuronal processes contribute to the LFPs. It is now widely recognised that local field potentials can index processes and events that are causal to action potentials (Kajikawa and Schroeder 2011), and LFP changes in amplitude and frequency can reflect the changes of local neuronal activity (Buzsaki et al. 2012; Einevoll et al. 2013).

2.3.1 Signal analysis of Local field potentials

Neuronal oscillations are thought to play a key role in processing neural information. Oscillatory activity of a neuronal ensemble can respond to stimulus inputs by changes in frequency and amplitude or show a temporary interruption, which is referred to as phase resetting (Einevoll et al. 2013). Therefore, we analysed recorded signals' amplitude change by calculating their power spectra using the Fourier transform, which provides spectral power that identifies the amplitudes of sine functions of various frequencies. Moreover, neuronal oscillations are dynamic, with frequency content changing over time, so the short-time fast Fourier transforms were performed to do time-frequency analysis, mapping a one-dimensional signal in the time domain into a two-dimensional time-frequency representation of the signal.

One assumption of the Fourier transform is that the data analysed are stationary, the statistics of the data do not change over time. This is clearly not the case for LFP data: the frequency structure of neurophysiological activity changes over time. Violations of stationarity can decrease the peakiness of the result of the Fourier transform, which makes the spectral peaks for the nonstationary data less well defined, and limits its ability to estimate low frequency power. Therefore, we should be careful when reporting the results of low frequency power calculation. Wavelet approaches might have provided better short time scale low frequency estimates because the stationarity assumption of wavelet convolution is that the signal is stationary only during the time period in which the wavelet looks like a sine wave (Cohen 2014). But I also did temporally localized frequency decomposition methods, (Hilbert transform and short time fast Fourier transform) for the limitations of the Fourier transform.

Comparing oscillations between different brain regions offers insight into how distributed neuronal oscillations work together to complex brain functions. Therefore, regarding the functional connectivity between the sensory thalamus and the periaqueductal grey, we simultaneously recorded local field potentials from these two areas, and calculated spectral coherence to show their functional connectivity.

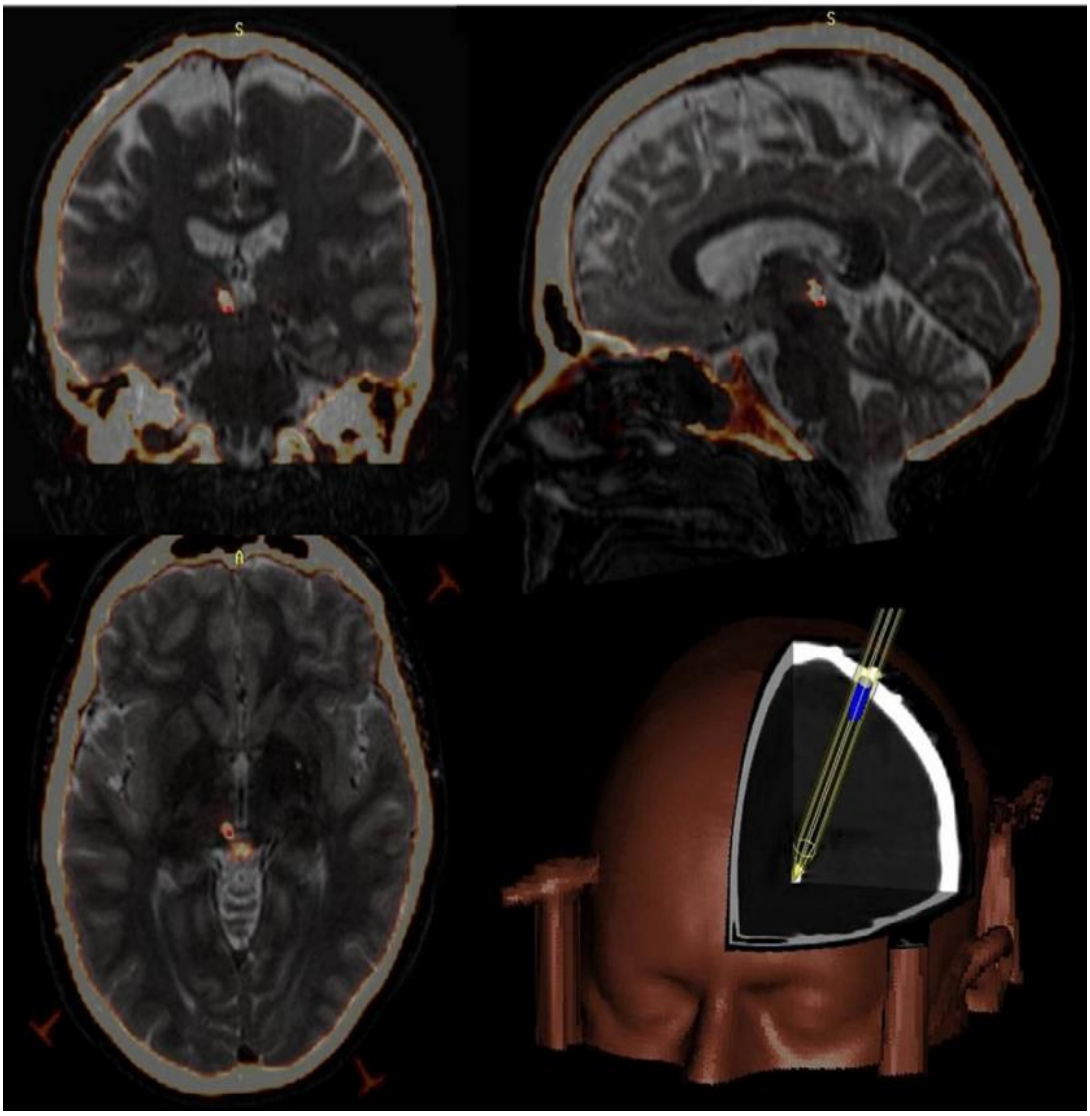


Fig 2.2 Fused MRI and CT images highlighted using heat mapping showing left PAG electrode placement.

2.4 Functional Connectivity vs Anatomical Connectivity

Cerebral connectivity can be referred to a pattern of anatomical links ("anatomical connectivity") or of statistical dependencies ("functional connectivity") between distinct units within a nervous system, and the units can be individual neurons, neuronal populations, or anatomically segregated brain regions. Structural links such as synapses or fibre pathways form the connectivity pattern. Connectivity can also represent statistical relationships measured as spectral coherence. Connectivity constrains neural activity, and by extension neural codes. Brain connectivity is therefore vital to understanding how neurons and neural networks process information.

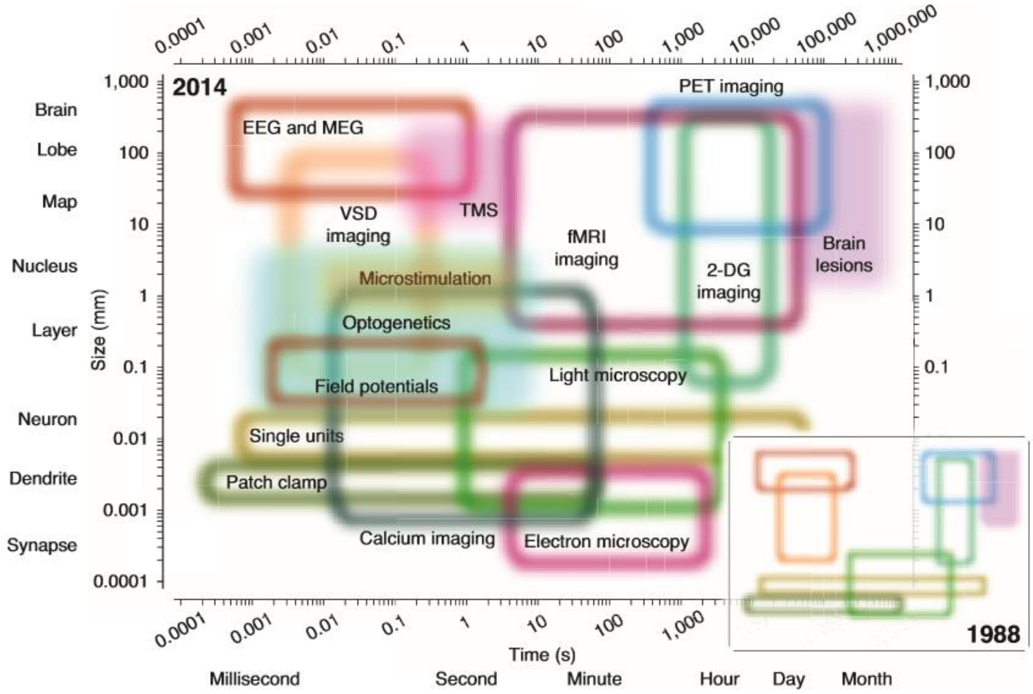
2.4.1 Methods to calculate functional connectivity

The Magnitude-Squared Coherence was used to analyse functional connectivity between the sensory thalamus and the periaqueductal grey. The power spectra, using the windowed FFT, is computed. Using the FFT-based weighted overlapped-segment averaging method, we estimate the magnitude squared coherence (MSC). Two signals x(t) and y(t) are divided into nd segments with an overlap of L; each segment is multiplied by a smooth weighting window, and then the averaged cross-spectral and the power spectral densities are obtained from the nd segments. The MSC is estimated as

$$\hat{\gamma}_{xy}^{2}(f) = \frac{\left|\sum_{i=1}^{n_d} X_i(f) Y_i^{*}(f)\right|^2}{\sum_{i=1}^{n_d} |X_i(f)|^2 \sum_{i=1}^{n_d} |Y_i(f)|^2}$$

Here, * denotes the complex conjugate, Xi(f) and Yi(f) are the FFT of the ith of the weighted segments of the stochastic processes x(t) and y(t) (Carter, 1987). $\sum_{i=1}^{nd} Xi(f)Yi\,(f)$ is the magnitude cross-spectrum between x(t) and y(t). The MSC is the normalised cross-spectral density by the power spectral density. Statistical evaluation on the significance of the coherence estimate was based on the independence threshold and the 90% confidence interval of the coherence estimates (Wang et al., 2004). The Magnitude-Squared Coherence was calculated for each recording to analyse functional connectivity between the sensory thalamus and the periaqueductal grey. This method is a phase-based connectivity analysis, which relies on the distribution of phase angle differences between two electrodes, with the idea that when neural populations are functionally coupled, the timing of their oscillatory processes, as measured through phase, becomes synchronised.

Calculation of functional connectivity using the Magnitude-Squared Coherence should give a better measure of functional connectivity than functional MRI, for the spatiotemporal resolution of fMRI is not as good as that of LFPs; from the figure below we can see that field potential studies provide a much better spatiotemporal resolution than fMRI (Sejnowski et al. 2014).



The spatiotemporal domain of neuroscience and of the main methods available for the study of the nervous system in 2014. Each colored region represents the useful domain of spatial and temporal resolution for one method available for the study of the brain. Open regions represent measurement techniques; filled regions, perturbation techniques. Inset, a cartoon rendition of the methods available in 1988, notable for the large gaps where no useful method existed[9]. The regions allocated to each domain are somewhat arbitrary and represent our own estimates. EEG, electroencephalography; MEG, magnetoencephalography; PET, positron emission tomography; VSD, voltage-sensitive dye; TMS, transcranial magnetic stimulation; 2-DG, 2-deoxyglucose.

Regarding the limitation of the thesis’s method, one is that the EEG was not recorded as well as the electrode LFPs. The aim of the thesis is to detect the functional connectivity between the sensory thalamus and the PAG in pain modulation, therefore when the experiments were planned, EEG was not considered to be recorded. However, EEG should have been measured because more information of the pain matrix would have gathered and that would have helped make the argument and experiments better.

Chapter 3 Result (1): Reciprocal interactions between the sensory thalamus and periaqueductal grey may be important for pain perception

3.1 Summary

Background. PAG stimulation can decrease pain but it is not clear whether it only decreases the incoming nociceptive signals at the level of the spinal dorsal horn. However, pain mechanism involves the brain and the spinal cord to maintain homeostasis, and with the fact that the DTI indicates the PAG and the thalamus have functional connections, so we postulated that the PAG may also modulate pain by inhibiting the sensory thalamus, and that these may also reciprocally influence the PAG.

Methods. Four patients with deep brain stimulation treatment for pain relief were studied. Each had a PAG deep brain stimulator and a second electrode inserted into the sensory thalamus. Local field potentials were recorded from the PAG electrodes when the thalamus was being stimulated, and vice versa. Power spectra of the signals were obtained and averaged evoked potential analysis was performed.

Results. Stimulating the PAG decreased the thalamic LFP delta power most, followed by theta, alpha and beta, but did not change gamma power. Stimulating the sensory thalamus increased the PAG delta LFP power most, followed by theta, but did not change alpha, beta or gamma power. The stimulation frequency with the most powerful effect in modulating the LFPs of the PAG or sensory thalamus corresponded to that eliciting the most pain relief. PAG stimulation resulted in an evoked potential in the sensory thalamus, and thalamic stimulation resulted in an evoked potential in the PAG with short latencies.

Conclusions. The PAG and the sensory thalamus interact reciprocally at short latency, which may be related to pain modulation.

3.2 Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the local Ethics Committee. Four male patients undergoing DBS for alleviation of chronic neuropathic pain gave informed consent.

Subjects

Table 3.1 shows the demographics of the patients, the aetiology of the pain and the DBS targets. Their mean age was 42.5 years. Each patient had a deep brain stimulator in the ventrolateral PAG and a second electrode in the sensory thalamus, either in the thalamic ventral posterolateral (VPL) or ventral posteromedial (VPM) nucleus.

Gender	Age	Diagnosis	Trial targets	Responsive targets	Best responsive targets	VAS before op	VAS after permanent implant	History of pain	Pain distribution
Male	44	Phantom limb, left leg	R PAG R VPL	R PAG R VPL	R VPL 30&50 Hz	7	2	5 years	Left phantom leg
Male	39	Trigeminal neuralgia	R PAG RVPM	R PAG R VPM	R PAG 20Hz	6	2	13 years	Left supra-orbital
Male	49	Failed back surgery	L PAG L VPL	L PAG L VPL	L VPL 50 Hz	8	4	8 years	Back Right lower leg
Male	38	Phantom limb, left arm	R PAG R VPL	R PAG R VPL	R PAG 20 Hz	8	1	5 years	Left phantom arm

Table 3.1. Summary of patient demographics, diagnoses and stimulation parameters. Trial targets- the anatomic sites where DBS electrods were inserted and stimulated. Responsive targets - patients felt pain relief when these nuclei were stimulated. Best responsive targets - the most effective site for pain-relief, where the permanent DBS electrode was implanted. VAS= visual analog scale for pain (10: most severe; 0: no pain). VPL= thalamic ventral posterolateral nucleus. VPM=thalamic ventral posteromedial nucleus. PAG=periaqueductal grey.

Measurements

We recorded LFPs simultaneously from both the thalamus and the PAG at rest, and during stimulation at either site. These recordings were phase-locked so that we could determine temporal relationships between areas. Each electrode has 4 circumferential contacts placed linearly, and therefore each electrode can yield three bipolar recordings. We did bipolar recordings in all the patients, and in two of them we also made unipolar recordings, using left or right mastoid as the reference. In total we report results from 12 bipolar and 8 unipolar recordings. The stimulation parameters were: frequency, 5Hz to 50Hz; pulse width, 200 to 450µs; and amplitude, 0.5 to 3V.

Before experiments a full range of combinations of contacts, frequencies, pulse width and amplitude were trialled to determine the contacts and parameters providing best subjective analgesia. The deep brain stimulator was initially turned off for at least 10 min prior to experiments. We started with 5-min LFP recordings from PAG and VPL/VPM without stimulation. Then we recorded thalamic LFPs with PAG stimulator on at the contact determined and with various frequencies. In each frequency state we recorded for 5 min, and between each state we stopped recording for 5 min. We recorded PAG's field potentials with the same method.

These experiments are separate to the 'externalised trial' to assess pain relief which was carried out over a period of one week. During this period, different combinations of contacts, and electrical parameters were assessed in relation to immediate pain relief (within 5 -10 minutes). 'Effective' settings were generally tried for several hours or even 1-2 days to confirm pain relief prior to the implantation of the pulse generator.

Signal processing and statistical analysis

LFPs were first amplified × 10,000, and then digitised (CED 1401 mark II, Cambridge Electronic Design, Cambridge, UK) at a sampling rate of 20k Hz.

Power spectra were calculated using the fast Fourier transform (sliding time-window 5s with 3s overlap). In each recording, three 25-second segments were analysed and the three spectra were averaged.

Power spectra were calculated from the LFP recordings for five frequency bands: delta (0-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz) and gamma (30-90Hz). Large respiratory and blood pressure artefacts contaminated power at 0-1.5 Hz and were therefore removed (Xie et al. 2006). We removed power at 19-21 Hz when we calculated beta power of PAG and thalamic LFPs under 20Hz thalamic and PAG stimulation respectively. In addition, we removed power at 39-41Hz, 59-61Hz and 79-81Hz when calculating gamma power under 20 Hz stimulation condition, and removed power at 49-51Hz when calculating gamma power under 50Hz stimulation condition. Power during stimulation periods was normalized by dividing it by resting state power. Thus a normalized power of greater than 1 indicates that stimulation increased it, and less than 1 indicates a decrease.

In addition to the spectral analysis, we also performed evoked potential analysis to determine the latency of peak responses to stimulation. Continuous LFP data were analysed. LFP epochs containing the deep brain stimulation artefacts were extracted. We removed trials contaminated by artefacts due to gross movements. To identify evoked potential components, LFP sweeps time-locked to the stimulation artefacts were averaged. Each LFP recording had thousands of trials, depending on the stimulation frequencies.

Statistical significance was assessed using paired t-tests or the Wilcoxon signed ranks test, if the data were not normally distributed, as assessed by the Kolmogorov-

Smirnov test. In the PAG none of the normalised powers were normally distributed; therefore the Wilcoxon signed ranks test was used. However, the normalised powers in the thalamic local field potentials in all but the beta and gamma bands were normally distributed, so paired t-tests were performed. The Wilcoxon signed ranks test was used for the beta and gamma bands.

Averaged evoked potential analysis was performed using Spike II software® (version 5, Cambridge Electronic Design, Cambridge, UK).

Computation was carried out using MATLAB® (R2010b SP1, Math-Works Inc., Ma, USA) and SPSS (Version 20, SPSS Inc., IL, USA). All p values are two-tailed and adjusted in accordance with the numbers of comparisons (significant p value =0.05/n).

3.3 Results

Effective DBS targets in pain relief

Stimulation in either the PAG or sensory thalamus relieved pain to different degrees in different patients. Two experienced the most relief with PAG stimulation at 20 Hz, and less with thalamic stimulation. The other two had the most relief with thalamic stimulation at 30 or 50 Hz, and benefitted less from PAG stimulation (Table 3.1). PAG stimulation at 50 Hz made the pain worse in these four patients.

Power spectrum analysis

Because the patients felt 20 Hz PAG stimulation best and 50 Hz worst in pain relief, so we compare the LFP changes between these two frequencies. PAG stimulation at 20 Hz decreased the power of thalamic LFPs in the delta (1.5-4Hz, paired t-tests, n=20, P=0.001), theta (4-8Hz, paired t-tests, n=20, P<0.001), alpha (8-13Hz, paired t-tests, n=20, P<0.001) and beta band (13-30Hz, Wilcoxon signed ranks test, n=20, P=0.014) but not gamma, with the largest changes occurring in the delta band (Fig 3.1b, 1d). But at 50 Hz PAG stimulation only decreased the power of thalamic LFPs significantly in the theta and alpha bands (paired t-tests, n=20, p = 0.003 and 0.002, respectively). 20 Hz stimulation of the PAG was significantly more powerful in suppression of thalamic LFPs than 50 Hz in all frequency bands that we examined except beta and gamma (Fig 3.1b, 1d, paired t-tests, n=20, P=0.004, 0.002, 0.017, respectively).

Sensory thalamus stimulation at 50 Hz significantly increased the power of PAG local field potentials in both the delta and theta bands (Wilcoxon signed ranks test, n=20, p = 0.002); the greatest increase was in the delta band (Fig 3.1c, 1e). Moreover, 50 Hz stimulation was significantly more effective than 20 Hz in the delta, theta and alpha bands (Fig 1c, 1e, Wilcoxon signed ranks test, n=20, P=0.03, <0.001, =0.006, respectively).

Peak response latency of averaged evoked potentials

PAG stimulation resulted in an evoked potential in the sensory thalamus, and thalamic stimulation resulted in an evoked potential in the PAG. These latencies were about 5 milliseconds (Figure 3.2), with no significant differences between targets and stimulation frequencies (PAG stimulation: at 20 Hz, mean 4.39, s.e.m

0.84; at 50Hz, mean 4.26, s.e.m 1.02; sensory thalamus stimulation: at 20Hz, mean 4.85, s.e.m 0.84; at 50Hz, mean 4.15, s.e.m 0.59).

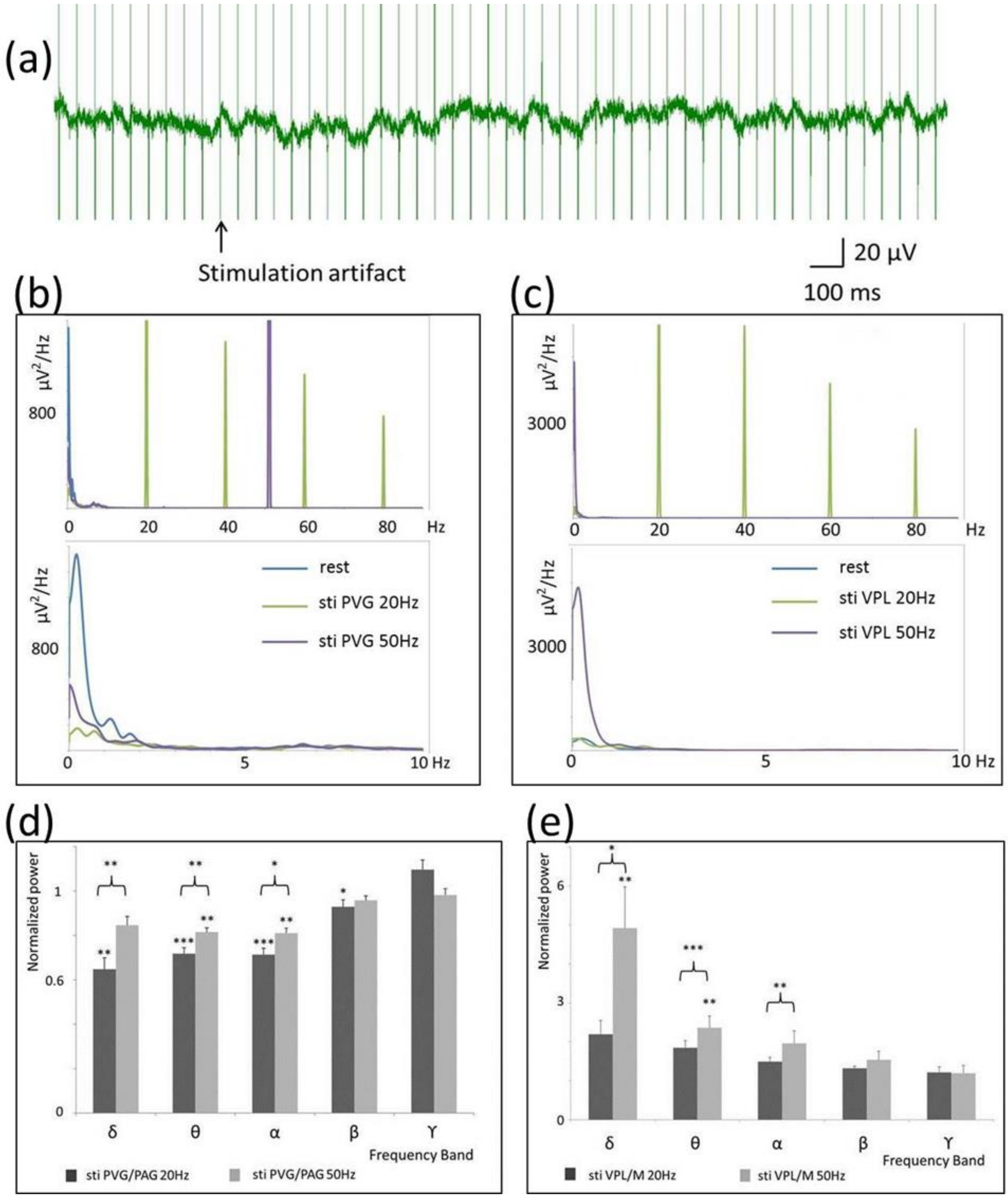


Fig 3.1 Power spectra analysis of local field potentials of the sensory thalamus and the periventricular/periaqueductal grey. (a) shows an example of LFP recording of

the PAG during sensory thalamus stimulation at 20 Hz. Thalamic stimulation artefacts were superimposed on the recording. (b) Example presentation of power spectra change of sensory thalamus LFPs during PAG stimulation at various frequencies. Upper part: power at 0 to 90 Hz; lower: 0 to 10 Hz. (c) Example presentation of power spectra change of PAG LFPs during sensory thalamus stimulation at various frequencies. Upper part: power at 0 to 90 Hz; lower: 0 to 10 Hz. (d) Mean of normalized power recorded from the sensory thalamus during PAG stimulation at 20 or 50 Hz. Paired t-tests, except beta band (Wilcoxon signed ranks test). (e) Mean of normalised power in the PAG recorded during sensory thalamus stimulation at 20 or 50 Hz. Wilcoxon signed ranks test. n=20. * P<0.05. ** P<0.01. *** P<0.001, two tailed. Error bars, s.e.m.

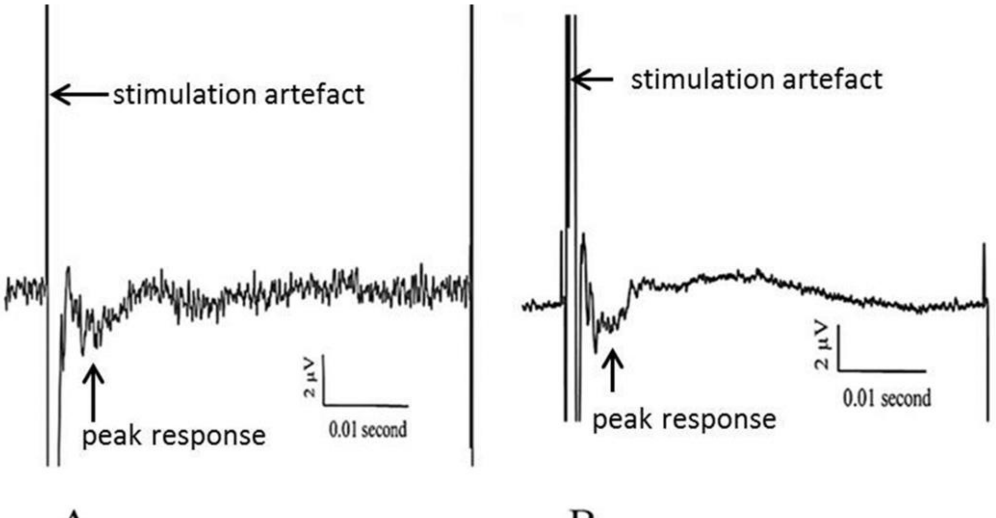


Fig 3.2. Examples of averaged evoked potentials, from the patient with left phantom leg. A. Thalamic evoked potential following PAG stimulation at 20Hz. B. PAG evoked potential following thalamic stimulation at 20 Hz.

Chapter 4 Result (2): Functional connectivity between the sensory thalamus and the periaqueductal grey in pain modulation

4.1 Summary

Background. DBS of the PAG and the sensory thalamus has reveal the reciprocal interaction between these two areas in pain modulation under DBS condition. But we wanted to ask, without exogenous modulation by DBS, how the interaction between these two areas goes with various pain conditions.

Methods. Eight patients with deep brain stimulation treatment for pain relief were studied. Each had a PAG deep brain stimulator and a second electrode inserted into the sensory thalamus. Local field potentials were recorded from both electrodes, when the patients were at rest or having peripheral ice stimulation to induce pain.

Results. 100-150 Hz gamma spectral coherence between the PAG and the sensory thalamus was noted, and this gamma coherence was diminished with increased pain. Time-frequency analysis of the LFPs did not show significant power change along with pain variation.

Conclusions. Functional connectivity between the sensory thalamus and the PAG can be revealed in usual physiological state without DBS. The decrease in the gamma spectral coherence with increased pain suggests the possibility that the PAG and the sensory thalamus usually maintain a stable interaction for homeostasis, but when in sudden pain situation, the coherence diminishes, which suggests the balance breaks

and the body will initiate proper responses to counter the imbalance, to regain homeostasis.

4.2 Methods

Measurements

We had eight chronic neuropathic pain patients taking part in the study. We recorded LFPs simultaneously from both the thalamus and the PAG at rest, and during ice stimulation at patients' painful sites. We did bipolar recordings in all the patients. The stimulation parameters were: frequency, 5Hz to 50Hz; pulse width, 200 to 450µs; and amplitude, 0.5 to 3V.

Experiment Protocol

Experiments took place in a quiet room, kept at a constant 22 degrees Celsius. Patients sat in a comfortable chair in a semi-recumbent position. Local field potentials were simultaneously recorded with bipolar configuration from the adjacent four circumferential contacts of each DBS macroelectrodes. Signals were filtered at 0.5-500 Hz and amplified (x10,000) using isolated CED 1902 amplifiers and digitised using CED 1401 Mark II at a rate of 4K Hz (Cambridge Electronic Design, Cambridge, UK). LFPs were then displayed online and saved onto hard disk using Spike II software® (version 5.0, Cambridge Electronic Design). Each experiment was started with 10 minutes of rest prior to recording. Recording of LFPs then lasted 10 minutes during which time Visual Analogue scale of pain was recorded every 60 seconds.

Patients were sitting comfortably for 10 minutes before recording started. First patients had several minutes of rest, and then external physical stimuli by ice were applied to patients' pain areas to intensify or induce their pain, and then stopped the stimuli to relieve the induced pain. Therefor the recording has sections of patients' pain and rest states.

Signal analysis

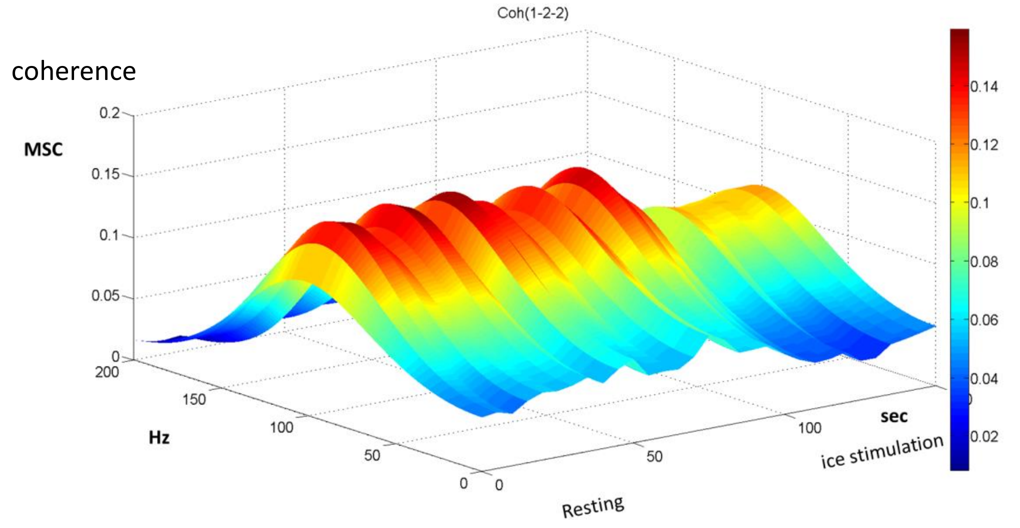
The Magnitude-Squared Coherence (see chapter 2) was calculated and STFT time-frequency power spectral analysis was also performed for each recording to analyse functional connectivity between the sensory thalamus and the periaqueductal grey and power change along time and various pain state.

Computation was carried out using MATLAB® (R2010b SP1, Math-Works Inc., Ma, USA).

4.3 Results

The most obvious spectral coherence between the sensory thalamus and the periaqueductal grey is in gamma frequency range, around 100-150 Hz. It is noted that when patients were having increased pain due to ice stimulation on painful sites, their gamma spectral coherence decreased, compared with that during the resting state. The finding was consistent throughout the eight patients.

The following figures are those eight patients' spectral coherence time-frequency images, which demonstrates that the aforementioned pain-induced coherence decrease is consistent in those eight patients.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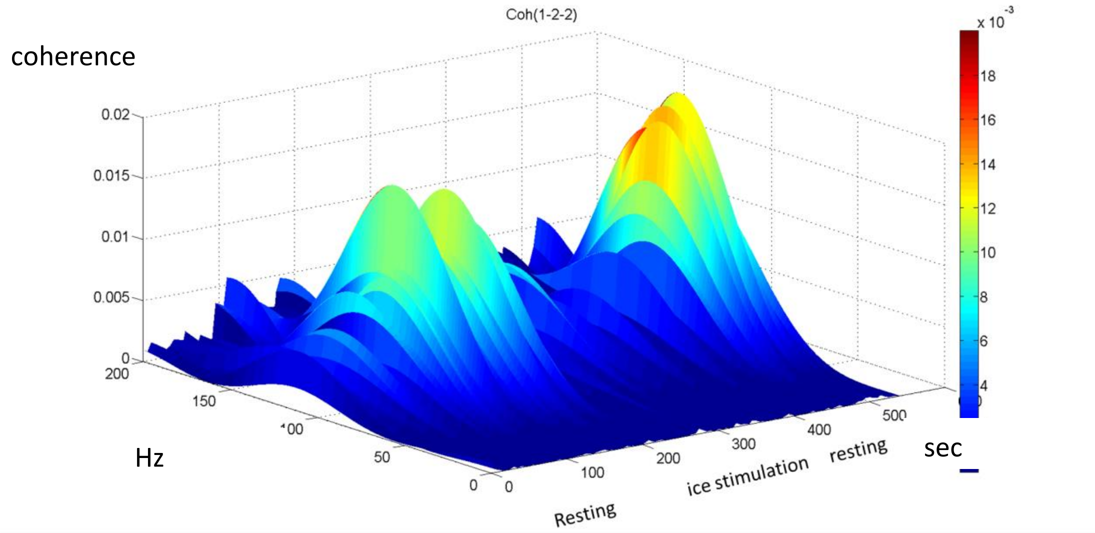
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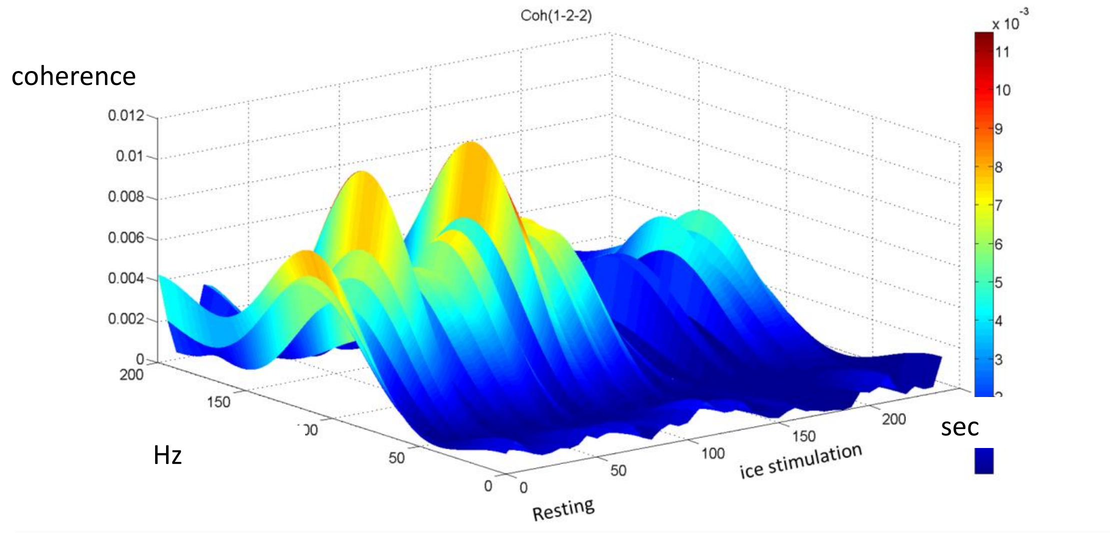
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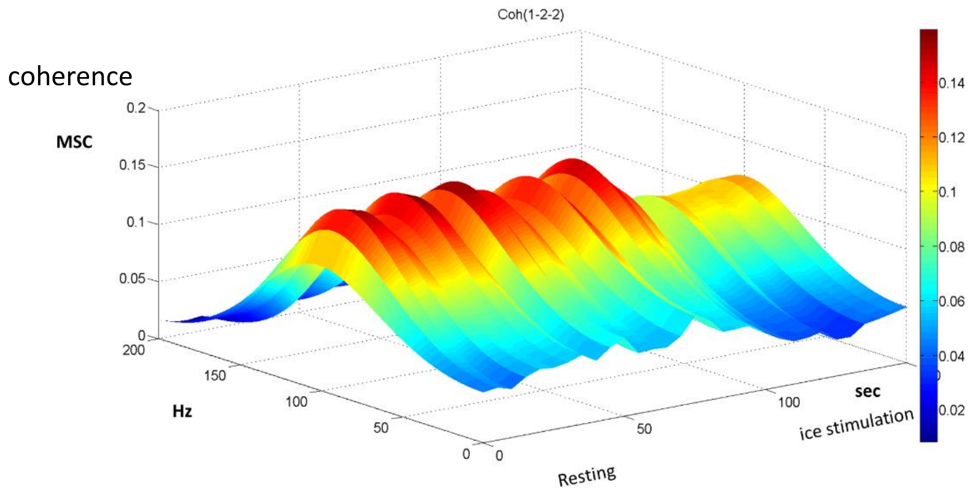
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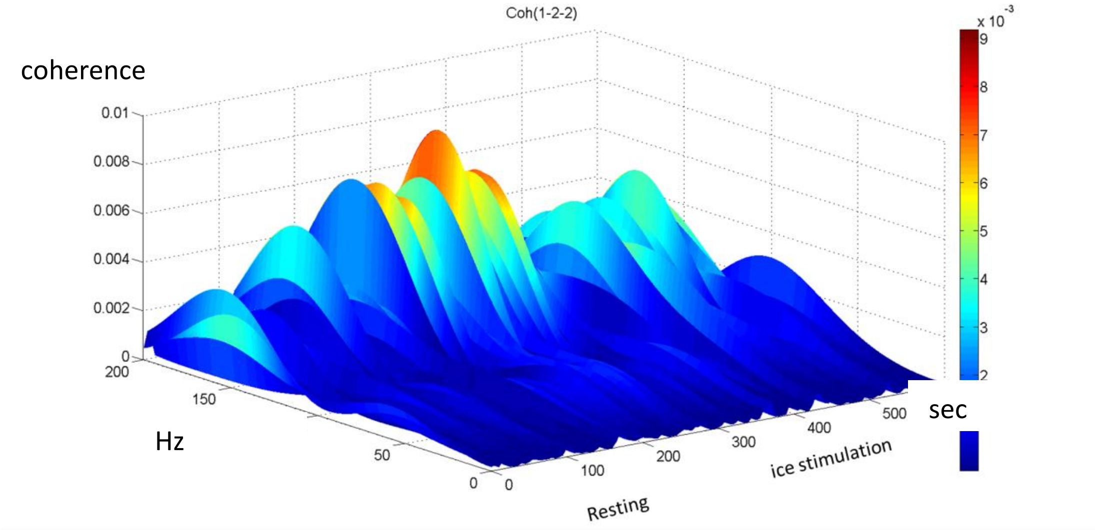
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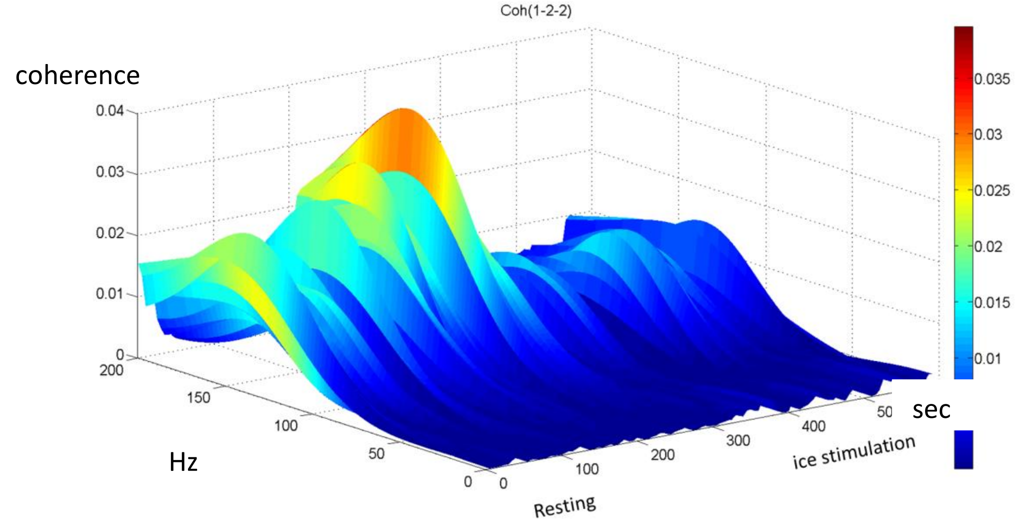
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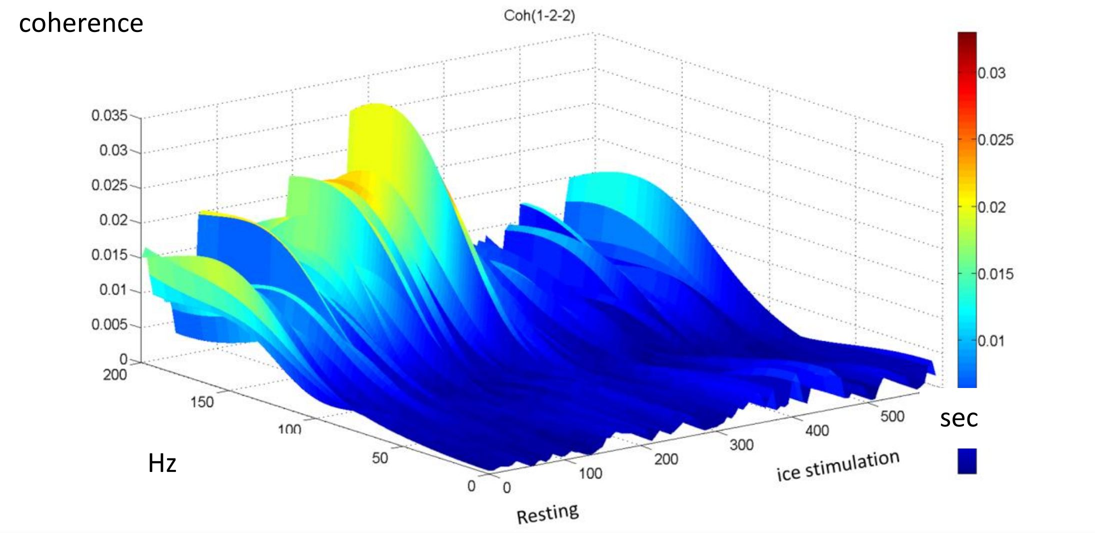
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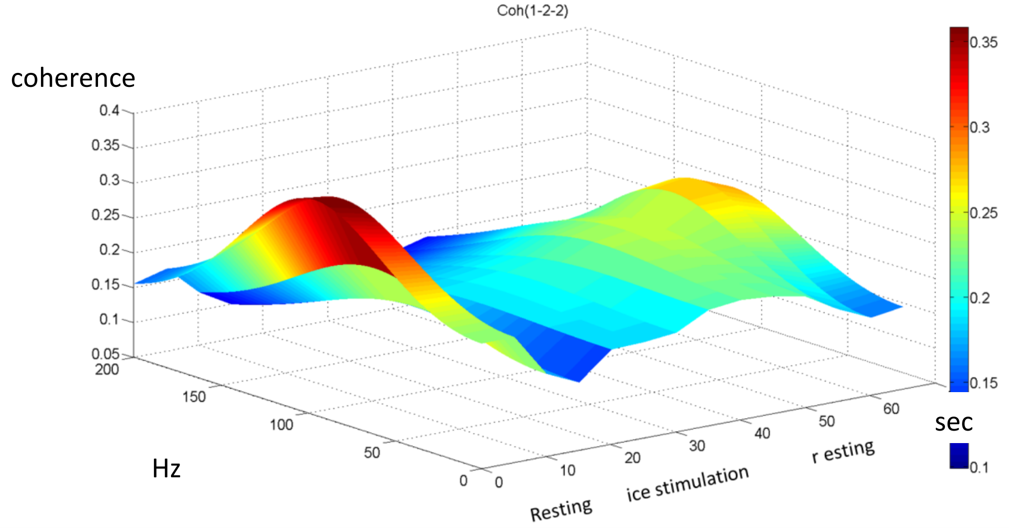
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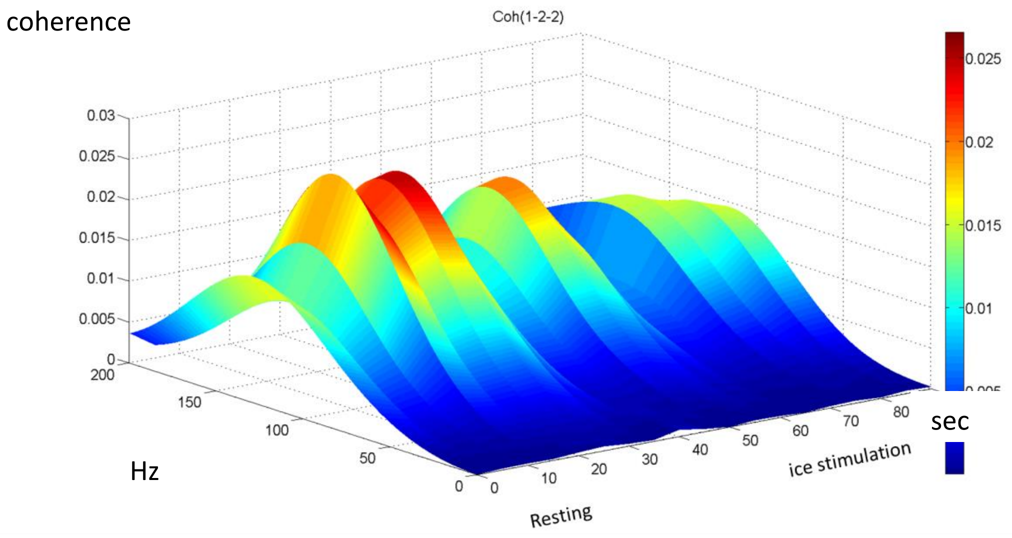

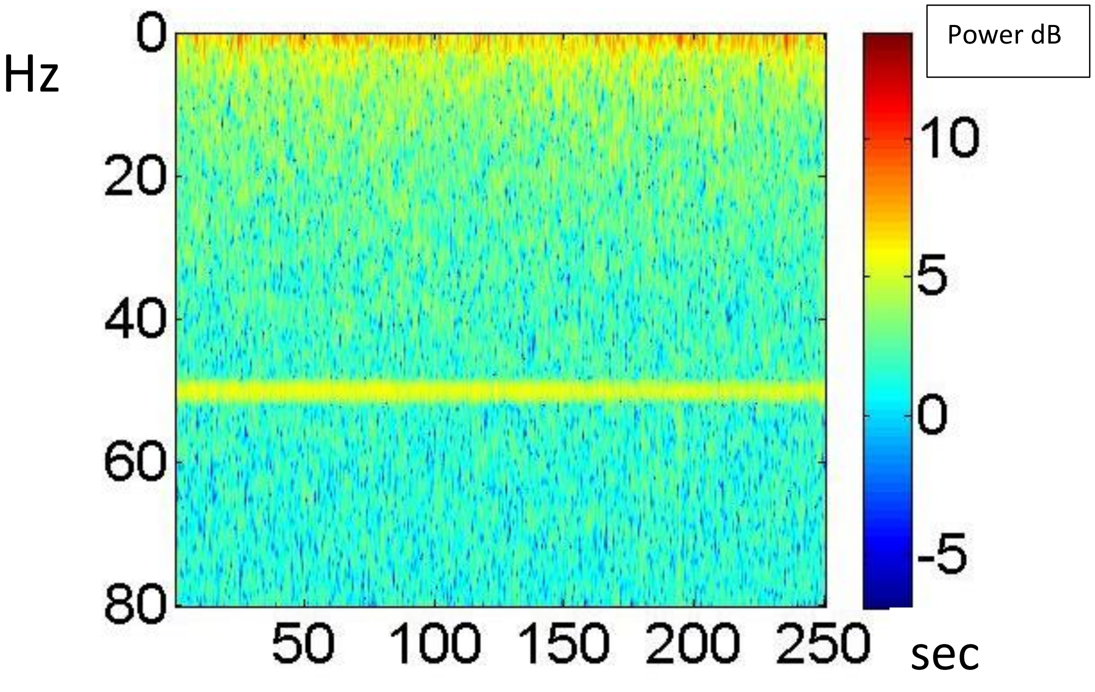

An example of STFT time-frequency image showing power change along time. From the figure we did not find power change during the period of experiment.

Chapter 5 Result (3): The effect of general anaesthetic propofol on the functional connectivity between the sensory thalamus and the periaqueductal grey

5.1 Summary

Background. Our previous experiments, in chapter 3 and 4, suggest that the interaction between the sensory thalamus and the PAG might be important in pain modulation and maintaining homeostasis. Moreover, literature of anaesthesiology has shown that the consciousness and pain sensation can be separable. Thus we wanted to test if the pain-related functional connectivity between the sensory thalamus and the PAG would be affected by general anaesthetic propofol.

Methods. We analysed a set of local field potential data, recorded, during the induction period of general anaesthesia using propofol, from two patients' PAG and sensory thalamus electrodes and two patients' bilateral anterior cingulate cortex electrodes. We did time-frequency power spectral analysis to see power change along time, and we also compared the difference between the ACC and PAG in terms of power change to propofol.

Results. Spectral coherence analysis shows that gamma coherence between the PAG and the sensory thalamus was not only maintained, but also enhanced. Time-frequency analysis shows that propofol increased alpha and decreased gamma synchronisation in the areas we studied. Statistics show that, between these regions, the ACC had a significantly different increased alpha activity from other regions.

Conclusions. Our result suggests that the functional connection between the sensory thalamus and the periaqueductal grey will not be inhibited during the induction period of general anaesthesia using propofol, which is compatible with the fact that consciousness mechanism and pain processing could be separable, and propofol enhances GABA inhibitory activity.

5.2 Introduction

In previous chapters we have demonstrated the importance of the interaction between the sensory thalamus and the periaqueductal grey in pain modulation, and have shown how their functional connectivity responded to different pain state. In this chapter we want to test whether the functional connectivity between the PAG and the sensory thalamus would change if the participants are not conscious. Our participants needed to have general anaesthesia to receive the second stage of DBS operation so we had the opportunity to investigate the effect of general anaesthetics on these two areas' functional connectivity.

Regarding the mechanism of general anaesthesia, it is still not totally clear how general anaesthetics affect the brain and then cause loss of consciousness. It has been argued that consciousness depends on intact function of brainstem-thalamocortical arousal circuits, and anaesthetic-induced unconsciousness may be caused by disruption of neuronal information integration, in which the thalamocortical system plays an important role (Alkire et al. 2008; Emery N. Brown et al. 2010; Emery N. Brown et al. 2011b; Franks 2008; G. A. Mashour 2013a). The general anaesthetic *propofol* is thought to enhance GABA-mediated postsynaptic inhibition in the cortex, the thalamic reticular nucleus, and the arousal centres in the midbrain and pons, to cause unconsciousness (Alkire et al. 2008; Emery N. Brown et al. 2010; Emery N. Brown et al. 2011b; Franks 2008).

It is suggested that the binding of propofol to the $GABA_A$ receptor is the "root" cause of unconsciousness, while impaired corticocortical connectivity, induced by the root cause, could be the "proximate" cause, which more directly results in the observed unconsciousness (G. A. Mashour 2013a). Recent studies reveal that propofol induces a frontal alpha rhythm at dose levels enough for loss of consciousness (Ching et al. 2010; Feshchenko et al. 2004; Vijayan et al. 2013), and blocking of intracortical communication by hypersynchronous alpha ongoing activity is a key mechanism for the loss of consciousness (Supp et al. 2011), which suggests that increased alpha activity plays an important role in the "proximate" cause. That said, it is not clear how electrical oscillation activities in subcortical areas, such as the thalamus or PAG, is affected by propofol.

Animal studies (Behbehani et al. 1990) showed that, when injection of GABA into the PAG, its descending pain inhibition activity increased. So in this study we wanted to see if propofol, a GABA against, could affect the PAG and its functional connectivity with the sensory thalamus

5.3 Methods

The experimental procedures and protocols used in this study were reviewed and approved by the local Ethics Committee. Ten patients undergoing DBS operations gave informed consent.

This study involved recording and analysing LFPs to measure changes induced by propofol. The nuclei were those suitable for deep brain stimulation surgery for patients with chronic medicine-refractory pain.

Study Participants

We studied four patients with chronic neuropathic pain who underwent DBS surgery. From these patients we obtained LFPs from the sensory nuclei of the thalamus, the ACC and the PAG.

Surgical Technique and Targeting

In brief, patients had a Magnetic Resonance Image brain scan prior to the day of surgery. Slice thickness was 2.0 mm. Targeting was based on T1-weighted axial images. On the day of surgery, the Cosman-Roberts-Wells^{TM} stereotactic frame was applied to the patients' scalp under local anaesthetic (10ml 0.25% bupivacaine mixed with 10 ml 2% lidocaine with 1:200000 adrenaline). A stereotactic Computed Tomography (CT) scan was then performed (1.9mm slice thickness, zero gantry tilt). The pre-operative MRI was fused to the stereotactic CT using the Radionics Image Fusion^{TM} and Stereoplan^{TM} software (Radionics, Burlington, MA), and the electrode trajectory was selected to avoid possible penetration of the surface vessels on the cortex and the lateral ventricle. After preparing the patient's scalp with cetrimide® and alcoholic chlorhexidine® solution, 20 ml of local anaesthetic (as above) was injected into the scalp on the side of targeting. Curved incisions were made over the coronal sutures, with the base of the flap posteriorly. Next, a 2.7 mm twist-drill craniostomy was performed, passing through skull and dura. A Radionics^{TM} electrode of 1.8 mm diameter and 2.0 mm exposed tip was slowly passed to the target while the impedance values were monitored for a sudden drop in impedance value from 500-600 Ω to under a few tens of Ω, suggesting possible penetration of a ventricle. The Radionics^{TM} electrode was replaced by a Medtronic 3387® electrode (Medtronic Inc., Minneapolis, USA). Test stimuli were applied at <3.0 V in amplitude, 120 μs in pulse width and 10-50 Hz in frequency to check for a warm feeling or paraesthesia in the area of pain or pain suppression, and abnormal eye movements. Once clinical efficacy had been established, the DBS electrode was fixed onto the skull with a titanium bioplate^{TM} and externalised for further investigation. The wounds were then sutured. If the patient had experienced good clinical effect, the whole stimulation system was then internalised one week later in a second procedure under general anaesthesia. This involves insertion of extension leads (Medtronic) and an implantable pulse generator (IPG – kinetra^{TM}, Medtronic) that is placed in a subcutaneous pocket in the chest wall or abdomen. The laboratory-based pain experiments were performed in the week between these two procedures. We localised all electrodes on post-operative CT fused to pre-operative MRI to confirm location.

Experimental Protocol and Measurements

We started LFP recording while patients were still fully conscious, before induction was initiated (Fig. 5.1). The recordings lasted until the patients were intubated, so each recording contains patients' brain signals in different consciousness states.

Each DBS electrode has 4 circumferential contacts (placed linearly and spaced 1.5mm apart), and therefore each electrode can at most yield three bipolar recordings and four unipolar recordings.

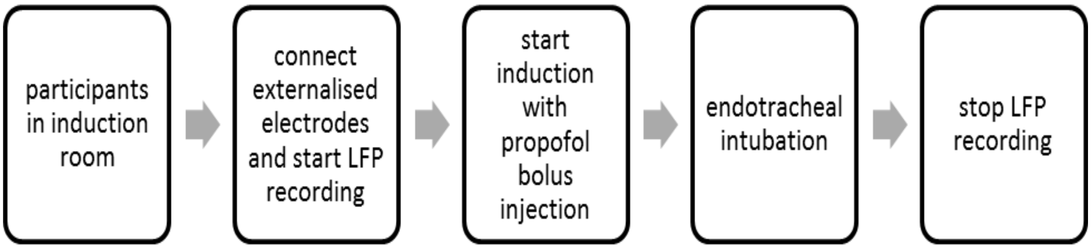

Fig. 5.1 Flow chart of the experiment. The LFPs were recorded from the period when participants were conscious to the period when they were intubated with loss of consciousness.

Signal processing and statistical analysis

All signals were sampled at 2048 Hz, and amplified and digitised using TMSi Porti and its respective software (TMS International). Data were analysed MATLAB (MathWorks)-based porgrammes.

To do time-frequency analysis of power spectra of the signals, we used the short time Fourier transformation to analyse the whole length of each recording. We also calculated power spectra density using the fast Fourier transform (sliding time-window 5s with 3s overlap). Power in various frequency bands studied was obtained by calculating the area under the curve of the spectrum.(Xie et al. 2006). Time-frequency images showed changes in certain frequency bands between different periods of time, and we then calculated the powers in these bands and periods and statistically analysed (see below). In addition, powers of these bands were normalized by dividing them by the total power of each signal recording (whole areas under the spectrum curve), and then the normalised powers were compared between various subcortical areas to examine if there was any difference of certain oscillation bands between these areas.

We also performed instantaneous frequency analysis using Hilbert transformation, with extraction of 8-90Hz oscillation from each signal recording to analyse frequency modulation during induction of general anaesthesia.

In order to calculate the functional connectivity we performed spectral coherence (magnitude squared coherence) analysis, based on FFT with a Hanning window of length 2s and overlap of 0.5s, to measure the degree of synchrony in the neuronal oscillations of two brain regions.

Statistical significance of power differences between pre-induction and induction period of time in each of the brain regions were assessed using paired t-tests or the Wilcoxon signed ranks test, if the data were not normally distributed, as assessed by the Kolmogorov-Smirnov test. Statistical significance of the normalised oscillation power differences between brain areas were calculated using one-way ANOVA.

Statistics was carried out using SPSS (Version 20, SPSS Inc., IL, USA). All p values are two-tailed and adjusted in accordance with the numbers of comparisons (significant p value =0.05/n).

5.4 Results

Time-frequency analysis of signals

Using the short-time Fourier transform we produced a time-frequency image showing the transition of power change in oscillation frequency against time (Fig 5.2, 5.3). From these images it can be seen that propofol increased α and decreased γ power in those brain areas studied, but it is not clear whether γ power decrease followed α power increase or not. Regarding the time interval between the start of propofol induction and the emergence of alpha activity, there was no obvious difference between brain regions. We also calculated instantaneous frequency by Hilbert transform, which showed that propofol modulated LFP oscillation frequencies and induced alpha hypersynchronisation (Fig 5.2).

Statistics of power changes

We calculated powers of alpha and gamma frequency bands in the time period before propofol infusion and in the time period with increased alpha activity, after propofol induction. Statistical analysis showed that the spectra changes revealed from time-frequency images were statistically significant (Fig 5.4). We also calculated and compared the normalised alpha power and gamma power from the time periods aforementioned to see if there were any power differences between the various regions (Fig 5.5). Before induction, when patients' consciousness was still intact, the normalised alpha power between the brain areas did not show significant differences. However, during propofol induction, when alpha oscillation was induced, the ACC showed significantly different induced alpha activity (Fig 5.5). On the other hand, before induction, the ACC's normalised gamma power was significantly different from other regions, but this difference disappeared after propofol induction.

Spectral coherence change

It was observed that spectral coherence between the PAG and the sensory thalamus increased in high gamma range during the propofol induction period (Fig 5.6A). On the other hand, the coherence between bilateral ACC was more stable along the induction period, and did not show changes as substantial as those of PAG/thalamus. Also the bilateral ACC demonstrated a difference frequency range of spectral coherence (0-80Hz) (Fig 5.6B).

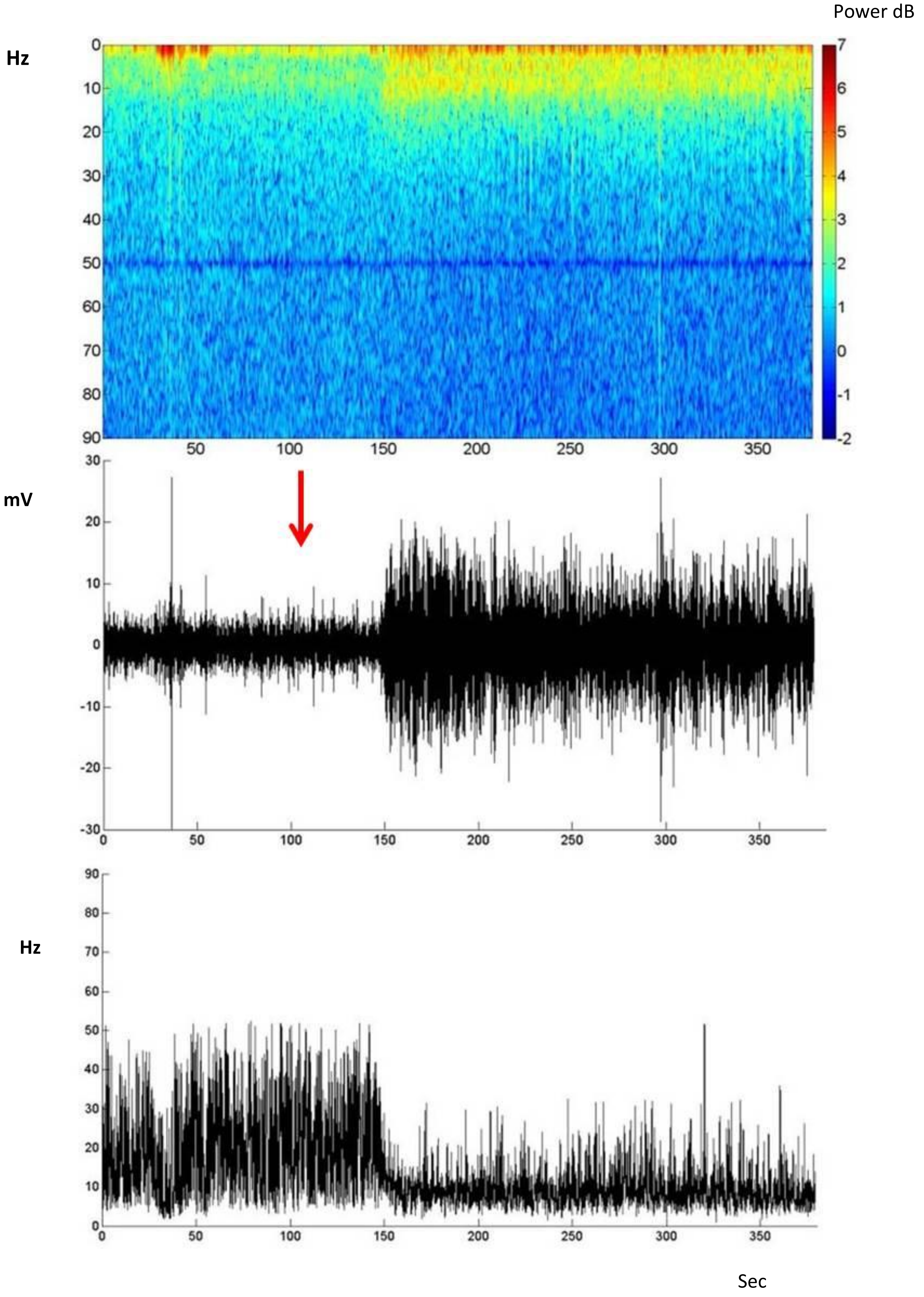
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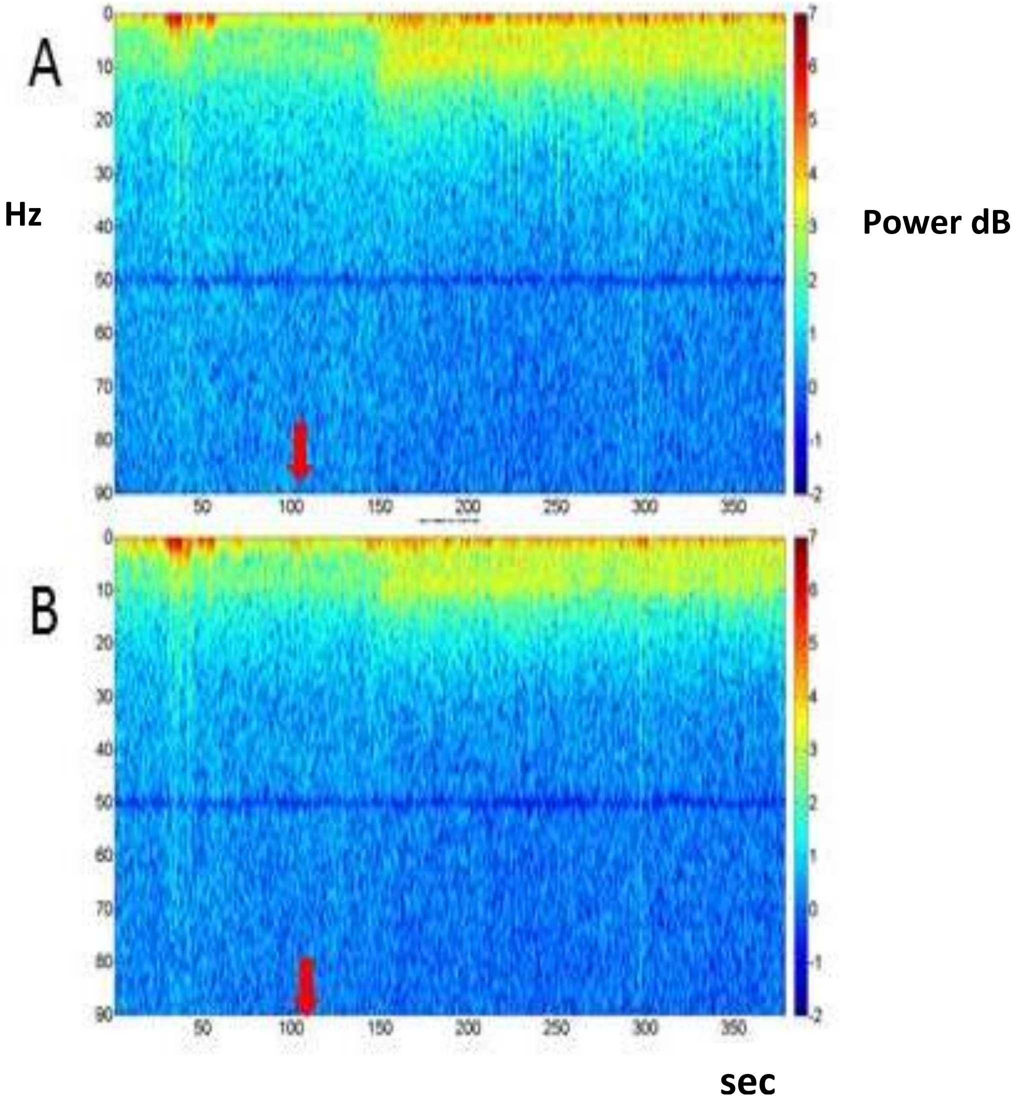
Fig 5.2. Example of time-frequency analysis of LFPs recorded from vPAG. The x-axis indicates time (sec), and the red arrow indicates the time of propofol bolus injection. The top picture shows the power spectra change against time, revealing an obvious alpha power increase occurring within 50 sec after propofol injection. The picture in the middle is the extraction of 8-90 Hz frequency range of the same LFP signal. We used the extraction to perform instantaneous frequency analysis (Hilbert transformation) to get the result, shown as the bottom picture, showing that the oscillation frequencies of the signal were modulated from a status of wide frequency range to an alpha dominant stage.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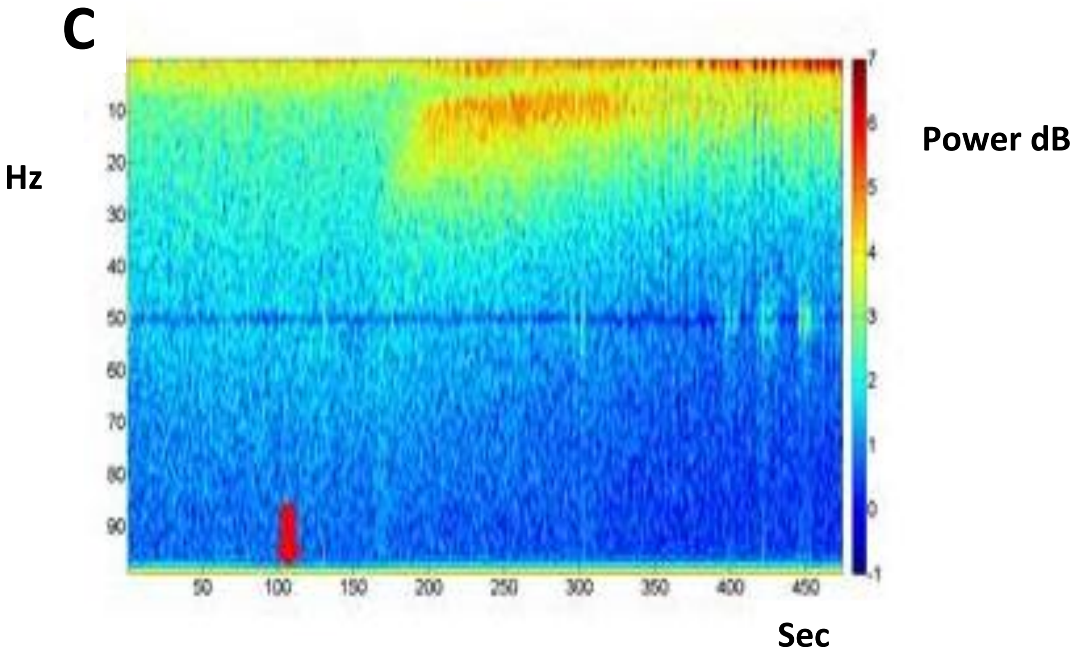
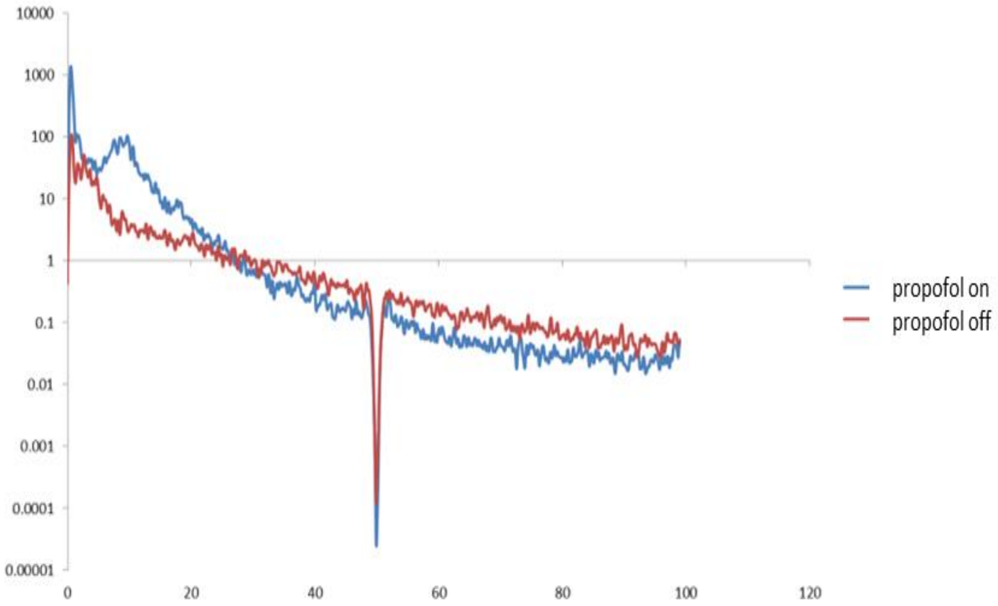


Fig 5.3 Time-frequency images of LFP changes in various brain regions. A) PAG; B) sensory thalamus; C) ACC. Red arrows indicate the time when propofol induction started. x axis: time (sec); y axis: frequency (Hz)

A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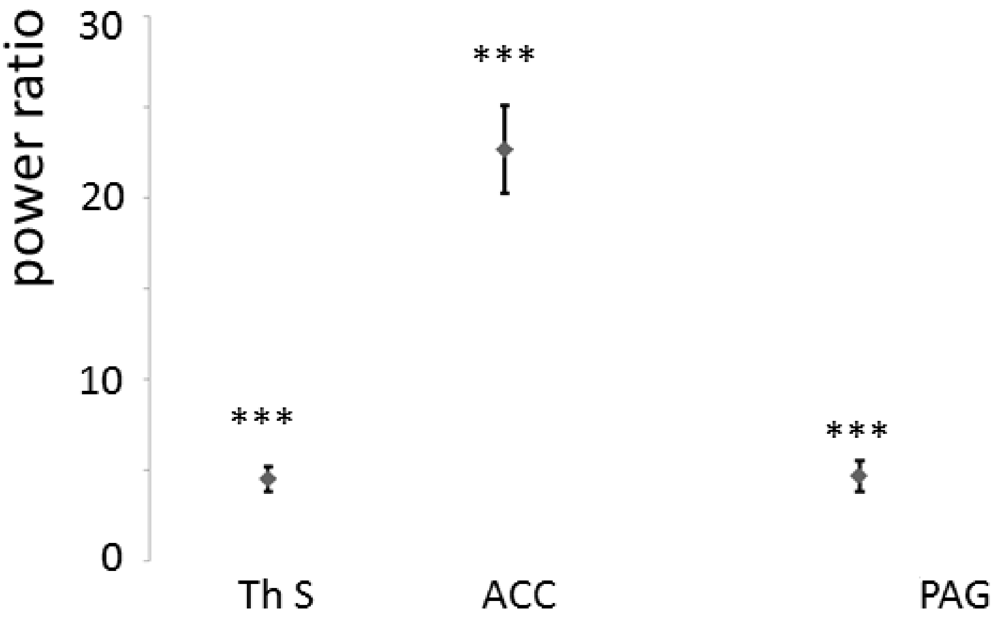
B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PAG

C

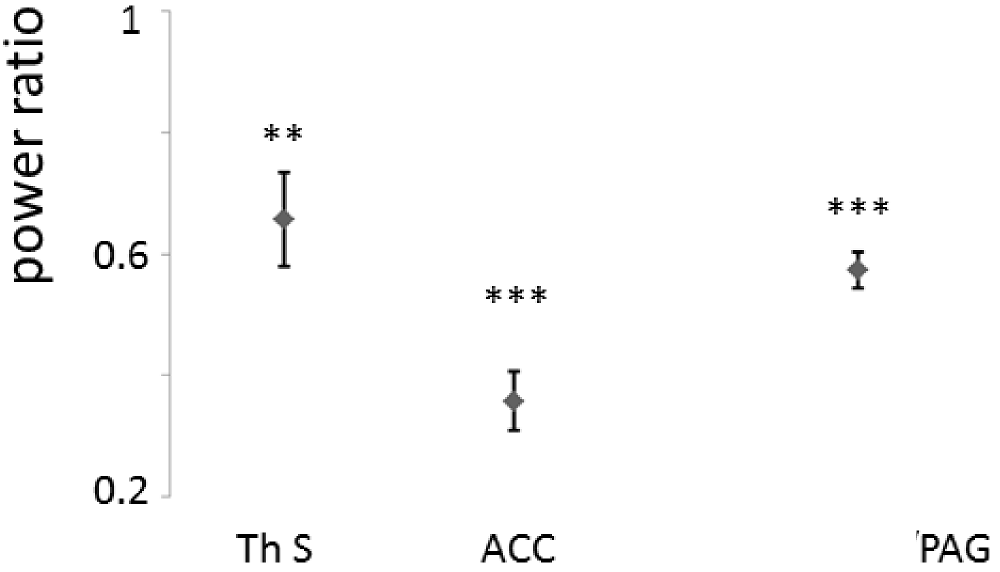
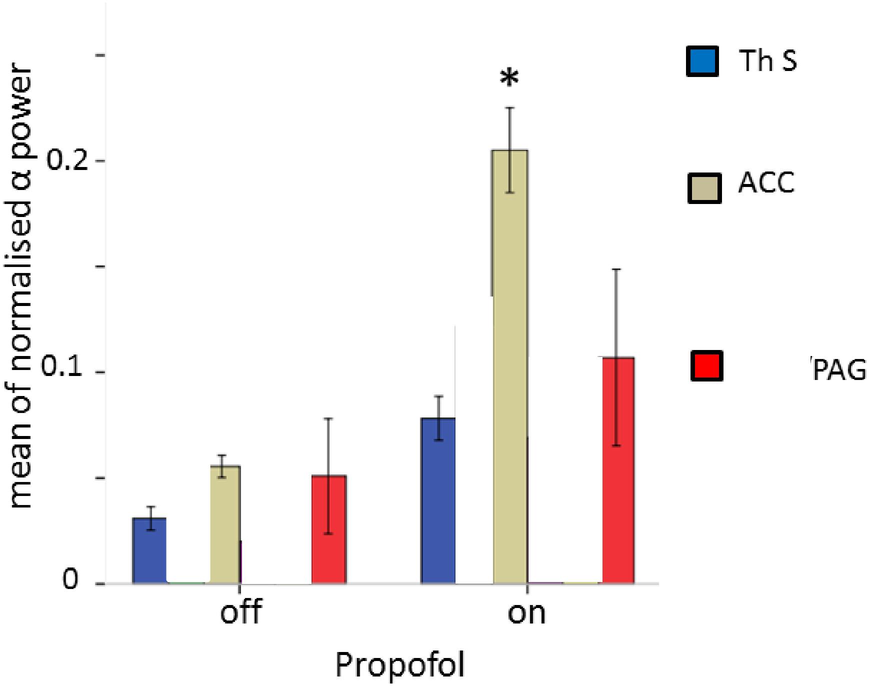


Fig 5.4 Statistic analysis of power spectra change. A) shows an example of power spectra density calculation of LFPs recorded from the vPAG. From the spectra curve we calculated the area under the curve to get the power of various frequency bands. B) shows the statistically significant alpha power increases induced by propofol in various brain areas (ThS, n=17, p<0.001; ACC, n=26, p<0.001; PAG, n=14, p=0.001. Wilcoxon signed ranks test for Ths; others, paired-t tests). C) shows the significant decrease of gamma power, induced by propofol (ThS, n=17, p=0.003; ACC, n=26, p<0.001; PAG, n=14, p<0.001. Wilcoxon signed ranks test for ThS; others, paired-t tests). ThS, sensory thalamus; ACC, anterior cingulate cortex; PAG, periaqueductal grey. * p<0.05; ** p<0.01; *** p<0.001, two tails. Error bars, SEM.

Power ratio=power with propofol/power without propofol (<1 means decreased, >1 means increased by propofol).

A



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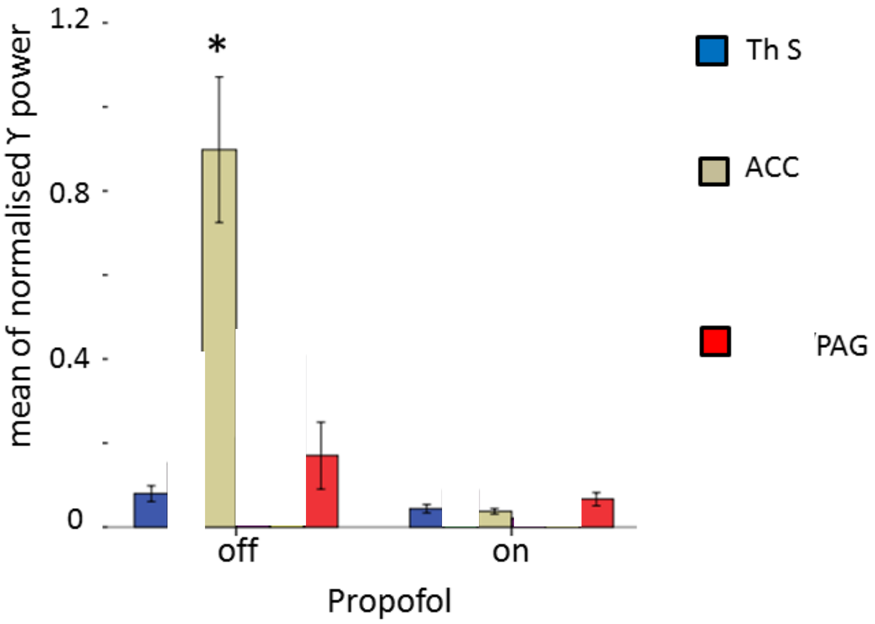
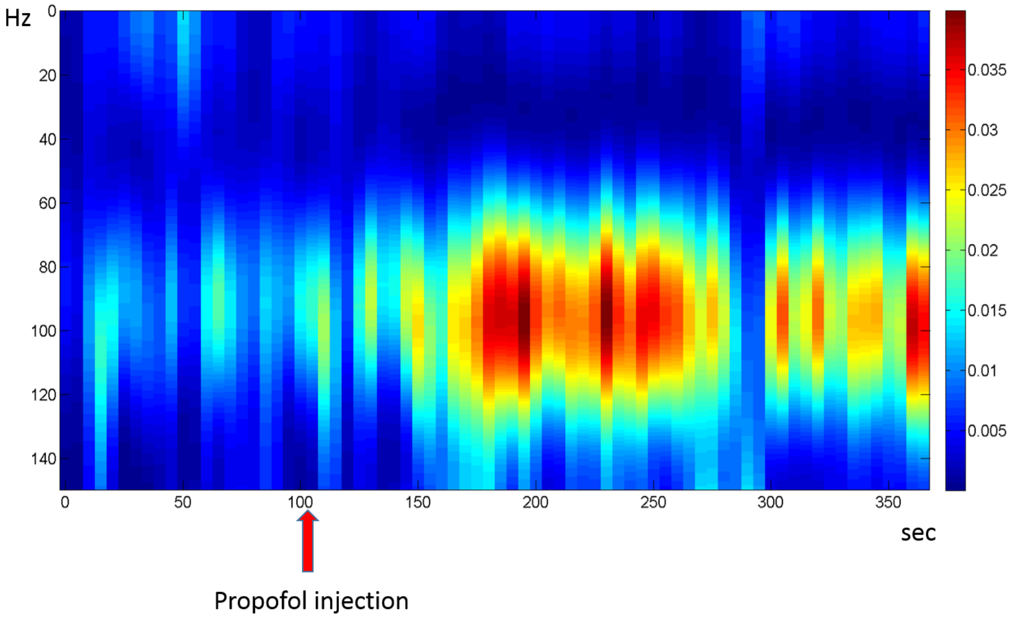


Fig 5.5 Differential power responses to propofol induction between various brain areas. A) α power changes. Before propofol induction (propofol off), there were no differences in alpha power between regions (one-way ANOVA, F(5,99)=1.269, p=0.283). During propofol induction, ACC demonstrated a differential α power response (one-way ANOVA, F(5,99)=7.366, p<0.001; post hoc: Bonferroni). B) γ power changes. Before propofol induction (off), ACC had different normalised γ power from other areas (one-way ANOVA, F(5,99)=9.251, p<0.001; post hoc: Bonferroni). During induction the ACC's γ difference disappeared (one-way ANOVA, F(5,99)=5.475, p<0.001; post hoc: Bonferroni). .

A



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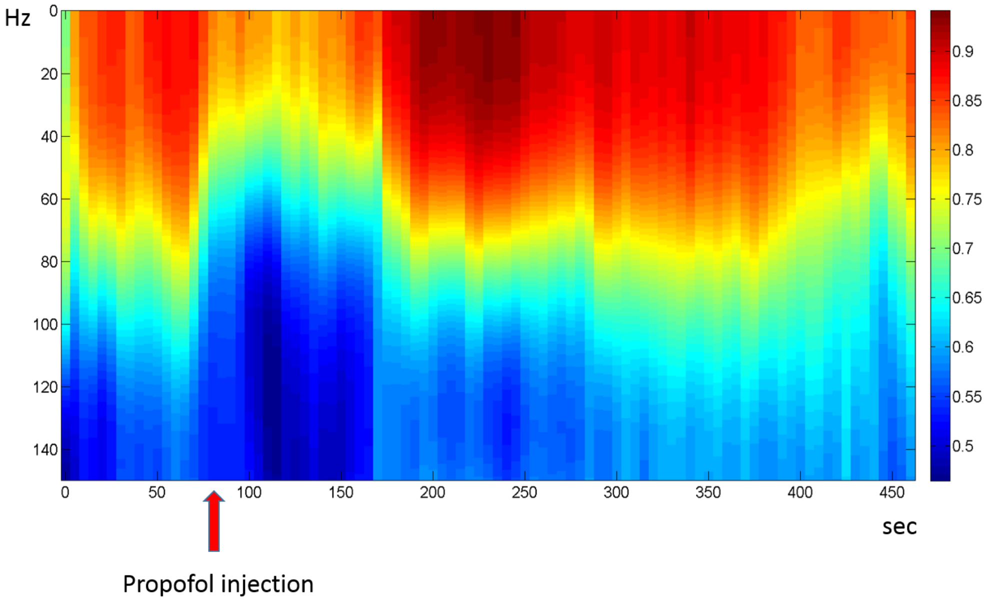

Fig 5.6. Spectral coherence A) between the PAG and sensory thalamus, and B) between bilateral ACC. Red arrows indicate the time when propofol induction started

Chapter 6 Conclusions and discussions

6.1 Summary of the experimental findings

DBS of the sensory thalamus and the periaqueductal grey: (chapter 3)

We have shown that 1) 20 Hz PAG DBS inhibited the sensory thalamus with decreasing thalamic delta, theta, alpha and beta power, but did not change gamma power, and 2) 50 Hz sensory thalamus DBS excited the PAG with increasing PAG delta and theta power, but did not change alpha, beta or gamma power. We have also demonstrated that the PAG and the sensory thalamus interact reciprocally at short latency, which may be related to pain modulation.

The spectral coherence between the sensory thalamus and the periaqueductal grey (chapter 4)

The most obvious spectral coherence between the sensory thalamus and the periaqueductal grey was in high gamma frequency range, around 100-150 Hz, which would be inhibited by pain.

The effect of general anaesthetic propofol on the functional connectivity between the sensory thalamus and the PAG (chapter 5)

Time-frequency analysis shows that propofol increased alpha and decreased gamma synchronisation in the PAG, sensory thalamus and ACC. Statistics show that, among these regions, the ACC had the most significant increased alpha activity. Spectral coherence analysis shows that high-gamma coherence between the PAG and the sensory thalamus was increased during induction period which is compatible with the hypothesis that propofol induces loss of consciousness via its GABAergic activity.

6.2 Discussions

The functional connections between the sensory thalamus and the PAG, revealed by our three experiments

Our DBS study results show that the PAG and the sensory thalamus can affect each other reciprocally at short latency. Stimulating the ventrolateral PAG *decreased* the thalamic LFP delta power most, followed by theta, alpha and beta. On the other hand, stimulating the sensory thalamus *increased* the PAG delta LFP power most, followed by theta. Accordingly, it can be suggested that the PAG and the sensory thalamus influence each other in opposite directions. The evoked potential results show that this may involve relatively short pathways leading to activation at short latency. Since Denker et al. (Denker et al. 2011) demonstrated that LFPs reflect surplus spike synchrony and LFP oscillations are reflections of the activation of neuronal assemblies, it could be suggested that PAG stimulation might inhibit the sensory thalamus by a short latency route, whilst sensory thalamus stimulation might excite the PAG. That said, it cannot be certain if the evoked potentials were caused totally by the interaction between the PAG and sensory thalamus only, because pain modulation mechanism involves many parts of the brain and spinal cord, and both structures also have many connections with other areas.

It is argued that sensory systems are simply mapping from stimuli to responses (Carpenter and Reddi 2012) (ie sensory processing is for organism to make proper responses to maintain homeostasis), pain signals can be modified if needed. Moreover, pain is a perceptual experience; noxious stimulation produces signals that enter an active nervous system that is already the substrate of past experience, culture, anticipation, anxiety and so forth. These brain processes actively participate in the selection, abstraction and synthesis of information from the total sensory input. Therefore, it can be argued that the sensory thalamus could be no longer regarded as a passive relay centre, but is thought to play an active part in preprocessing incoming information, before transfer it to the cerebral cortex. Also its activity can be modulated. Our experiments support this view by showing that thalamic stimulation can modulate the activity of the PAG, and vice versa, to change pain perception.

The results of the functional connectivity study show that the spectral coherence between the sensory thalamus and PAG was changing with pain. When the patients were stimulated by ice and had increased pain symptoms, the gamma coherence was decreased. It shows that the functional connectivity we found between the sensory thalamus and the periaqueductal grey might be related to pain. The results also suggest that PAG and the sensory thalamus might maintain a close relationship to observe and keep body’s homeostasis; induced pain broke the balance, and the pain induced a series of physiological responses to counter the change, which might decrease the coherence between those two areas because the PAG have the ability to inhibit the sensory thalamus.

The high gamma coherence was enhanced during propofol induction, as revealed by the general anaesthesia study, even though the patients had already lost their consciousness. It has been know that consciousness and pain can be dissociated and pain processing ability can be preserved if the level of general anaesthesia is not deep enough (E. N. Brown et al. 2011a). The spectral coherence between the sensory thalamus and PAG was not inhibited by propofol during induction, which supports the point. Moreover, our data shows that propofol even increased the spectral coherence, which is compatible with the hypothesis that propofol has mechanisms of action through potentiation of $GABA_A$ receptor (Alkire et al. 2008; Emery N. Brown et al. 2010; E. N. Brown et al. 2011a). Animal studies (Behbehani et al. 1990) showed that, when injection of GABA into the PAG, its descending pain inhibition activity increased, which indicates that the PAG may contain a tonically active GABAergic network that operates, at least in part, through $GABA_A$ receptors. This GABAergic system may modulate activity in descending pain inhibitory pathways emanating from the PAG. Our result is consistent with the theory that propofol enhances GABA activity.

There have been reports suggesting that during general anaesthesia, despite unconsciousness, not all of the brain activity will be decreased; average neuronal spike activity persists or is even increased and cognitive modules such as primary sensory cortex may be relatively maintained but cortical-cortical and cortico-subcortical connectivity are disrupted (George A. Mashour 2013b). Here our data demonstrates that the interaction between the PAG and the sensory thalamus could be preserved during propofol induction, even though the patients were already unconscious.

The possible routes connecting the sensory thalamus and PAG

Animal studies have shown that the ventrolateral PAG has direct projections to various thalamic nuclei. Krout and Loewy (Krout and Loewy 2000b) demonstrated that, in rats, the ventrolateral PAG projects to all the intralaminar nuclei and many midline thalamic sites. Mantyh (Mantyh 1983b) showed, in non-human primates, that the ventrolateral PAG has projections to many thalamic areas, including the nucleus reticularis thalami, the nucleus medialis dorsalis, the midline thalamic nuclei and the intralaminar thalamic nuclei. Rinvik and Wiberg (Rinvik and Wiberg 1990) further demonstrated a reciprocal connection between the ventraolateral PAG and the thalamic reticular nucleus in cats. The thalamic reticular nucleus contains only GABAergic, inhibitory neurons. Thus, even though it is speculative, PAG stimulation might inhibit the sensory thalamus via the thalamic reticular nucleus, whereas sensory thalamus stimulation probably excites the PAG, which could then return inhibition to the thalamus.

It is well known that the PAG can inhibit the activity of the nociceptive neurons in the spinal dorsal horn via the descending pain modulation pathway (Basbaum and Fields 1984; Budai et al. 1998; Heinricher et al. 2009; Millan 2002). Thus it can be argued that the neurophysiological effect of PAG stimulation on the sensory thalamus is via changes in the dorsal horn. The fastest conduction velocity of the human spinothalamic tract is 21 m/s (Cruccu et al. 2000; Rossi et al. 2000; Tran et al. 2002), ie a signal would take c. 50 msec to ascend from the spinal cord to the thalamus, whilst the descending pathway from the PAG to the dorsal horn would likely take even longer. The latency we have shown between the PAG and sensory thalamus is therefore far too short to allow for the spinal cord loop. Nandi and colleagues also showed that periventricular grey stimulation immediately affected thalamic LFPs (Nandi et al. 2002; Nandi et al. 2003). Thus it is possible, as discussed earlier, that the PAG can affect the sensory thalamus via a shorter route than the dorsal horn.

Manipulation of some nuclei in the medial thalamus, including stimulation of thalamic nucleus submedius (Roberts and Dong 1994; Tang et al. 2009) and lesion of the central lateral nucleus (Lenz et al. 1989; Lenz et al. 1994), has shown benefit in pain treatment. Our understanding of how PAG and thalamic stimulation affects other circuitry within the thalamus as well as other parts of the pain matrix is limited. It could be that the reduction in thalamic power in some frequency bands accounts for changes in pain perception or it could be that these changes alter the function of the overall matrix (Erlick A. C. Pereira et al. 2007). Further studies are needed to address these issues.

The inseparable connection between pain and autonomic nervous system: maintaining homeostasis

We cannot be sure that the interactions we found between the sensory thalamus and PAG are necessarily related to pain modulation. Both sites are involved in pain perception and their stimulation reduced pain in our patients. In particular, the stimulation frequency with the most powerful effect in terms of modulating the LFPs of the PAG or sensory thalamus corresponded to that eliciting the most pain relief.

Thus, the most likely explanation is that the reciprocal effects demonstrated here relate to pain modulation. However, the functions of the PAG are not confined to analgesia. The PAG is involved in responses under the rubric of homeostasis, many of which are influenced by its hypothalamic connections (Bandler et al. 2000; Benarroch 2012). This raises the possibility that the interactions identified by us could represent processes unrelated to analgesia but related to autonomic effects for homeostasis. That said, it is also possible that pain modulation and autonomic function responses cannot be separated, because pain perception mechanism also involves the autonomic nervous system to modify respiration, heart rate, blood pressure and muscle tone to prepare the body for emergency and escape.

Knyazev (Knyazev 2012) suggested that delta activity is related to pain *and* homeostatic processes. Delta oscillations are strongly related to motivation and are triggered by both biological rewards and dangers. Thus they are involved in many functions critical for survival, such as synchronization of brain activity with autonomic functions, motivational processes related to reward and defensive mechanisms, and cognitive processes connected with attention and the detection of motivationally salient stimuli in the environment. Craig (A. D. Craig 2002; A. D. B. Craig 2003) argued that pain perception is essentially to maintain homeostasis. Our results show that the largest LFP changes occurred in the delta frequency band, which suggests that modulation of delta power may be important in pain modulation. We know that manipulation of the PAG alters autonomic function (Bandler et al. 2000; Green et al. 2005; Hyam et al. 2012) but human stimulation of sensory thalamus has not been shown to alter autonomic function (Green et al. 2006a). Therefore, although pain and autonomic function are inextricably linked to survival, whether the functional connection between PAG and thalamus is connected to autonomic function is entirely speculative.

Propofol induced alpha activity: what did this study also help to reveal, in addition to the functional connectivity between the sensory thalamus and PAG

Our propofol study shows that propofol increased alpha and decreased gamma synchronisation in the brain areas studied. Moreover, among these areas the anterior cingulate cortex had the most significant induced ongoing alpha activity. Before propofol induction, there were no significant differences in normalised alpha power between the brain regions. However, when the patients were unconscious during induction, the ACC's normalised alpha power was significantly different from others'.

It has been known that propofol induces ongoing alpha activity in the human frontal cortex (Feshchenko et al. 2004). Our study shows that not only cortex but also subcortical areas demonstrate increased alpha synchronisation. Modelling studies provide a possible mechanism for the propofol-induced alpha synchronisation in the frontal cortex (Ching et al. 2010; Vijayan et al. 2013). It is suggested that propofol increases the strength and decay time of GABAergic projections from cortical interneurons onto cortical pyramidal cells and from thalamic reticular neurons onto thalamic relay cells. The enhanced GABAergic inhibition leads to a thalamic inhibitory-excitatory rhythm locking to the cortical drive in the alpha frequency range. Our data shows that subcortical areas would also be affected by propofol to present increased alpha activity, including PAG and the sensory thalamus. But the origin of the induced alpha activity cannot be determined.

Alpha activity is thought to be related to functional inhibition (O. Jensen and Mazaheri 2010). During wakefulness information is gated by inhibiting task-irrelevant regions, thus routing information to task-relevant regions. Increased alpha activity in task irrelevant regions provides pulsed inhibition reducing the processing capabilities of the regions (O. Jensen and Mazaheri 2010). Alpha oscillation is also related to the production of unconsciousness. Dynamic causal modelling indicates that propofol-induced unconsciousness is associated with a selective breakdown of corticocortical backward connectivity (Boly et al. 2012), and Supp and colleagues demonstrated that hypersynchronous alpha activity blocks intracortical communication, which plays an important role in the mechanism for the loss of consciousness (Supp et al. 2011). Even though we do not know whether the alpha changes we found in various brain areas were just the epiphenomenon of unconsciousness, our study suggests that the effect caused by propofol leading to functional inhibition via increased alpha activity may be prevalent, not only at the cortical level. That said, it was the cortical area (ACC) that demonstrated the most significant increase in alpha power.

Lee and colleagues demonstrated that after induction with propofol, mean information integration capacity was reduced in all oscillation frequency bands, and most prominently in the gamma band of the EEG. They also demonstrated that loss of consciousness is reflected by the breakdown of the spatiotemporal organisation of gamma waves (Lee et al. 2009). Our data show that gamma power was inhibited by propofol. But whether the gamma power inhibition was caused by increased alpha synchronization needs further investigation.

Different brain areas have different sensitivity to propofol; some areas show more functional inhibition than others. Experiments on cats demonstrated that propofol depresses cortical, thalamic and reticular formation neurons (Andrada et al. 2012). PET studies demonstrated that propofol preferentially decreases cerebral blood flow in brain regions related to arousal, performance of associative functions, and autonomic control (Fiset et al. 1999; Kaisti et al. 2003). Some functional MRI studies demonstrated a breakdown of thalamo-cortical and corticocortical connectivity (Boveroux et al. 2010; Schroeter et al. 2012); some showed the thalamocortical system isolated from sensory stimuli, whereas internal thalamocortical exchange persisted (Mhuircheartaigh et al. 2010; Ni Mhuircheartaigh et al. 2013). Moreover, anaesthetics may exert a differential effect on specific and nonspecific thalamocortical systems as they suppress consciousness (Liu et al. 2013). Our data show the different response to propofol between cortical (ACC) and subcortical regions (The PAG and sensory thalamus).

Limitations of the experiments in the thesis

During PAG DBS, the ventrolateral part of the PAG is targeted, based on our previous experience that this is the part of the PAG that is associated with reduction in blood pressure and better pain relief (Green et al. 2006a). Another theoretical rationale for targeting ventrolaterally is the observation that ventrolateral PAG can excite the descending pain modulation pathway to inhibit the incoming pain signals at the level of the spinal dorsal horn (Bandler and Shipley 1994; Basbaum and Fields 1984; Budai et al. 1998; Heinricher et al. 2009; Millan 2002). However, in practice, we cannot be sure that our electrode is only stimulating the ventrolateral PAG as the PAG is a small structure and it is likely that there is current spread to adjacent parts. Nevertheless, we position our electrode anterolateral to the aqueduct to maximise ventrolateral PAG stimulation.

There are some more limitations in the DBS study. One is that exogenous electrical stimulation of deep brain nuclei does not represent normal physiological conditions, both because of the stimulation parameters (continuous train of stimuli at several mA) which are unlike those occurring naturally and also because the electrical current may spread beyond the targets of study, which would confound the establishment of connections between structures. Moreover, responses evoked by continuous stimuli are different from those evoked by a single stimulus (Montgomery and Gale 2008). Therefore, the functional connections, proposed by the observations during DBS in our study, suggest putative mechanisms of how DBS may work to relieve pain, but we should be cautious about drawing conclusions regarding how these nuclei interact in the pain state and about directionality of the connections.

Regarding the general anaesthesia study, one limitation is that we did not follow the change of patients' propofol concentration in the blood against time, which prevents us from correlating the induced alpha activity and the increased gamma coherence with propofol concentration. Another limitation is that we did not record EEG simultaneously with LFP recording. It is still not clear whether propofol affects the thalamus earlier or later than cortical or other subcortical areas, which may suggest the thalamus's position in producing unconsciousness (Gili et al. 2013; Mhuircheartaigh et al. 2010). With EEG as a reference we can compare the speed of emergence of alpha activity between brain areas, which would help answer the aforementioned question.

Delta oscillations are associated with the state of consciousness (Knyazev 2012), which could be a confounding factor to our pain studies. In the DBS study and the functional connectivity study, all subjects were awake and alert enough to report their pain and comply with the relatively short protocol. We did not, however, objectively measure alertness as this may have been a distractor for the pain and influenced the pain scores.

6.3 Conclusions

This thesis suggests that the sensory thalamus and the periaqueductal grey have a reciprocal connection with each other. Functionally this connectivity might be related to pain modulation. Also this functional connectivity might represent the fact that the transmission and processing of pain signals is always monitored and modified by central pain modulation systems so that organisms can respond properly to the incoming signals.

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