

Microrecordings within the posterior nucleus of the hypothalamus in pain and aggressive behaviour

By

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Microrecordings within the posterior nucleus of the hypothalamus in pain and aggressive behaviour

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Abstract

In the last decade there has been new interest in the posterior nucleus of the hypothalamus (PIH) as the target for the placement of deep brain stimulation to improve pain and psychiatric symptoms. This has brought the possibility to study single-unit activity from PIH. Very scanty information is available regarding the firing discharge of human's PIH neurons. The aim of this study is to describe the firing discharge properties of PIH neurons in neurological and psychiatric disorders. Continuous physiological extracellular recordings were obtained in awake and sedated humans. Firing rate analysis, time domain and frequency domain analyses were used to characterize the firing pattern of PIH neurons. 19 PIH neurons from 7 patients were further studied (5 patients with Trigeminal Autonomic Cephalalgias, 1 aggressive behavior associated with epilepsy, and 1 aggressive behavior associated with head injury). During wakefulness PIH neurons displays tonic firing discharge at around 25Hz, while during sedation the firing rate is 12Hz and the firing pattern more variable. In aggressive behaviour and epilepsy the firing discharge is phasic and rhythmic with oscillations locked at around 7-8Hz. Regular and irregular tonic discharge is noticed in aggressive behaviour and head injury. Spontaneous activity in awake TACs patients is similar to what has been reported in animal models. Interestingly, in aggressive behaviour with epilepsy the observed pattern is bursting and rhythmic at around 7-8Hz. In the patient with head injury no specific pattern is found in PIH neurons. At this stage of knowledge these data are a novelty in the literature, thus it is not possible to exclude that all these observations represent normal features of PIH neurons. However the differences noticed between pathologies may suggest that PIH neurons discharge rates and patterns are associated to the underlying neurological and psychiatric condition.

Riassunto

Nel corso dell'ultima decade c'è stato un nuovo interesse nel nucleo posteriore dell'ipotalamo (PIH) come target per la stimolazione cerebrale profonda nel trattamento del dolore e di sindromi psichiatriche. Questo ha dato la possibilità di studiare l'attività di una singola cellula del PIH. Pochissime informazioni sono disponibili sull'attività di scarica di questi neuroni nell'uomo. Lo scopo di questo studio è descrivere le proprietà di scarica di neuroni del PIH in disordini neurologici e psichiatrici. Registrazioni fisiologiche extra-cellulari sono state campionate in pazienti svegli o anestetizzati. Sono stati analizzati, la frequenza di scarica, il dominio temporale ed il dominio di frequenza.

19 neuroni dal PIH di 7 pazienti sono stati studiati (5 pazienti con Trigeminal Autonomic Cephalalgias-TACs-, 1 con comportamento aggressivo associato ad epilessia, 1 con comportamento aggressivo associato a lesioni cerebrali traumatiche) . In stato di veglia in neuroni del PIH mostrano una scarica tonica di circa 25Hz, mentre in stato di sedazione la frequenza scarica è di 12Hz e patterns variabili. Nel paziente aggressivo con epilessia il pattern di scarica è fasico e ritmico con oscillazioni nelle frequenza della banda theta (7\8Hz). Attività tonica di tipo regolare ed irregolare è descritta nel paziente aggressivo con lesioni traumatiche. L'attività spontanea nei pazienti svegli con TACs è simile a quanto riportato in modelli animali. E' interessante il dato nel paziente aggressivo con epilessia che il pattern è di tipo fasico e ritmico. Nell'altro paziente aggressivo nessun specifico pattern è stato riportato. Al momento questi dati costituiscono una novità nella letteratura scientifica, quindi non è possibile escludere che essi descrivano normali caratteristiche dei neuroni del PIH. Comunque le differenze osservate suggerirebbero che il pattern di scarica di questi neuroni è associato alla sottostante patologia.

Contents

| | |
|--|-----------|
| <i>Abbreviations</i> | 8 |
| Introduction | 10 |
| 1. Literature review | 13 |
| 1.1. The limbic system | 13 |
| 1.2. The hypothalamus | 15 |
| 1.3. Posterior Nucleus of the Hypothalamus | 16 |
| 1.4. PIH in TACs and aggressive behaviour | 22 |
| 1.4.1 Trigeminal Autonomic Cephalagias (TACs) | 22 |
| 1.4.2 Aggressive behaviour | 27 |
| 1.5. Aims and hypothesis | 30 |
| 2. Methods | 32 |
| 2.1. Patients descriptions | 32 |
| 2.1.1 TACs | 32 |
| 2.1.2 Aggressive behaviour and epilepsy | 33 |
| 2.1.3 Aggressive behaviour and head injury | 33 |
| 2.2. Operative technique and data sampling | 34 |
| 2.3. Data analysis | 36 |
| 3. Results | 40 |
| 3.1. Comparison between behavioural states | 40 |
| 3.2. Neuronal activity in TACs | 43 |
| 3.3. Neuronal activity in aggressive behaviour | 47 |
| 3.3.1 Global population | 47 |
| 3.3.2 Aggressive behaviour and epilepsy | 47 |
| 3.3.3 Aggressive behaviour and head injury | 50 |
| 4. Discussion | 57 |

| | |
|--|-----------|
| 4.1. Methodological considerations | 57 |
| 4.2. Towards a posterior hypothalamic neurophysiological mapping | 58 |
| 4.3. Behavioural states | 60 |
| 4.4. TACs | 61 |
| 4.5. Aggressive behavior | 64 |
| 4.6. Conclusions | 68 |
| 4.7. Future directions | 70 |
| References | 72 |

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Index of Figures

| | |
|--------------|----|
| FIGURE 1. 1 | 14 |
| FIGURE 1. 2 | 18 |
| FIGURE 1. 3 | 25 |
| FIGURE 1. 4 | 26 |
| | |
| FIGURE 2. 1 | 39 |
| | |
| FIGURE 3. 1 | 41 |
| FIGURE 3. 2 | 44 |
| FIGURE 3. 3 | 45 |
| FIGURE 3. 4 | 46 |
| FIGURE 3. 5 | 49 |
| FIGURE 3. 6 | 52 |
| FIGURE 3. 7 | 53 |
| FIGURE 3. 8 | 54 |
| FIGURE 3. 9 | 55 |
| FIGURE 3. 10 | 56 |

Index of Tables

| | |
|------------|----|
| TABLE 3. 1 | 42 |
| TABLE 3. 2 | 48 |

Abbreviations

AC anterior commissure
ACTH adrenocorticotropic hormone
ADHD attention deficit and hyperactivity disorder
CCH chronic cluster headache
CNS central nervous system
CRH corticotropin-releasing hormone
CT computed tomography
DBS deep brain stimulation
FFT fast fourier tranform
GABA gamma aminobutirric acid
Gp globus pallidus
HPA hypothalamopituitary-adrenal axis
ISI inter-spike interval
ISIH inter-spike interval histogram
MRI magnetic resonance imaging
PC posterior commissure
PD Parkinson's disease
PH paroxysmal emicrania
PIH posterior nucleus of the hypothalamus
RPO nucleus reticularis pontis oralis
SNR signal to soise ratio
STN subthalamic nucleus
SUNCT short unilateral neuralgia with conjuntival injection and tearing
SWS slow wave sleep
TACs trigeminal autonomic cephalalgias

To F. and I.

Introduction

The posterior nucleus of the hypothalamus (PIH) is one of the many nuclei that make up the hypothalamic region of the brain, and is part of the limbic system. PIH has been classically linked to the control of behavioural states. In animal model it has been reported that during wakefulness PIH neurons' fire at around 25 spikes/s, and during slow wave sleep at around 15Hz. The discharge pattern is tonic regardless in both states. Since the last decade there is new interest in the PIH as the target for the placement of deep brain stimulation leads to treat neurological and psychiatric disorders, such as the trigeminal autonomic cephalalgias, and aggressive behaviour. This has brought the possibility to study neuronal activity sampled within this neuro-anatomical district. Very scanty information is available regarding the firing characteristics of PIH neuronal activity in humans, and due to the lack of data the aim of this study is to describe the firing discharge properties of PIH neurons in neurological and psychiatric disorders. It has been hypothesized that as well as in animals PIH neurons in awake humans display a spontaneous tonic discharge at 25 spikes/s, and PIH discharge properties differ between neurological and psychiatric patients.

The target's coordinates were determined by stereotactic CT scans, fused with MRI. Stereotactic coordinates of the PIH were 3mm behind and 5mm below the midcommissural point, and 2mm lateral to the anterior commissure-posterior commissure line. Continuous physiological extracellular recordings were obtained in awake and sedated humans. Data samplings started as soon as the microelectrode is between 5 and 10mm to the presumptive target's coordinates.

The microelectrode, with a tip of 250 μ m, was extruded through the brain by steps of either 0.5, or 1mm. Response properties of the isolated neurons were obtained by superficial and deep tactile stimulation. Post-operative volumes rendering 3D reconstructions of the microelectrode trajectories were done to identify the correct position of the recorded units. Only sampled data showing stable recordings with good signal to noise ratio for at least 10 seconds were analyzed. The firing rate was calculated by dividing the total number of spikes by the time of the recordings. Inter-Spike Interval Histogram were plotted to inspect the properties of the spike trains. To assess the periodicity of the firing discharge autocorrelograms and power spectra were plotted to perform respectively, a time-domain and a frequency-domain analyses.

Five patients had diagnosis of TACs, one had aggressive behaviour associated with epilepsy, and one had aggressive behaviour associated with head injury. Nineteen neurons that were placed in the posterior nucleus of the hypothalamus, as deduced by the 3D reconstructions, were further analyzed. None of them showed either activation or inhibition to tactile and pin-prick stimulation, with the exception of one in a TAC patient. *Trigeminal autonomic cephalalgias*: five cells recorded at the target site were analyzed. The average firing rate is 16 \pm 12Hz. Interestingly two patients had a pain attack during the surgery and received drugs to control it. It has been possible to compare the discharge properties between the group pain-free and the group with-pain. The former group had an irregular tonic discharge and a firing rate of 24Hz, while the latter group had regular tonic discharge pattern and a firing rate of 5Hz. All neurons showed no rhythmic discharge patterns. Only one cell showed a periodicity of 1Hz reflecting a cardiovascular artefact. *Aggressive behaviour and epilepsy*: 4 neurons were

analyzed, and were mainly concentrated within 1mm from the target, although above this landmark there was either lack of activity or the recorded units were not stable and not suitable for further studies. The average firing rate was $19\pm 13\text{Hz}$. Interestingly all neurons had rhythmic discharge patterns at around $7\text{--}8\text{Hz}$. *Aggressive behaviour and head injury*: spontaneous neuronal activity from 10 cells has been recorded along the final 4mm from the target. The distribution of firing rates and patterns was random. The average firing rate for these neurons was $10\pm 10\text{Hz}$ (range 2-32). Three cells exhibited high discharge rates (average 23Hz), and seven low firing rates (average 4Hz). All the studied neurons were firing in tonic mode and either in regular, or irregular discharge. All but one was firing randomly. The only exception showed oscillatory activity assessed at around 25Hz.

Spontaneous activity in TACs patients is similar to what has been reported in animal models, thus tonic and around 25Hz. Patients that received analgesic drugs had lower firing rates. More variability is noticed in psychiatric patients. Interestingly in aggressive behaviour with epilepsy, in all the studied neurons the discharge pattern is bursting and rhythmic around 7-8Hz. No specific patterns have been found within the PIH of patient with head injury. A few neurons are firing at very high firing rates, whilst the majority at lower rates. All cells were firing in a tonic fashion, although the patterns were grouped in irregular and regular. At this stage of knowledge these data are a novelty in the literature, thus it is not possible to exclude that all these observations represent normal features of PIH neurons. However the differences noticed between pathologies may suggest that PIH neurons' discharge rates and patterns are associated to the underlying neurological and psychiatric condition.

1. Literature review

1.1. The limbic system

The limbic system is biologically/evolutionarily an old part of the brain. The term limbic system is derived from the Latin word *limbus*, meaning a border, and was introduced by the neuroanatomist Pierre Paul Broca to describe the ring of gyri that surround the brain stem. Later on James Papez suggested the limbic system as the anatomical site where the emotions are formed (Greenstein Greenstein, 2000). Its management of fight or flight chemicals is an evolutionary necessity for lizards as well as modern humans.

The limbic system is made by several structures both cortical and subcortical, including the posterior nucleus of the hypothalamus (PIH), the septal area, and the nucleus accumbens (part of the striatum), all surrounding the diencephalon (Figure 1.1). Other structures such as the amygdala, fornicate gyrus, hippocampus, mammillary bodies, and orbitofrontal cortex are part of this system.

The limbic system operates by influencing the endocrine system and the autonomic nervous system. This system is involved in emotional states and direct physiological changes accompanying emotional states. In particular it is involved in aggressive behaviour, sexuality, pleasure, learning and memory, and the expression of emotional states in response to external sensorial stimulation.

The limbic system is highly interconnected with a structure known as the nucleus accumbens, commonly called the brain's pleasure center. The limbic system is also tightly connected to the prefrontal cortex. Some scientists contend

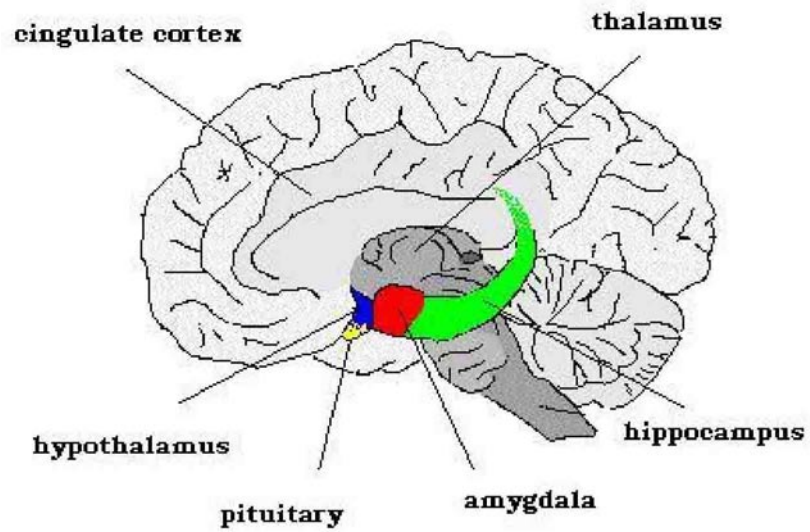


Figure 1. 1

Drawing showing a few structures of the limbic system.

that this connection is related to the pleasure obtained from solving problems. To cure severe emotional disorders, this connection was sometimes surgically severed, a procedure of psychosurgery called a prefrontal lobotomy. Patients who underwent this procedure often became passive and lacked all motivation.

1.2. The hypothalamus

The hypothalamus is the brain area that controls much of the body's endocrine secretions, through its portal blood link with the anterior pituitary gland (adenohypophysis) and its neuroendocrine links with the posterior pituitary gland (neurohypophysis). The hypothalamic nucleus lies at the base of the diencephalon beneath the thalamus (Figure 1.1), from which is separated by the hypothalamic sulcus. The rostral boundary is taken to be at the level of the lamina terminalis, while the caudal end is at the level of the mammillary bodies (Greenstein & Greenstein, 2000).

Modern studies have demonstrated that the hypothalamus functions to integrate autonomic response and endocrine functions with behaviour, especially with behaviour related with the basic homeostatic requirements of everyday life (Iversen *et al.*, 2000). It serves this integrative function by regulation of five physiological functions:

- It controls blood pressure and electrolytic composition;
- It regulates the body temperature;
- It controls energy metabolism;
- It regulates reproduction;
- It controls emergency response to stress.

To do this the hypothalamus contains an array of specialized cell groups with different functional roles. Although the hypothalamus is a small part of the whole central nervous system (CNS), it is packed with a complex array of cell groups and fibers pathways. It can be divided into three anatomical regions: anterior, middle and posterior. These three regions are associated with different group of nuclei, or groups of cell bodies (Greenstein & Greenstein, 2000; Iversen *et al.*, 2000).

The hypothalamus, although small, has extensive afferent and efferent connections with the rest of the brain, and some of these are well defined, while others are more diffuse and harder to define. Amongst the afferent fibers that have been identified are worth of mention the hippocampo-hypothalamic fibers, the amygdalo-hypothalamic fibers, and brainstem reticular afferents, and the retinohypothalamic afferents (Greenstein & Greenstein, 2000)

1.3. Posterior Nucleus of the Hypothalamus

The posterior nucleus of the hypothalamus (PIH) is one of the many nuclei that make up the hypothalamic region of the brain (Figure 1.2). It is located above the mammillary bodies at the side of the third ventricle. The PIH contains a homogeneous population of small to medium sized cells, with occasional large neurons scattered throughout the rostrocaudal extent of the nucleus. Cell packing density is low relative to neighboring hypothalamic structures, and fiber tracks course through and around the PIH along its rostrocaudal extent (Abrahamson and Moore, 2001). A chemoarchitectural analysis has shown four main cell types. The majority of PIH cells are glutamatergic, followed by melanin concentrating hormone cells, tyrosine hydroxylase cells, and hypocretins cells.

Nevertheless there are also GABAergic cells, neuropeptide Y cells, enkephalin cells, serotonergic and dopaminergic neurons.

The most important neurotransmitter produced by PIH neurons are the orexins, also called hypocretins, the common names given to a pair of highly excitatory neuropeptide hormones, and despite are produced by a very small population of cells in the lateral and posterior hypothalamus, they send projections throughout the brain.

Major fiber tracks and cell morphology, and packing density differences of adjacent structures demarcate the boundaries of the area dorsally (thalamus, fasciculus retroflexus, periaqueductal gray), ventrally (dorsal premammillary nucleus, dorsomedial tuberomammillary nucleus, supramammillary decussation), caudally (periaqueductal gray and mesencephalic reticular formation), and laterally (lateral hypothalamic area, zona incerta, fornix, mammillothalamic tracks). The periventricular hypothalamic nucleus and fiber systems separate the PIH from the ependyma of the third ventricle. The rostral border of PIH appears to extent as a uniform structure rostrally to the level of the dorsal hypothalamic nucleus (Abrahamson and Moore, 2001). The PIH receives afferents from cortical, subcortical, and brainstem structures involved in autonomic regulation. These include the insular cortex, septal nuclei, amygdala, subiculum, bed nucleus of stria terminalis, central grey, parabrachial nucleus, nucleus of the solitary tract and brainstem reticular nuclei. In addition the PIH

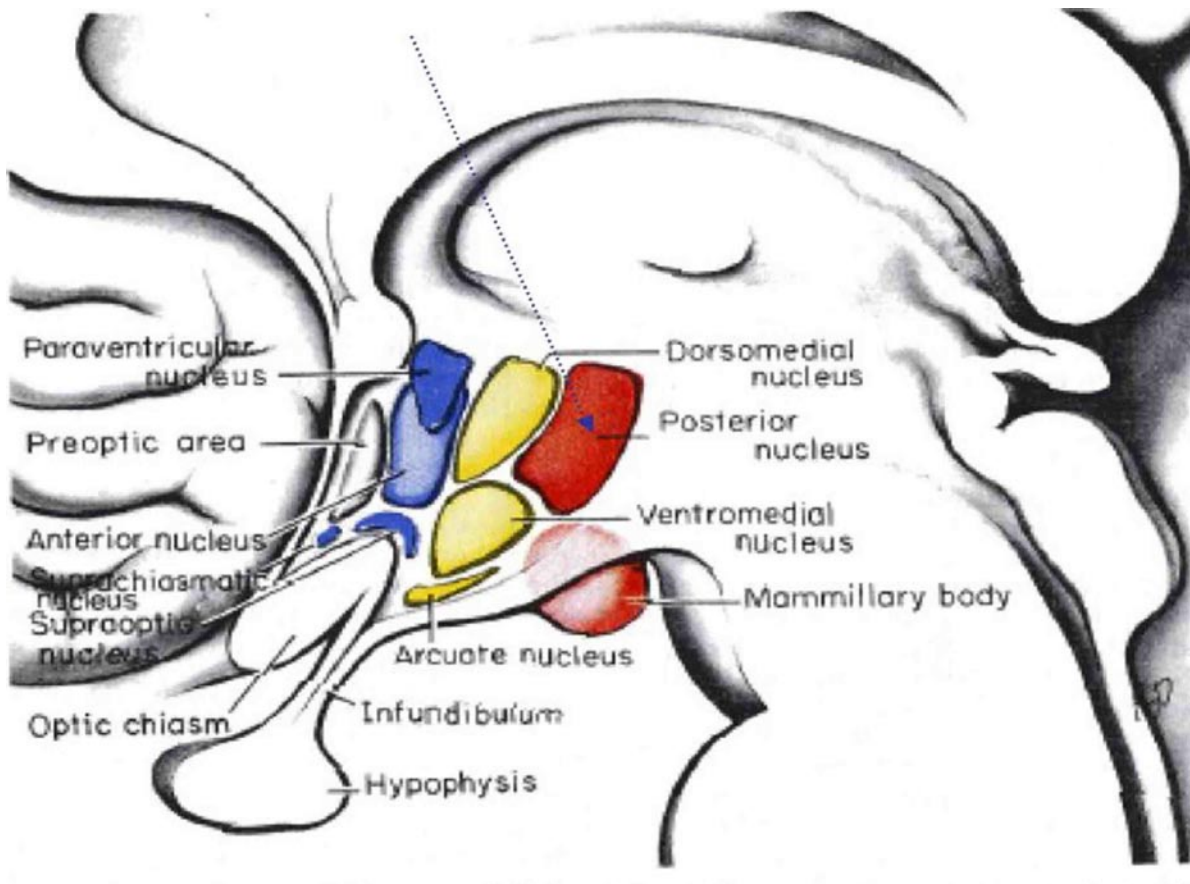


Figure 1. 2

Drawing showing the sagittal view of the hypothalamic nuclei. The posterior hypothalamus is indicated by the arrow (Modified from Carpenter, 1976).

receives inputs from structures such as the cingulate, frontal, parietal and insular cortices (Abrahamson and Moore, 2001; Cavdar *et al.*, 2001).

In older literature the posterior hypothalamus has been mentioned as a controlling center for the sympathetic system and consciousness. The posterior hypothalamic area was once also reported to show cell loss in a case of Cushing's disease (Swaab, 2003). Cushing's disease occurs when the pituitary gland (an organ of the endocrine system) makes too much of the hormone ACTH (adrenocorticotrophic hormone). ACTH stimulates the adrenal glands to produce cortisol.

This area was also linked to functions such as respiration, cardiovascular activity, locomotion, antinociception and arousal/wakefulness. Extensive descending projections as described in the rat may serve a role in these functions (Vertes and Crane, 1996). Experimental findings in rats have pointed out that the posterior hypothalamus also receives spinohypothalamic input, probably involving somatosensory and visceral sensory information (Swaab, 2003).

The posterior hypothalamus has been linked to the control of behavioural states (Lin *et al.*, 1989; Sallanon *et al.*, 1989). Early studies have demonstrated that experimental lesions of the PIH induced long lasting somnolence in monkeys and rats (Davison and Demuth, 1946; Fulton and Bailey, 1929; Nauta, 1946; Ranson, 1939). Moreover muscimol injections in the posterior hypothalamus in animal models have reinforced the idea of the importance of the PIH in maintaining the arousal (Lin *et al.*, 1989; Vanni Mercier *et al.*, 1984). Single unit recordings, performed in animal models, have been reported that during wakefulness, cats' posterior hypothalamic neurons' spontaneously discharge at around 25 spikes/s, during the slow wave sleep (SWS) the firing rate is lowered at

around 15Hz (Parè *et al.*, 1989), with a tonic discharge pattern regardless the behavioural state, and around 13Hz in anesthetized rats (Beatty *et al.*, 2005).

Karplus and Kreidl (1948) have extensively examined the effect of posterior hypothalamic stimulation to cardiorespiratory and behavioural responses. These authors described changes in cardiopulmonary functions and behavioural alteration following PIH lesions. Subsequent studies have reported the increase in mean arterial blood pressure, heart and respiratory rate and escape\defence behavior following the electrical (Lammers *et al.*, 1988) and chemical stimulation of the PIH, through glutamate (Ohta *et al.*, 1985), acetylcholine agonists (Martin, 1996a; 1996b) or neuropeptide Y (Martin *et al.*, 1988; 1989; 1991), and GABA antagonists (DiMicco and Abshire, 1987; Shekhar and DiMicco, 1987).

Single units recordings were sampled both *in vivo* and *in vitro* within the PIH of spontaneously hypertensive rats (Beatty *et al.*, 2005). The authors compared the neuronal firing rate between a group of rats with free access to a running wheel (exercise hypertensive rats) and a group with no access to the running wheel (no-exercise hypertensive rats). In the *in vivo* experiments the average firing rate for the exercise group was lower (8,5Hz) compared to the no-exercise group (13,7Hz). These results were also confirmed *in vitro*, although the overall firing rate is lower compared to the *in vivo* experiments. All units have shown no rhythmic firing pattern.

These findings suggest that PIH maintains behavioural and sympathetic activation characteristic of escape\defence behaviour. It has been suggested that the role of the PIH in defence behavior and its associated physiological correlates is related to the PIH involvement in the maintenance of the arousal (Abrahamson and Moore, 2001).

PIH is also involved in the generation of large amplitude, sinusoidal electrical oscillations, the theta (θ) oscillations, in the hippocampus of awake behaving rats, and in rats undergoing REM sleep episodes. PIH is part of a neuronal network along with the oral pontine reticular formation, supramammillary nucleus, medial septum and diagonal band of Broca (Vertes and Kocsis, 1997). In particular it has been shown the role of PIH in tonically modulating neuronal activity in the medial septum during hippocampal theta activity (Bland *et al.*, 1990; Oddie *et al.*, 1994). In addition, this nucleus receives descending inhibitory inputs from the medial septum during theta oscillations in the hippocampus. This suggests that PIH neurons are important in modulating θ rhythms, and in turn may be modulated by hippocampal activity through the inhibitory projections from the lateral septum (Kirk *et al.*, 1996). It has been demonstrated the existence of topographically organized projections from the hippocampus to the lateral septum (Abrahamson and Moore, 2001). Single-units discharge has been recorded from the rats PIH, during hippocampal theta oscillations elicited from stimulation of the reticular nucleus pontis oralis (Kirk *et al.*, 1996). All PIH neurons were classified as tonic theta-ON, namely an increased tonic discharge rate during the elicited hippocampal θ . The authors also reported bursting discharge in neurons located within the supramammillary and the medial mammillary nuclei.

Besides the role in the control of the behavioural states, experimental findings in rats have shown two distinct fiber tracts convey sensory information from the skin surface to the posterior hypothalamus, namely the trigeminohypothalamic tract and the reticulohypothalamic tract. The former conveys nociceptive inputs only from cephalic region to the hypothalamus; the latter conveys sensory inputs from both cephalic and extra-cephalic regions (Malik *et al.*, 2000). It is important

to mention that the trigeminohypothalamic pathway displays receptive fields only from the contralateral side, instead the reticulohypothalamic pathways displays more complex receptive fields comprising both side of the body.

In humans, electrical stimulation of this nucleus has evoked cognitive responses (anxiety\phobia), but not vegetative modification (Franzini *et al.*, 2003). An earlier report (Sano, 1970) has described that the stimulation of the posterior area of the hypothalamus evoked an increase in heart rate, blood pressure, pupillary dilatation, and EEG de-synchronization. However the discrepancies between the two reports might be consequence of the diversity in target coordinates and the available technology.

In the last decade there has been resurgence of attention in the posterior area of the hypothalamus as the target for the placement of DBS leads in the treatment of disorders such as trigeminal autonomic cephalalgias (TACs), and aggressive behaviour (Franzini *et al.*, 2003; Franzini *et al.*, 2005; Leone *et al.*, 2004; Leone *et al.*, 2007; Schoenen *et al.*, 2005).

1.4. PIH in TACs and aggressive behaviour

1.4.1 Trigeminal Autonomic Cephalalgias (TACs)

Chronic cluster headache (CCH), SUNCT (short unilateral neuralgiform headache with conjunctival injection and tearing), along with the paroxysmal hemicrania (PH), are part of the Trigeminal Autonomic Cephalalgias (TACs). These syndromes are characterized by a sudden and fast onset of unilateral pain sited around the eye, temple and cheeks (Figure 1.3) (International Headache Classification, 2004), all anatomical districts that are innervated by the trigeminal nerve's ophthalmic

branch (V cranial nerve). The TACs differ in attack duration and frequency as well as response to therapy. CCH has the longest duration and relatively low frequency; PH has intermediate duration and frequency; SUNCT has the shortest duration and the highest frequency (Cohen *et al.*, 2007). All these syndromes are described, as the most unpleasant form of pain experienced by human being and it is often accompanied by autonomic symptoms (Figure 1.3). In fact the clinically differentiating factors for the TACs, as a group, is the prominence of cranial autonomic activation, manifest as lacrimation, conjunctival injection, eyelid oedema, or rhinorrhea (May & Leone, 2003).

It has been suggested to refer to TACs as “neurovascular headaches” (Goadsby, 2002), in which the vascular change that is seen in the cranial circulation is driven by the trigeminal-autonomic reflex (trigeminal-facial nerves). Stimulation of the trigeminal ganglion in cats (Lambert *et al.*, 1984) or monkeys (Goadsby *et al.*, 1986) leads to a decrease in carotid resistance, which increased flow and facial temperature, predominantly through a reflex mechanism. The afferent limb of this reflex is the trigeminal nerve, and the efferent is the facial\greater superficial petrosal nerve (parasympathetic) dilator pathway (Goadsby, 2002). The trigeminal neural innervation of the cerebral circulation is somatotopically selective. In humans, painful stimulation through the administration of capsaicin, produces dilatation of the internal carotid artery when administered into the skin innervated by the first (ophthalmic) division of the trigeminal nerve (May *et al.*, 1998). However when capsaicin is injected into the skin innervated by the third (mandibular) division, or into the leg, there is no response in the ipsilateral carotid artery despite the experience of pain (Pareja *et al.*, 2001).

The circadian timing with two significant peaks of bouts in July and January (Kudrow, 1987), along with the alterations in plasma melatonin, cortisol, testosterone, gonadotrophins, prolactin, growth hormone and thyrotropin have been documented in TACs (Leone & Bussone, 1993). Taken together, these results strongly suggest a hypothalamic involvement in TACs pathophysiology.

In recent years, the knowledge on TACs' central mechanisms has greatly improved due to new neuroimaging data. Recently, neuroimaging techniques have shown the activation of the ipsilateral, to the painful side, posterior hypothalamus during CCH (May *et al.*, 1998; Sprenger *et al.*, 2004), SUNCT (Sprenger *et al.*, 2005), and paroxysmal hemicrania (Matharu *et al.*, 2006) attacks. This activation may be specific in these patients since it is not reported in other painful conditions such as migraine. Moreover the PIH is activated in nitroglycerin-evoked TACs bouts, and is not activated when the subjects were pain-free (May *et al.*, 1998).

The evidences that TACs bouts do not disappear after multiple thermorizotomies support the central origin of this pain (Leone *et al.*, 2004; Matharu & Goadsby, 2002). Thermorizotomy is the radiofrequency lesion, through the use of heat, of that part of the trigeminal nerve, which is the cause of pain.

From these findings, at the National Neurological C. Besta in Milan, the neurosurgical team guided by Prof. Broggi and Dr. Franzini in collaboration with the department of cerebro-vascular disease and facial algias, coordinated by Prof. Bussone and assisted by Dr. Leone, have implanted deep brain stimulation electrodes (Figure 1.4) in the posterior hypothalamic area of patients suffering of chronic cluster headache and later in a SUNCT patient (Franzini *et al.*, 2005;



Figure 1. 3

In TACs pain is usually reported around the eye and the temple, and is associated with autonomic symptoms such as lacrimation, conjunctival injection and rhinorrhea.

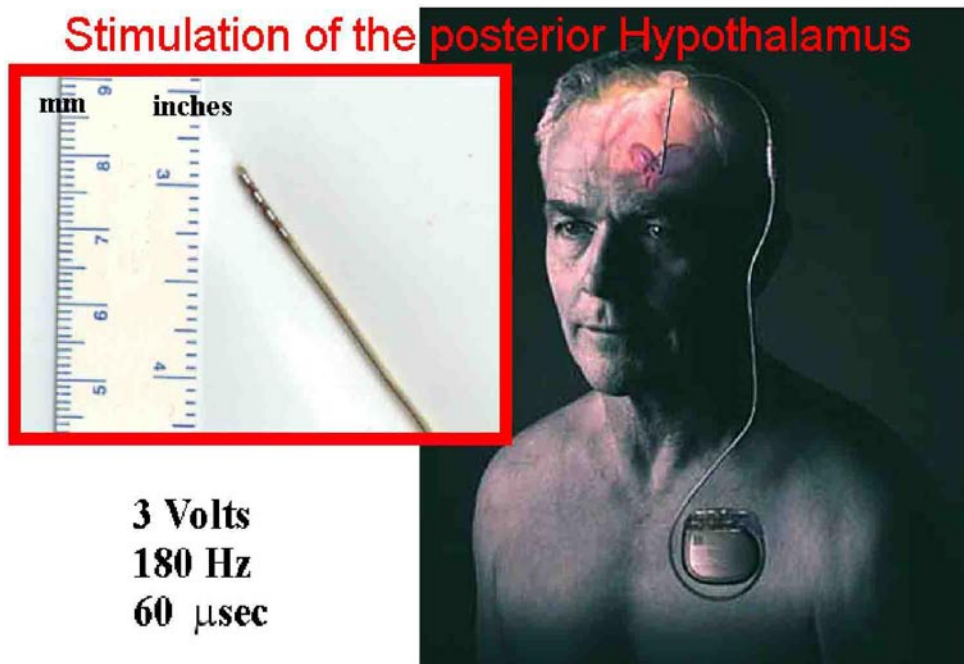


Figure 1. 4

Deep brain stimulation lead inserted in the deep cerebral structures and connected to a subclavicular pulse generator. Inset shows the quadripolar stimulating contacts. Also, there are indicated the stimulating parameters used in TACs.).

Leone *et al.*, 2003). This has been the first time where the anatomical target to place the stimulating lead has been chosen on radiological data. The clinical results in the mid- and long-term periods have shown the efficacy of this procedure (Leone *et al.*, 2003). Subsequently other authors have confirmed these results in CCH (Schoenen *et al.*, 2006; Rasche *et al.* 2006; Starr *et al.*, 2007.)

1.4.2 Aggressive behaviour

Genetic, developmental and environmental developmental factors play a role in later levels of aggression.

Various developmental factors may determine the later degree of aggression. Minor physical abnormalities in newborns that result from some form of genetic transmission or insult during early pregnancy predict short attention span, peer aggression, and impulsivity at three years of age, and recidivistic violent behaviour later.

In children with gelastic seizures and hypothalamic hamartoma, high rates of aggression are found. Gelastic epilepsy is defined as laughter that is inappropriate, stereotyped and not precipitated by either a specific humorous or non-specific stimulus. It is more common in infants than in adults. Convulsive laughter thus lacks an affective component. Gelastic epilepsy has been found to be associated with general temporal lobe epilepsy. It is not clear how the temporal lobe attacks may be related to altered hypothalamic functions. During the laughing attacks there can be a loss of consciousness. Gelastic seizures spread from the hypothalamus through hypothalamus-amygdala connections and spread to mesial temporal structures, causing complex partial seizures and a pattern of symptomatic generalized epilepsy with tonic, atonic and other types of seizures in

association with slow spike and wave discharge and cognitive deterioration.

Usually the response of gelastic seizures to antiepileptic drugs is poor (Swaab, 2003).

Impulsive and violent behaviour is often associated with severe brain damage and mental retardation. These behaviours usually fall into the category of defensive rage and are linked to the limbic hypothalamic-midbrain-periacqueductal gray axis, although the structural basis is usually far from proven. A number of hypothalamic structures and neurotransmitter systems seem to be involved. Animal experiments have shown that, after cortical ablation, stimulation of the posterior lateral hypothalamus elicits sham rage, a combination of hissing, pilo-erection, pupil dilation and extension of the claws. Stimulation of the human medioposterior or caudolateral hypothalamus during neurosurgical procedures evoked cognitive symptoms such as fear or horror (Carmel, 1980).

A number of neuroendocrine changes indicating hypothalamic involvement have been reported following head injury. There appears to be a rapid but variable activation of the hypothalamopituitary-adrenal (HPA) axis, and experimental data indicate the exclusive presence of a corticotropin-releasing hormone (CRH) response (Swaab, 2003). A link between testosterone levels and aggressive behaviour in humans has often been presumed and some studies indeed claim such relationship (Dabbs *et al.*, 1987). It has been demonstrated that individuals who have a history of numerous antisocial life histories tend to have higher levels of testosterone. However the serum testosterone levels in high- and low-aggression individuals do not differ consistently (Swaab, 2003).

In these patients high-dose neuroleptics can control aggressive and acting-out patterns. In some patients the medical treatment is not effective and sometimes associated with severe extra-pyramidal side effects. The management of such cases still remains a challenge (Gillberg *et al.*, 1986; Dosen, 1993; Bauermeister *et al.*, 1994).

Since the pionioristic works of Egas Moniz, a Nobel Laurate, who was the first to introduce the psychosurgery in the early 1900, numerous procedures have been proposed and performed for managing severe psychiatric disorders. The most employed has been the stereotactic amygdalotomy (Fountas *et al.*, 2007). At approximately the same time, lesions of the posterior hypothalamus (posterior hypothalamotomy) to improve aggressive behaviour in humans were firstly employed by Spiegel and Wycis (1962), Sano (1962; 1970), and Sano and colleagues (1966). In particular, the Japanese neurosurgeon Sano reported that after posterior hypothalamotomy the patients became markedly calm, passive and tractable, showing decreased spontaneity. These changes were long-lasting, although spontaneously returned to a considerable extent in 1 month. Somnolence was also noticed in the first seven to ten days after the operation. It has been also reported an amelioration of the intelligent quotient in almost 50% of the treated cases, and this is partly due because the patients were more cooperative after the hypothalamotomy (Sano, 1970).

The resurgence of attention in the role of posterior hypothalamus in trigeminal autonomic cephalalgias, the early works by Sano, along with the clinical observation that cluster headache is the only facial pain where violent behaviour and psychomotor agitation could be developed during the pain attacks (Leone *et al.*, 2004; Torelli and Manzoni, 2003), have suggested to implant

neurostimulation leads in the posterior hypothalamus of aggressive patients (Franzini *et al.*, 2005). High frequency stimulation of the posteromedial hypothalamus in the treatment of patients with aggressive behaviour has showed similar results to those previously reported by Sano (Sano, 1962; Sano *et al.*, 1970).

1.5. Aims and hypothesis

Very scanty information is available regarding the firing characteristics of PIH neuronal activity in humans and animals (Beatty *et al.*, 2005; Kirk *et al.*, 1996; Parè *et al.*, 1989). In the seventies Sano (1977) has illustrates raw electrophysiological traces of humans PIH neuronal activity without characterizing either the discharge rate, or the firing pattern. It is only been reported that following pin-prick stimulation over the whole body there was either an activation, or an inhibition in the firing discharge. However Sano described the latency of the neuronal response to pin-prick stimulation. It was noticed that after the stimulation there was the occurrence of long latencies, between 400 and 1000ms. It has been speculated to be the result of the activation of the C fibers, the nociceptors. Also, it is interesting to report that in a few cases it has been possible to record, although not to further analyze, the spontaneous neuronal activity during the TAC attack. In these cases the administration of triptans halted the pain and the firing discharge (Leone *et al.*, 2003).

Due to the lack of data regarding the firing rate and firing pattern of PIH neurons in humans, the aim of this study is to describe the intraoperative

mapping, and to characterize the firing discharge of single neurons sampled in the PIH in humans suffering of TACs and behaviour disorders.

In addition the study will test the following hypothesis:

- as well as in animal models PIH neurons in awake humans display a spontaneously discharge tonically at around 25 spikes/s;
- as well as in animal models PIH neurons in sedated humans display a spontaneously discharge tonically at around 13 spikes/s;
- as well as in animal models PIH neurons firing rate is modulated by superficial and deep tactile stimulations of the three trigeminal branches;
- TACs and aggressive behaviour differ in firing rate and firing pattern.

2. Methods

These series of studies were carried out by retrospective analysis of data from seven subjects, gathered during physiological exploration of the sites for the placements of either unilateral, or bilateral DBS electrodes to improve the symptoms in pain and aggressive behaviours. Five patients had diagnosis of TACs, four with chronic cluster headache and one with SUNCT, and two with aggressive behavior (one with associated epilepsy, and the other one with associated head injury). The studies were approved by the institutional review board, in accordance to the guidelines prescribed in the declaration of Helsinki and all patients gave written informed consent prior the procedure.

2.1. Patients descriptions

2.1.1 TACs

All TACs patients underwent to unilateral PIH DBS implants, and were awake throughout the whole microrecordings sessions. In this neurosurgical procedure it is important that the patients are collaborative in order to avoid unwanted side effects to electrical stimulation. At the time of the surgery patient 1 aged 43, with 10 years of CCH, and pain in the left side; patient 2 aged 47, with three years of CCH, and pain in the right side; patient 3 was 66, 14 years of SUNCT, with pain in the right side; patient 4 was 30, 7 years of CCH, with pain in the right side; patient 5 was 46, 10 years of CCH, with pain in the right side. There were three males and two females. All patients were not taking prophylactic drugs since the day before the implantation. Patients 4 and 5 both had a cluster attack during the neurosurgical procedure, and received analgesic drugs (triptans), to relieve the pain. Stimulating parameters were 180Hz, 60 μ s, 3V (see Figure 1.4).

2.1.2 Aggressive behaviour and epilepsy

This is a 19 years old male, whom symptoms were firstly noticed at the age of 2 with sudden laugh (gelastic epilepsy), psychomotor disturbances and absence states. The psychological evaluation highlighted the presence of attention deficit and hyperactivity disorder (ADHD), accompanied by hyperaggressivity crises. The episodes of aggressivity were both verbal and physical, and mainly directed towards objects, but also people. The symptomatology was unresponsive to drug therapy.

Since the patient could not cooperate, surgery and microrecordings were performed under general anaesthesia. Deep brain stimulation electrodes were placed bilaterally in the posterior nucleus of the hypothalamus as confirmed by post-op CT scan. Stimulation parameters were 130Hz, 90 μ s, 0.7V. No side effects were reported.

2.1.3 Aggressive behaviour and head injury

This 37 years old male had a motorcycle accident in 2004, and consequently was in coma for 1 month. CT scans showed bilateral lesions in the temporal poles. His relatives described that in parallel to the recovery from the coma state, the patient behaved violently, beating the paramedics, and subsequently the aggressiveness was triggered by environmental stimuli. The pharmacological therapy was uneffective in controlling the symptoms.

Since the patient could not cooperate, surgery and microrecordings were performed under general anaesthesia. Deep brain stimulation electrodes were

placed bilaterally in the posterior nucleus of the hypothalamus as confirmed by post-op CT scan. Stimulation parameters were 185Hz, 90 μ s, 1V. No side effects were reported.

2.2. Operative technique and data sampling

In the first part of the procedure stereotactic implants (Leksell G stereotactic frame; Elekta, Stockholm, Sweden) were placed with general anesthesia, using low doses of midazolam (0.05–0.1 mg/kg) or propofol (0.5–1 mg/kg). A few patients enrolled in the study did not take prophylactic drugs since the day before the implantation and remained awake throughout the surgical session. Antibiotic treatment was administered to all patients during the perioperative period. Preoperative magnetic resonance imaging (brain axial volumetric fast spin echo inversion recovery) was used to obtain high-definition anatomic images, which allowed precise determination of the anterior commissure-posterior commissure line. The three dimensional coordinates of the anterior (AC) and the posterior (PC) commissure were determined from magnetic resonance imaging (MRI) scans fused with 2-mm-thick computed tomographic (CT) slices obtained under stereotactic conditions. This was obtained by using an automated technique based on a mutual-information algorithm (Frame-link 4.0, StealthStation; Medtronic Sofamor Danek, Inc., Memphis, TN). The workstation also provided stereotactic coordinates for the target, 3 mm behind the midcommissural point, 5 mm below the midcommissural point, and 2 mm lateral to the midline.

In the second part of the procedure, a rigid cannula was inserted through a precoronal paramedian burr hole. The burr hole was placed in the skull in the region of the coronal suture at the approximate laterality of the target to enhance the probabilities that all the electrode trajectories were made in the parasagittal plane. The rigid cannula containing the microelectrode was positioned up to 10 mm from the target. A manually driven mechanical micro-drive attached to the stereotactic frame was used to extrude the microelectrodes into the brain, usually using 0.5\1mm steps.

In the third part of the procedure, continuous physiological recordings began as soon as the microelectrode extruded into the brain. Signal recordings were amplified, band pass-filtered (500-5KHz) by means of a *Medtronic Leadpoint™* system (Medtronic Inc., Minneapolis, MN, USA). Microrecordings were obtained using high impedance (up to 1.5 MOhm) microelectrodes (250µm tip) that allow isolation of single neurons, and the collection of signals from one cell for a time long enough to permit a postoperative off-line analysis. Intra-operatively action potentials were discriminated using a level discriminator, with its output fed into an audio monitor. In order to investigate the neuronal response properties in well isolated neurons, were systematically employed stimuli such as light touch, pressure to the skin, and pin-prick to the trigeminal branches, and to entire body. Neurophysiological recordings were employed as an exploratory tool, thus the collected information were not used to alter the target's stereotactic coordinates. In one patient with aggressive behavior associated with gelastic epilepsy, EEG recordings through subdermal needle electrodes were performed by means of the Nicolet Viking system (Nicolet Inc., Madison, WI, USA). According to

the international standard 10-20 system active electrodes were placed in T3, T4, Fp1 and Fp2, and reference electrode in Cz.

In the final part of the procedure, the rigid cannula was used as a guide for placement of two four-contacts definitive electrodes (DBS-3389; Medtronic Inc., Minneapolis, MN, USA). Figure 1.4 displays an example of the four-contact lead. Macrostimulation through the DBS contacts (1–7V amplitude, 60 μ s pulse-width, 180 Hz frequency) was used to evaluate potential side effects. Two subclavicular pulse generators (Solettra, Medtronic Inc., Minneapolis, MN, USA) were placed and then connected to the brain electrodes, tunnelled, and brought out percutaneously, for subsequent trial stimulations. Bilateral continuous monopolar (case-positive) 185Hz, 1V, 60 μ s stimulation was started the day after the surgery using the deepest contacts positioned at the target. The post-operative stereotactic CT showing the microelectrode position (Figure 2.1) was merged with the pre-operative MRI to confirm the correct electrode placement.

2.3. Data analysis

Post-operative volume rendering 3D reconstructions of the microelectrode trajectories was done to identify the correct position of the recorded neurons. They were performed by the Dextroscope® system (Dextroscope, Bracco Inc., Singapore) loading volumetric postoperative MRI and CT images merged together. Neurons with stable activity recorded for at least 10 seconds were further analyzed. Off-line analysis of the spontaneous activity of neurons sampled intra-operatively was performed by the Spike2 analysis package (CED, Cambridge, UK). Single unit events were discriminated, and confirmed to arise from a single

neuron, using template-matching spike sorting software. The results were inspected for the accuracy of spike identification, with inappropriate identified spikes reclassified individually or the spike sorting repeated. The firing rate was calculated by dividing the total number of the isolated spikes by the length of the recording. All data were expressed as the mean \pm SD. Inter-spike interval histograms (ISIH; 5 ms bin width and lag up to 100 ms) were plotted to inspect the firing pattern's properties of the spike train. A spike train with a high proportion of inter-spike intervals (ISI) shorter than 5ms, was evaluated as expression of high frequency firing discharge, and classified as bursting discharge. A spike train with a high proportion of ISIs higher than 5ms, was classified as tonic firing discharge. In each neuron the presence of rhythmicity of the spike trains was assessed using auto-correlation analysis. Correlation histograms with a bin width of 5 ms and lags up to 1000 ms. The recurrence of peaks and troughs at regular interval was expression of rhythmicity of the spike train. The oscillation's frequency was determined by calculating the reciprocal of the peak-to-peak time interval of two consecutive peaks. Random discharge was identified when the autocorrelogram exhibited no regularity in the occurrence of peaks and troughs. Spectral analysis was performed on oscillatory neuronal activity to characterize its spectral components. Power spectrum measures power of intensity of the signal as a function of frequency. Spike trains were transformed from a series of events (sampled at 1000Hz) to a continuous function representing the density of the spike in time. The size of the transform used in the Fast Fourier Transform (FFT) analysis was 1024 points yielding a frequency resolution of 0.98Hz (i.e. waveform channel sampling rate divided by the FFT block size). Only cells that yielded at least 19 of non-overlapping power spectrum

blocks were used to calculate the power spectrum. Signal-to-noise ratio (SNR) was calculated by dividing the power of the spike train's "locked" frequency by the mean power across the spectrum. SNR is a measure of the extent to which power is concentrated at the "locked" frequency.

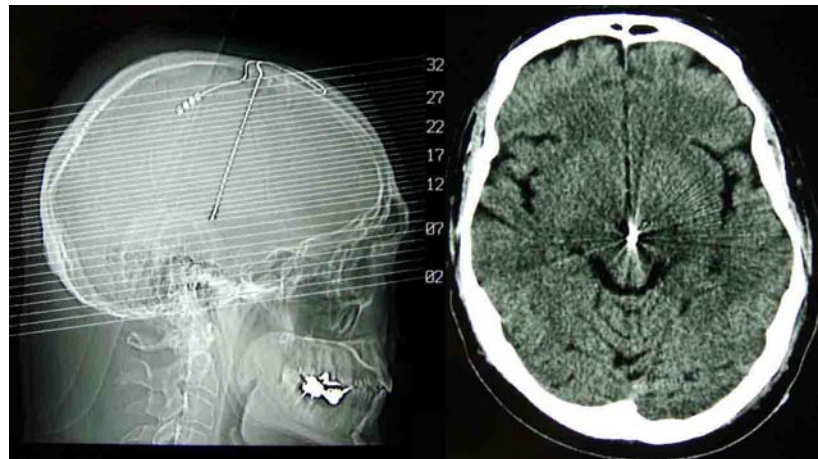


Figure 2. 1

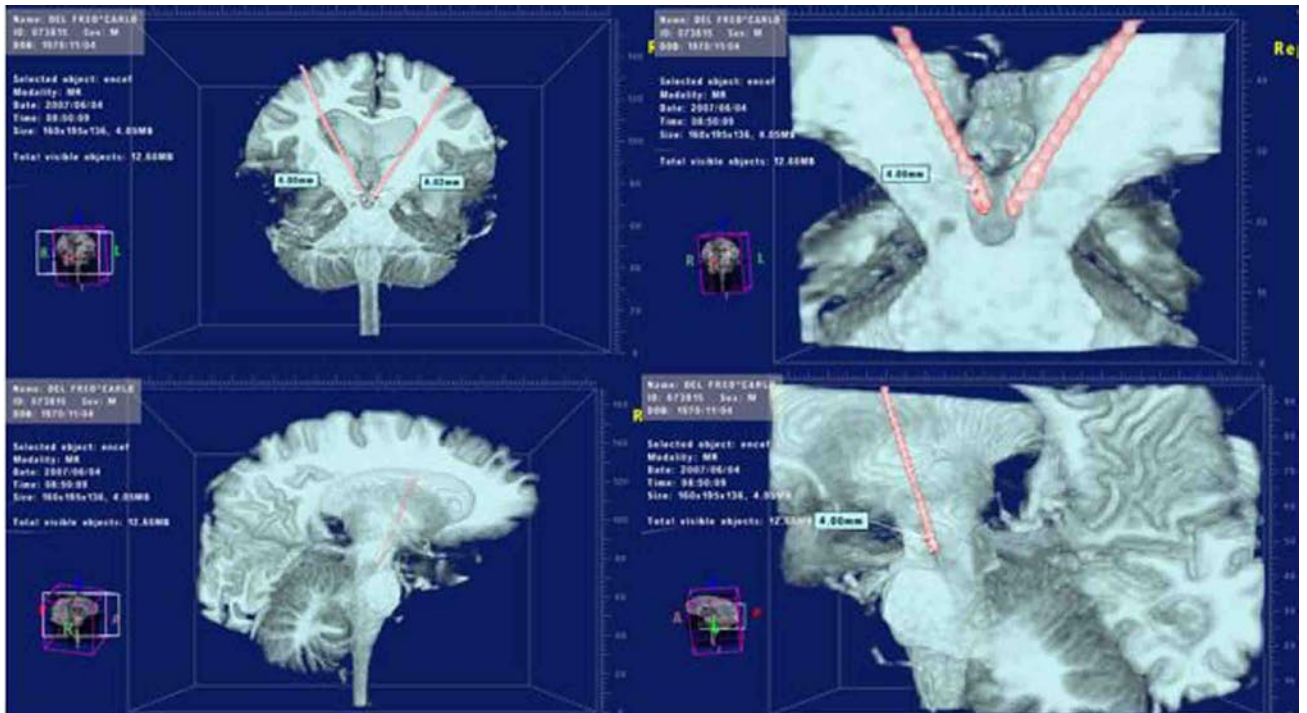
Post-operative CT scans showing the deep brain stimulation lead in the skull of the patient (left panel), and the most distant contacts placement in the diencephalon at the presumed stereotactic coordinates (right panel)..

3. Results

Nine microelectrode trajectories were performed, and nineteen neurons were sampled within the posterior nucleus of the hypothalamus, as deduced by the 3D reconstructions (Figure 3.1). Microrecordings were safe causing no morbidity. Intraoperatively none of the studied units has showed either activation, or inhibition following tactile and pin-prick stimulation with the exception of one in a TAC patient. Spike trains sampled were long enough to be further analyzed. Following surgical procedures, symptoms improved in all patients with no relevant side effects.

3.1. Comparison between behavioural states

Table 3.1 describes the firing characteristics of neurons sampled in either awake or sedated patients. Seventeen neurons were employed in this study. Two cells recorded in two awake TACs patients were not part of this study because both had cluster attack and received triptans during the procedure indeed sampled data are of difficult interpretation. Neurons recorded from three awake TACs patients displayed tonic and irregular firing discharge at around 24Hz. Neurons sampled from two sedated aggressive behaviour patients, displayed tonic and phasic discharge at around 12Hz. No awake neurons had rhythmic spike trains, while during sedated PIH states neurons showed both rhythmic and non-rhythmic discharge pattern.

**Figure 3. 1**

Microelectrode's trajectory 3D reconstruction. This method allows the correct placement of the sampled neurons.

Table 3. 1

Firing rate comparison between awake and sedated conditions.

| Behavioural states | N of cells | Firing rate (Hz) | Standard Deviation(Hz) | Min\Max (Hz) | Firing Pattern | Rhythmicity |
|--------------------|------------|------------------|------------------------|--------------|-------------------|----------------------|
| Awake | 3 | 24 | 8 | 15\32 | irregular | Random firing |
| Sedated | 14 | 12 | 11 | 2\32 | Regular\irregular | Random\Phasic firing |

3.2. Neuronal activity in TACs

Five cells (one in each patient) sited in the PIH as confirmed by post-implant MRI. The average firing rate is 16 ± 12 Hz (range 5-32). Interestingly two patients had a pain attack during the surgery and received drugs to control it, indeed it has been possible to compare the discharge properties between the group pain-free and the group with-pain. Figures 3.2 and 3.3 display the raw physiological traces recorded from TACs patients who were awake and pain-free throughout all the procedure. All neurons showed no rhythmic discharge pattern. Only one cell showed a periodicity of 1 Hz reflecting a cardiovascular artefact (Figure 3.3).

Three cells were sampled from the PIH of three pain-free patients. The average firing rate is around 24 spikes/s, and all neurons generated for the most of the recordings isolated action potentials, as the ISIHs have shown the highest concentration of intervals in the 10-15 ms range (Figure 3.2B), with 7.2% of ISI shorter than 5 ms, which reflect very high intraburst frequencies. The autocorrelograms displayed any regularity in the occurrence of peaks and troughs (Figure 3.2C), which indicates the lack of periodicity of the firing discharge. Only one autocorrelogram displayed regularity in the occurrence of peaks and troughs, with oscillatory pattern at around 1 Hz.

Two cells were sampled from the PIH of two with-pain patients. The average firing rate is 5 Hz, and both units had tonic discharge pattern.

In one patient, bilateral tactile stimulation of the ophthalmic branch showed that contralateral stimulation induced a reduction of firing rate; instead ipsilateral stimulation evoked a not discernible increase in neural activity (Figure 3.4).

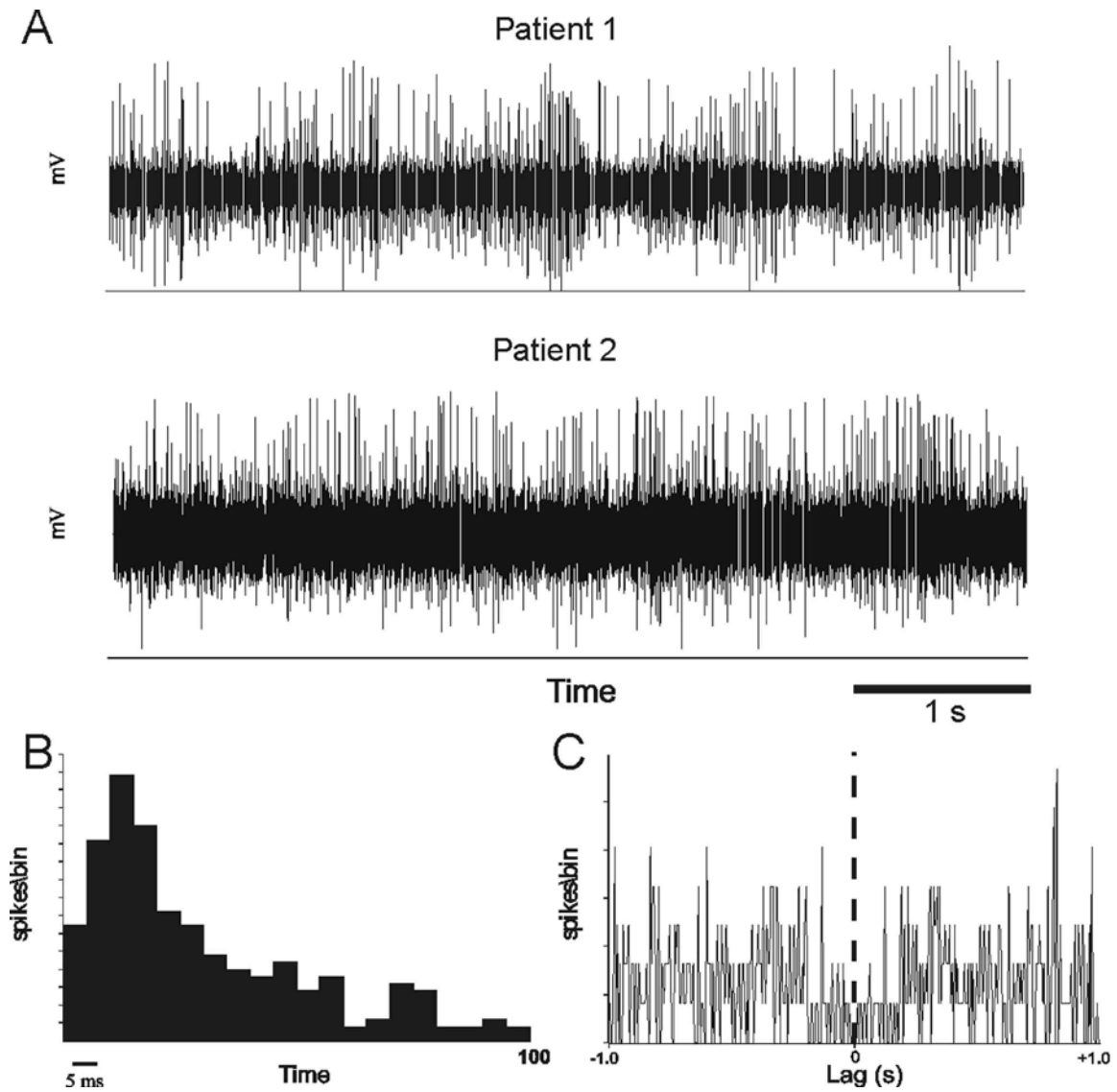


Figure 3. 2

A. Raw physiological traces from two awake pain-free TACs patients. B. ISIH showing the highest concentration of intervals in the 10-15ms range, and a low percentage of ISI shorter than 5ms. C. non-rhythmic autocorrelogram from the same cell as in B. No regularity in the occurrence of peaks and troughs denotes non-rhythmic discharge.



Figure 3. 3

Raw physiological traces sampled in one awake TACs patient showing a phasic discharge around 1Hz. This might be a pulsatile artifact, most likely due to the proximity of the microelectrode to a blood vessel.

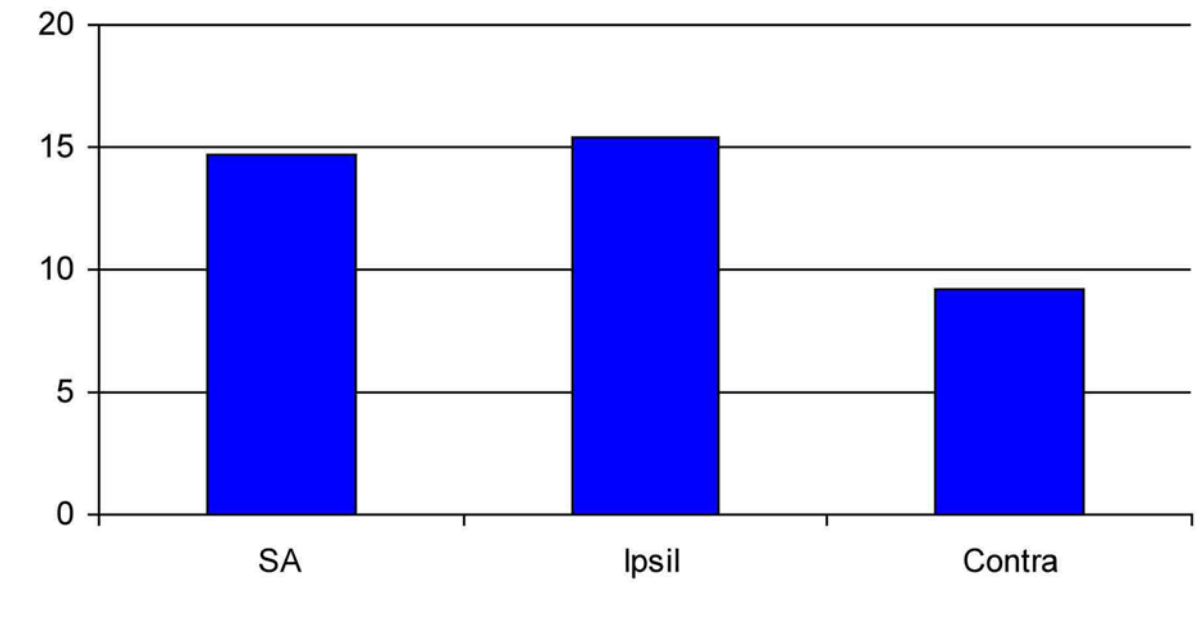


Figure 3. 4

Graphic showing the spontaneous firing rate (SA) and the evoked response to tactile stimulation of the ophthalmic branch in one patient. Contralateral stimulation inhibited the firing discharge, while no discernible changes are seen to ipsilateral stimulation.

3.3. Neuronal activity in aggressive behaviour

3.3.1 Global population

Spontaneous neuronal activity was recorded along 4 trajectories (two in each patients). All trajectories were planned to explore the targeted area, thus were not used to modify the target's coordinates. Along the trajectories it has been possible to identify several types of firing discharge rates and patterns. A total of 14 cells sited in the posterior hypothalamus, as deduced by the 3D reconstructions (Figure 3.1), were analyzed. None of them showed either activation or inhibition to tactile and pin-prick stimulation. The average firing rate for these cells is 13Hz (Table 3.2), although 9 cells (64%) showed a low frequency discharge at around 5Hz, and the remaining 5 cells (36%) discharged at higher frequencies (26Hz). Several firing patterns have been noticed: 4 cells exhibited tonic regular discharge, 4 cells tonic irregular discharge, 4 bursting discharge, and 2 had a sporadic firing. Periodicity was described in 5 units (4 bursting and 1 regular), but the remaining randomly fired.

3.3.2 Aggressive behaviour and epilepsy

In this patient 4 hypothalamic cells were analyzed, and were mainly concentrated within 1mm from the target, although above this landmark there was either lack of activity or the recorded units were not stable and not suitable for further studies. The average firing rate was 19 ± 13 Hz (Table 3.2). Two neurons were recorded from the right PIH (Figure 3.5 upper panel), and both had a low frequency discharge (6 and 9Hz respectively). Two were sampled from the left PIH (Figure 3.5 lower panel) showing higher firing rates, respectively 33 and 28Hz.

All ISIHs displays the highest concentration of ISI below 5ms (Figure 3.6A).

Table 3. 2

Firing discharge in psychiatric patients.

| Diagnosis | N. of cells | Mean firing rate (Hz) | Standard deviation (Hz) | Min\Max (Hz) | Firing pattern | Rhythmicity |
|--------------------------------------|-------------|-----------------------|-------------------------|--------------|-------------------|-------------|
| Aggressive behavior and epilepsy | 4 | 19 | 13 | 6\33 | phasic | 7-8Hz |
| Aggressive behaviour and head injury | 10 | 10 | 10 | 2\32 | Regular\irregular | random |
| Total | 14 | 13 | 12 | 2\33 | | |

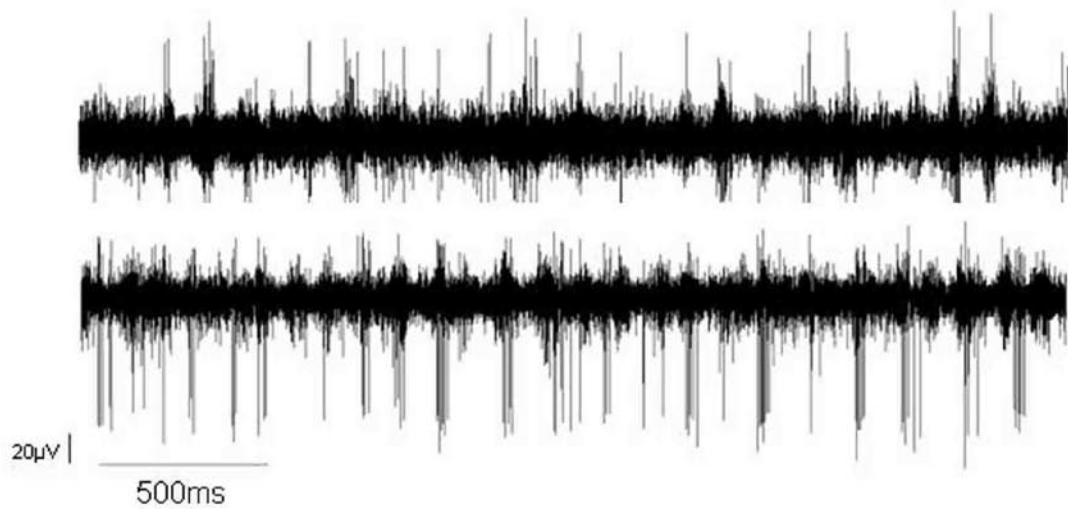


Figure 3. 5

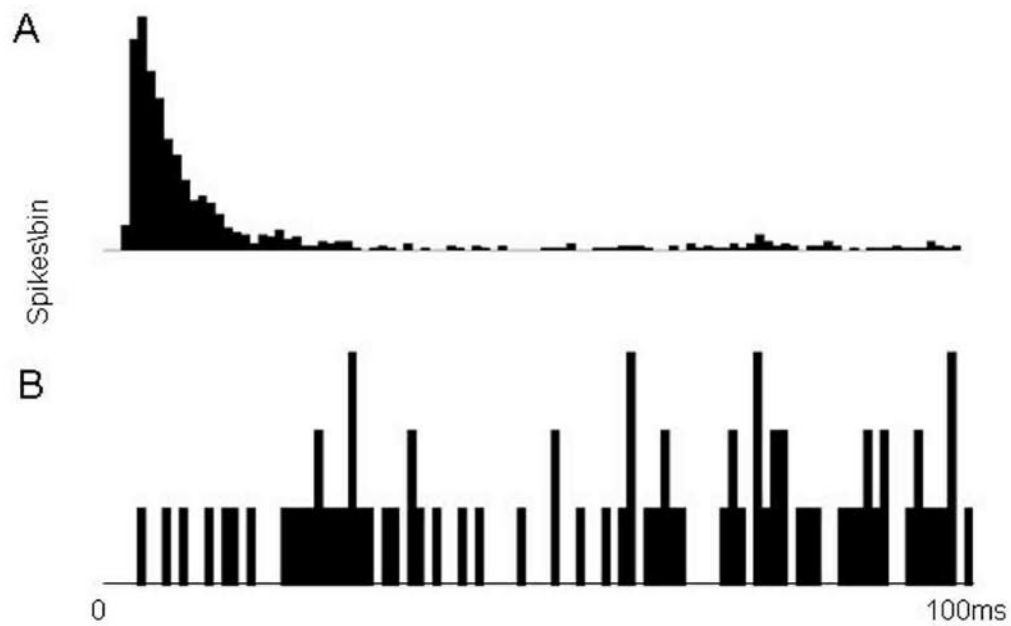
Raw physiological traces recorded within the PIH in aggressive behaviour and epilepsy. Upper panel, low firing discharge recorded from the right PIH; lower panel, high firing discharge recorded from the left PIH.

This suggests the presence of high frequency discharge, namely bursting activity. All studied neurons displayed autocorrelation histograms with the occurrence of peaks and troughs at regular intervals (every 125\140ms), suggesting the periodicity of the spike train. The rhythmicity has been assessed to be at around 7\8Hz (Figure 3.7A). The oscillation's frequency has been further confirmed by plotting power spectrum histogram, which displayed the locked frequency at 8Hz. Figure 3.8 displays the time domain and the frequency domain analyses for one of these neurons. Panel A shows the ISIH with a high proportion of ISI shorter than 5ms indicating bursting activity; Panel B: autocorrelogram for the same cell displays the regularity of the distribution of the action potential, indicating oscillatory activity; Panel C: oscillatory activity was further confirmed by power spectrum showing the locked frequency at 7Hz. This oscillatory band is associated with the EEG recording from bilateral sites showing a high amplitude low frequency pattern (theta band) intermingled with spike wave activity (Figure 3.9).

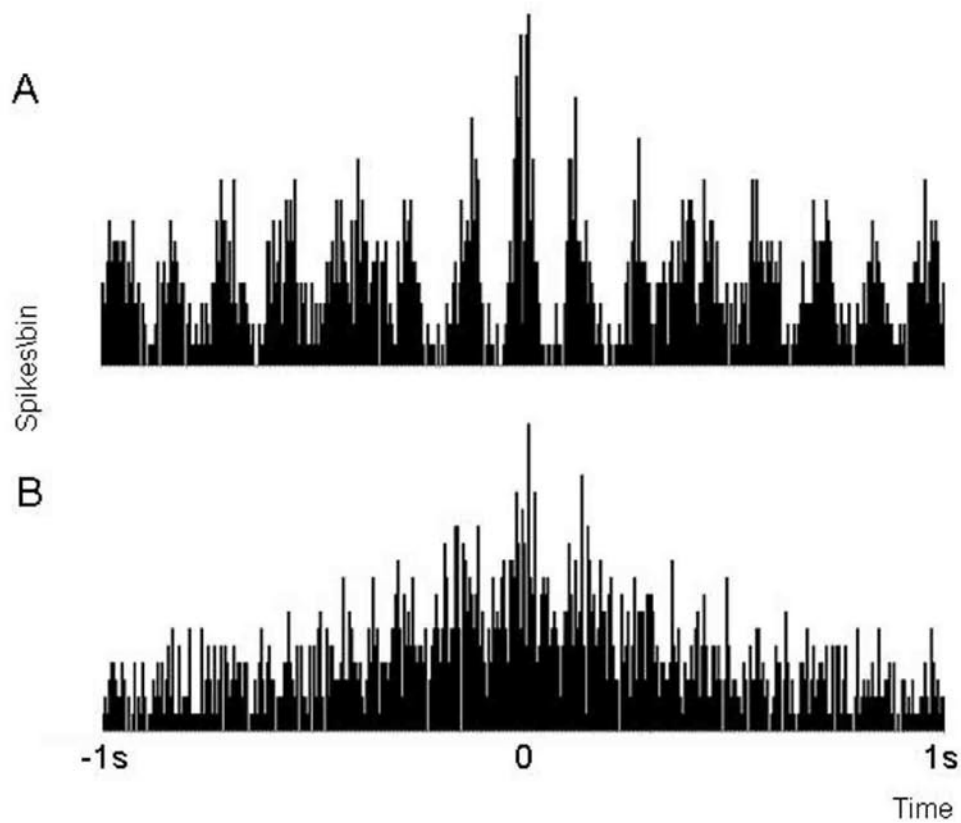
3.3.3 Aggressive behaviour and head injury

In this patient spontaneous neuronal activity from 10 cells has been recorded along the final 4mm from the target. The distribution of firing rates and patterns was random. The average firing rate for these neurons was 10 ± 10 Hz (Table 3.2). Three out of ten cells exhibited high discharge rates (average 23Hz, range 15-32 Hz), and the remaining seven low firing rates (average 4Hz, range 2-10Hz). Figure 3.10 displays the raw physiological traces reflecting both a high frequency (upper panel) and low frequency discharge (bottom panel).

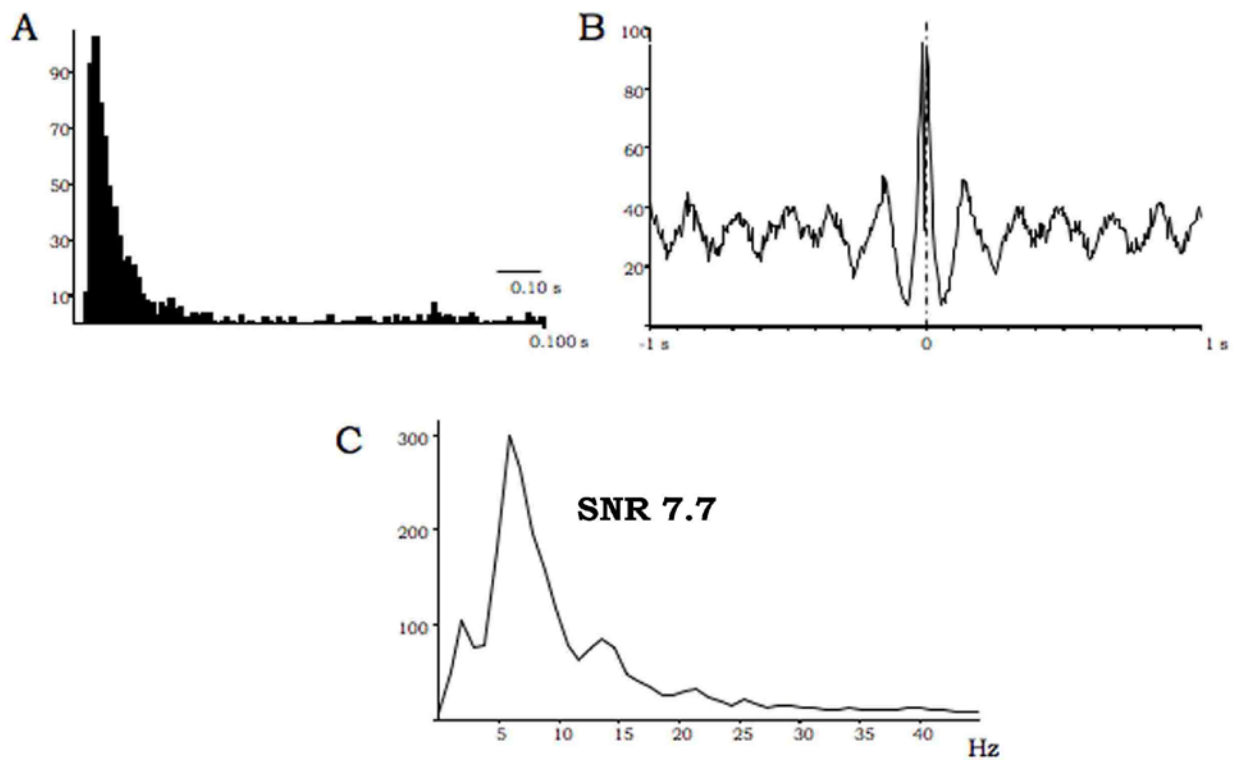
The majority of the analyzed ISIHs have shown random distribution of ISIs (Figure 3.6B), indeed irregular discharge pattern. 4 cells exhibited regular discharge, 4 cells irregular discharge, and 2 sporadic irregular activity. Moreover none of the autocorrelograms displayed the regular occurrence of peaks and troughs (Figure 3.7 lower panel), thus all the studied neurons were firing in tonic fashion. All but one was firing randomly. The only exception showed oscillatory activity assessed at around 25Hz (gamma band).

**Figure 3. 6**

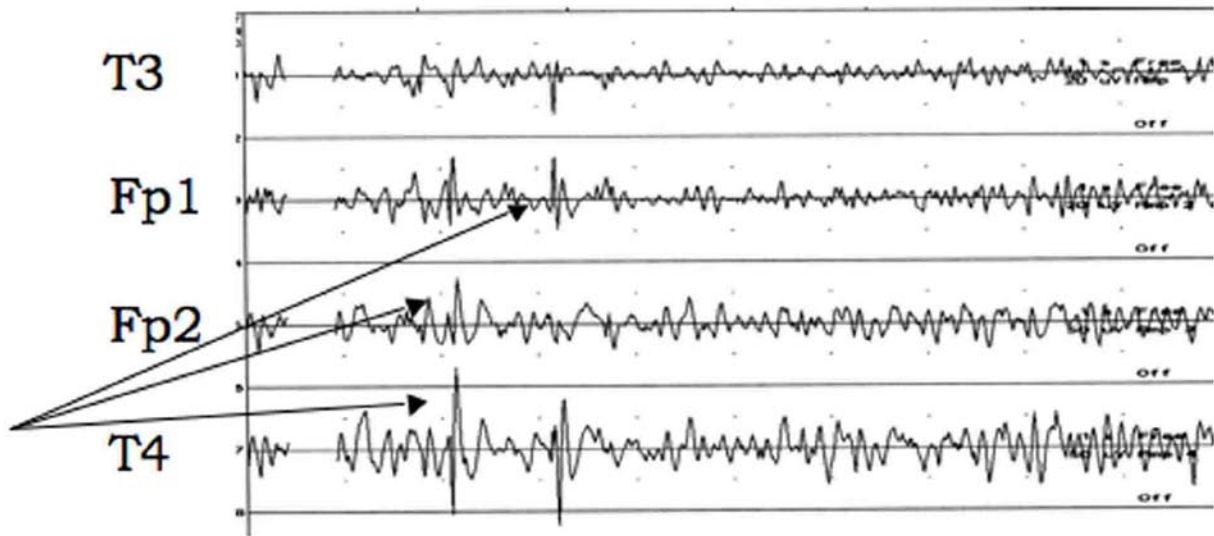
A. ISIH for one unit sampled in aggressive behaviour and epilepsy. The large majority of ISIs are concentrated at around 5ms. This indicates high frequency bursting discharge. B. ISIH for one unit sampled in aggressive behaviour and head injury. The distribution of ISIs is random, which indicates tonic discharge

**Figure 3. 7**

A. Autocorrelation histogram for one unit sampled in aggressive behaviour and epilepsy. The peaks and troughs occur at regular interval. B. Autocorrelogram for one unit sampled in aggressive behaviour and head injury. The peaks and troughs are randomly distributed.

**Figure 3. 8**

Time and frequency domain analyses for one unit sampled in aggressive behaviour and epilepsy. A. ISIH showing the highest concentration of ISIs at around 5ms. B. Autocorrelogram showing regularity in the occurrence of peaks and troughs. The interpeak intervals are around 140ms (7Hz). C. Power spectrum displaying the locked frequency at 7Hz.

**Figure 3. 9**

EEG recordings from bilateral temporal and frontal sites. Large amplitude low frequency activity (delta\theta band) is associated with the occurrence of spike and wave discharge (indicated by arrows).

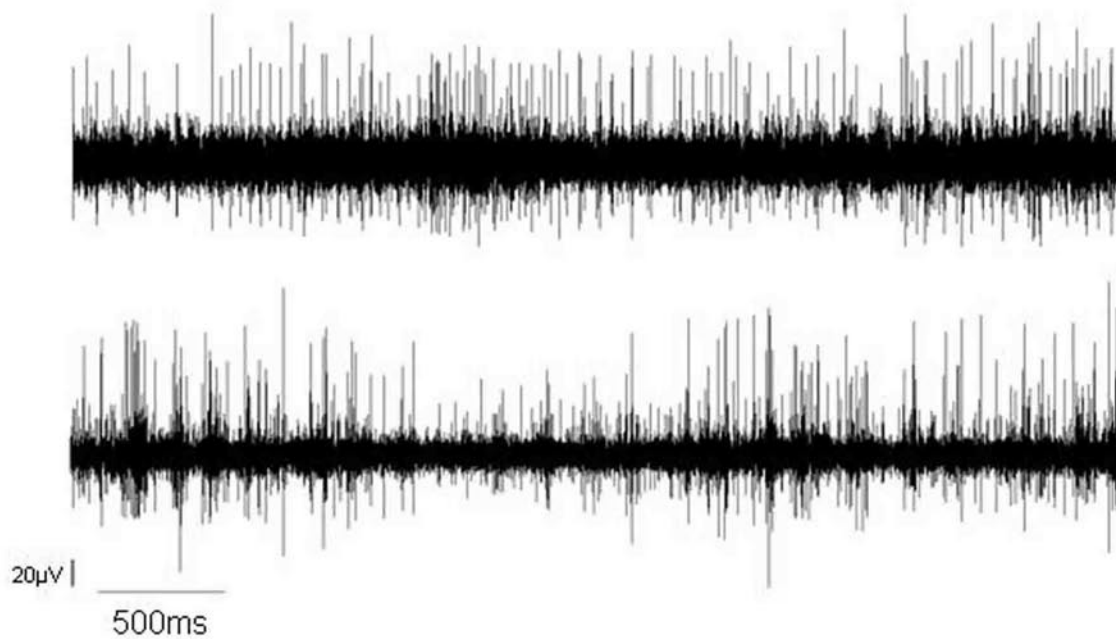


Figure 3. 10

Raw physiological traces sampled within the PIH of the aggressive behaviour and head injury patient. Upper panel: irregular high firing discharge; Lower panel: irregular low frequency discharge.

4. Discussion

This study reports the spontaneous neural activity sampled within the posterior nucleus of the hypothalamus in pain and psychiatric patients during the placement of deep brain stimulation leads. Following the neurosurgical procedure, the symptoms improved in all patients, suggesting that the electrodes were at a clinically effective site.

These studies represent the first attempt to quantify PIH neuronal activity in patients with TACs, and aggressive behaviour. The use of deep brain stimulation of the posterior hypothalamus has brought about a new opportunity to investigate the neuronal activity of this region in man with microrecordings. This technique allows the collection of signals from single neurons that are sampled during mapping of subcortical regions (Tasker *et al.*, 1998). Thus it can offer an opportunity to characterize the posterior hypothalamus neuronal activity, and may provide data to conceive a hypothesis of what is wrong with trigeminal autonomic cephalalgias, and aggressive behaviour.

4.1. Methodological considerations

Microrecordings data are obtained using high impedance microelectrodes that allow isolation of single neurons. The acquisition of valid data requires expertise, dedicated equipment and attention to the conditions of recording such the effects of drugs and the level of vigilance. Neuronal activity should be recorded in large number of cells and for epochs of at least 10 seconds to detect oscillatory activity. Microrecordings and isolation of single units are feasible in human hypothalamic area, with no morbidity in the series presented here.

Very scanty information is available on the firing characteristics of PIH neurons in humans. Thus this study represents the first attempt to characterize the firing rate and the firing discharge of neurons sited in the posterior nucleus of the hypothalamus in awake and sedated patients with pain and psychiatric pathologies.

A weakness of this report is the small number of analyzed cells, and the heterogeneity of the pathologies, and no clear physiological considerations or conclusion can be done. Nevertheless due to the scarcity of patients with TACs, and especially with aggressive behaviour who might be candidate to neurostimulation, it is justified by the interesting findings compared to the other populations of hypothalamic cells described in literature. In fact part of the data presented here have represented the first objective description of PIH neurons discharge pattern (Cordella *et al.*, 2007). Following this one more paper has dealt with microrecordings data recorded in pain patients (Starr *et al.*, 2007).

4.2. Towards a posterior hypothalamic neurophysiological mapping

Subcortical mapping through microrecordings techniques is consistently employed during the placement of DBS electrodes in Parkinson's disease (PD), dystonias, and pain (Tasker *et al.*, 1998). The neurophysiological mapping is used to corroborate the neuroradiological data on target coordinates. Usually the microrecordings offers the opportunity to record the firing discharge, and to evaluate the evoked responses to stimulation of neurons placed in a precise anatomical area. The information obtained through the description of the firing rate and firing pattern of the encountered single neuron is used to construct

neuroanatomical maps. From these maps, the neurosurgeon selects the best coordinates to place the stimulating leads. Various anatomical sites have been used as final target to stimulation, such as the subthalamic nucleus (STN) for PD (Pollack *et al.*, 2002), the globus pallidus (Gp) for dystonias (Lozano and Hutchison, 2002), and several thalamic nuclei for pain (Tasker *et al.*, 1987). In these neuroanatomical districts there is converging consensus about the firing discharge of each neuronal populations. An example could be the discharge pattern of thalamic neurons, with the presence of tonic and bursting firing discharge.

At the moment it is not possible to produce neurophysiological maps during PIH DBS neurosurgical procedures. Microrecordings data are not used to modify the target's coordinates. However data from this study, supported by others (Starr *et al.*, 2007) may provide several suggestions. First, neuronal activity should be expected when the microelectrode is within the PIH. Second, in awake pain-free patients high firing and tonic discharge pattern should be noticed. Third, in sedated condition an average low firing rate with either phasic, usually rhythmic, or tonic and arrhythmic discharge patterns. Fourth, a pulsatile neuronal activity, probably due to the close proximity of the PIH to the third ventricle, interpeduncular cistern, and basilar bifurcation. It is worth of mention that pulsatile neuronal activity synchronized with heart beat has been also described in rats (Beatty *et al.*, 2005). Fifth, lack of neuronal activity and abnormal impedance values should suggest that the exploring microelectrode is in the interpeduncular cistern. Starr and co-authors (2007) have described this latter point. Interestingly, they reported that neuronal action potentials were absent at the most ventral extent of the target region in one case. Post-operative

imaging in this case showed that the lowest contact was in the interpeduncular cistern. Penetration of the electrode tip into the interpeduncular cistern carries the risk of injury to vessels traversing this region.

PIH has a low density of spontaneous active cells when compared to either the STN, or Gp targets, as pointed out earlier, where neurons are routinely recorded during movement disorders surgeries, and no clear nuclear border identification could be done. The thalamic-hypothalamic transition is characterized by a continuum of progressively more sparse neuronal activity.

Nevertheless the limited number of published studies suggests caution to attempt the mapping of the PIH. As a consequence microrecordings within the PIH should be mainly employed as an explorative tool allowing the samplings of neuronal activity within this unexplored brain area.

4.3. Behavioural states

Classically the posterior hypothalamus has been linked to the control of behavioural states. In animal models hypothalamic neurons spontaneously discharge at around 25Hz in awake states, around 15Hz during slow wave sleep (Pare *et al.*, 1989), and around 13Hz in anesthetized rats (Beatty *et al.*, 2005). The firing pattern is described as tonic in all three conditions. In my study it has been possible to record spontaneous neuronal activity recorded in awake and sedated conditions. It is reported that in awake states human's PIH neurons discharge in a tonic fashion at around 24 spikes per second. On the other hand in sedated states human's PIH discharge in tonic and phasic fashion at around 12 spikes per second. Thus it appears that in both awake humans and cats,

posterior hypothalamic neurons spontaneously discharge at high firing rates, in tonic fashion with rare high-frequency bursts.

Despite the similar low frequency firing rates, humans and animal differ in discharge pattern during sedated states. In fact the firing rate is at around 12Hz in both animals and humans, however the firing pattern appears to be more complex in humans. In cats the firing discharge is tonic, while in humans tonic and phasic discharges were described.

4.4. TACs

In this studies it is described that the mean firing rate of PIH neurons in TACs is 16Hz. Starr and colleagues (2007) reported an average firing rate of 20Hz. Both studies did not detect any oscillatory activities. Several differences between the two studies need to be addressed. First, it is important to mention that in my series two patients had a TAC bout during the neurosurgical procedures, and received triptans to improve the symptoms. Both of them showed low frequency firing rates at around 5Hz. Thus the overall mean firing rate is lowered by these two neurons. In these two patients it is not possible to assess whether the low frequency is either a PIH neurons normal feature, or associated to the painful state. Starr and co-worker did not report TAC bouts in their series, thus any comparison is possible. Due to the difficult comprehension of the data, these two patients were excluded from the analysis. As a consequence the TAC series I have presented had an average firing rate of 24Hz. Second, the number of studied units differs between the two series. I have quantified data from five units instead Starr and co-workers described a larger

number of neurons. Third, I have sampled activity from the target site, Starr and colleagues performed explorative trajectories, and sampling data within various depth of the nucleus.

It is not certain that the neurons whose physiology has been described in the two studies are involved in the pathophysiology of TACs. It is well known that PIH grey matter is composed by neurons that send and receives projections to various brain structures (Abramson & Moore, 2001). It has also been demonstrate that PIH has neurons that carry the receptors for neuropeptides orexins A and B (Smart, 1999), which is demonstrated to mediate the regulation of autonomic, neuroendocrine and nociceptive functions (Bartsch *et al.*, 2004). Some of the neurons described in these two studies may be those expressing orexins receptors, thus potentially causal in the pathophysiology of TACs, but this is still far to be demonstrated.

Before the availability of modern neurostimulation devices, termocoagulation of the posterior medial hypothalamus in the treatment of facial cancer pain has been firstly applied by the Japanese neurosurgeon Sano (1977). Later on in experimental models it has been described: analgesia following the electrical stimulation of the hypothalamus (Lopez *et al.*, 1991); a monosynaptic pathway connecting the hypothalamus and the trigeminal nucleus (Malick *et al.*, 2000); and a differential modulation by hypothalamic neurons on trigeminal nucleus caudalis nociceptors (Bartsch *et al.*, 2005). Interestingly a recent PET study has investigated potential differences in the cerebral metabolic activity when the stimulator was on or off, in 10 patients with chronic cluster headache whom have received beneficial effects by PIH DBS (May *et al.*, 2006). The results pointed-out a stimulator-induced activation in the ipsilateral PIH (site of the

DBS tip), in the ipsilateral thalamus, somatosensory cortex, praecuneus, anterior cingulate cortex, the ipsilateral trigeminal nucleus and ganglion, and a reduced metabolism in the middle temporal gyrus, posterior cingulate cortex, bilateral inferior temporal gyrus, and contralateral anterior insula. Amusingly is that descending antinociceptive key structures such as the periaqueductal gray (PAG) and the periventricular gray (PVG) are not involved during the neurostimulation, despite the densely interconnections between these two areas and the PIH (Vertes and Crane, 1996). Thus it is feasible to exclude a pure antinociceptive mode of action by DBS, and to speculate that the efficacy of the DBS may be consequence of a direct modulatory effect by hypothalamic neurons on trigeminal nucleus nociceptors, or on the other hand, the positive effects may be consequence of the modulation of complex neuronal network, rather than consequence of a focal effect, since all the cerebral structures described by May and co-workers (2006) belong to the neuronal circuitries normally involved in pain transmission.

Sano (1977) reported, although did not perform quantitative measurements, that bilateral stimulation over the entire body evoked changes in discharge rates, and that pin-prick stimulation evoked neural responses with latencies similar to those of the C fibers. In one of our patients, tactile stimulation of the ophthalmic branch contralateral to the recording side led to a decrease in firing rates. Altogether these observations suggest that PIH neural activity may be modulated by afferent sensory input. Experimental findings in rats show that two distinct tracts relay sensory information to the posterior hypothalamus, the trigeminohypothalamic tract that conveys nociceptive inputs from cephalic region only, and the reticulohypothalamic tract, which conveys sensory inputs

from both cephalic and extra-cephalic regions. Interestingly, trigeminohypothalamic neurons exhibited receptive fields limited to regions contralateral the recording site while reticulohypothalamic neurons had large and complex receptive fields extended to the whole body. However Starr and colleagues (2007) did not describe modulation of PIH neuronal discharge by sensorial stimulation, thus the presence of these two fiber tracts in humans has not been demonstrated yet.

4.5. Aggressive behavior

This study describes the neuronal activity sampled within the posterior nucleus of the hypothalamus in two patients implanted with DBS electrodes to reduce aggressive behaviour. One patient had associated epilepsy, and the other had multiple traumatic lesions located in the temporal lobes. Both patients were sedated during the procedures due to the difficulties in controlling their conduct. To my knowledge there are no data in literature that deal with PIH firing discharge in psychiatric patients.

Various types of firing frequencies and patterns were noticed, especially in neurons sampled from the patient with aggressive behaviour secondary to head injury. Despite differences in the firing rates, all neurons recorded from the epileptic patient were firing in burst fashion, while in the head injured aggressive patient all cells were tonically firing without periodicity, with the exception of one.

However the most striking observation is the unique discharge pattern recorded in the epileptic patient. In this case, all hypothalamic neurons were

periodically firing in burst mode, at around 7\8Hz (EEG theta band). This pattern has not been noticed in the previously recorded units in TACs patients (Cordella *et al.*, 2007; Starr *et al.*, 2007). An important difference between the TACs series and the one reported here is that patients in the former group were awake throughout the procedures, whilst those in the latter group were under general anaesthesia. As a consequence the notable differences between the two population may be due to the anaesthetic agents used to induce the general anaesthesia. Nevertheless great differences were also noticed between the two sedated patients described in this report.

It is conceivable to hypothesize that these dissimilarities might be linked to the underline pathology. In particular, the posterior hypothalamic neurons in TACs tonically discharge at around 20\25Hz (Cordella *et al.*, 2007; Starr *et al.*, 2007), in aggressive behaviour secondary to head injury tonically and random at around 10Hz, and in aggressive behaviour associated to epilepsy periodically bursting, at around 17Hz. Still the differences in sedation regimens should be considered. Data from animal models reported that hypothalamic neurons spontaneously discharge tonically and at around 25Hz in awake states, tonic and around 15Hz during SWS (Parè *et al.*, 1989), and around 13Hz in anesthetized rats (Beatty *et al.*, 2005).

Nevertheless at this stage of knowledge it is not feasible to exclude the option that each described activity might represent normal features of distinct population of hypothalamic neurons.

The role of the hypothalamus in aggressiveness was firstly assessed by the Japanese neurosurgeon Sano, who treated these patients by lesioning this structure (Sano, 1970). The hypothalamus is a core structure of the limbic

circuit that connects two large limbic domains. One is related to the hippocampus, the other to the fronto-orbital cortex. The hypothalamus represents a key structure of the Papez circuit, having connections with the hippocampus, amygdala, limbic thalamus through the mammillary bodies and the fornix, the cingulate gyrus and the entorhinal cortex. The connections with the hippocampus, amygdala, cingulate gyrus and entorhinal cortex could explain the role of the hypothalamus in learning, memory, emotion, motivation, affiliative behaviour, and autonomic and endocrine functions (Mayanagi *et al.*, 1982).

It might be potential that hypothalamic neurons might have discharge patterns related to the underline pathology, thus oscillatory activity when the hippocampus and amygdala are involved in the disturbance, as happens in epilepsy. In experimental models the dependence of theta-related single unit discharge of cells in the posterior hypothalamic region on theta activity in the septo-hippocampal system was assessed (Kirk *et al.*, 1996). Recordings were obtained from the posterior nucleus, the supramammillary nucleus (SuM), and the medial mammillary (MM) nucleus during hippocampal theta activity elicited by nucleus reticularis pontis oralis stimulation (RPO). The results pointed out that cells sited in each nucleus were shown to have distinct theta-related discharge pattern. All theta-related cells recorded in the PIH were found to be of the tonic theta-ON type. This means that they discharged tonically at higher rates during hippocampal theta elicited by RPO stimulation. All theta-related cells recorded within the supramammillary and the medial mammillary nuclei were found to be of the phasic-ON type. This means that they discharge in rhythmic bursts phase locked with the hippocampal theta oscillation. It was

also found that theta-related cells in different nuclei were differentially affected by the abolition of the theta oscillation. The PIH cells tonic discharge survived after the theta oscillation suppression. Similarly the rhythmic bursts in the remaining two nuclei were still observed after the abolition of the hippocampal theta. Thus it could be concluded that no bursting discharge has been noticed in PIH neurons.

As previously stated, there are no data on the discharge characteristics of neurons recorded within the human's hypothalamus in psychiatric patients. Only one study has dealt with electroencephalographic recordings from the mammillary bodies of epileptic patients (van Rijckevorsel *et al.*, 2005). The mammillary bodies are part of posterior third of the hypothalamus. These recordings were obtained during the placements of DBS electrodes to improve symptoms in three patients with refractory epilepsy. The results pointed out a predominant electrophysiological pattern recorded during the different sleep-wake phases of low amplitude mixed delta-theta activity in all three patients.

On the other hand tonic discharge pattern were noticed in all neurons sampled within the PIH of the patient with head injury, although more variability is reported in the firing rate, displaying low and high firing discharges. It is possible that the differences in the firing pattern but not in the firing rate might be consequence of the head injury, where there was either the abnormal, or lack of connections from the temporal cortex and the amygdala to the posterior hypothalamus.

From these studies it appears conceivable to hypothesize that under normal circumstances PIH neurons discharge in tonic fashion either rhythmically or no-rhythmically, and that in epileptic patients they discharge in low frequency

(EEG theta band) rhythmic bursts. It is worth of mention that in this patient intraoperative EEG activity was assessed as low frequency with spikes and wave discharge. On the other hand it is not possible to exclude that the neuronal theta oscillations described in my series are not sampled within the PIH but within the adjacent mammillary bodies. However the different stereotactic coordinates between these two nuclei, the post-op neuroradiological check, and the 3D reconstructions merging post-op CT and pre-op MRI scans limited this eventuality, and gave strength to the fact that the microrecordings were effectively done within the targeted posterior hypothalamus.

However at this stage these are not hypotheses, but merely speculations, needing more data to be developed. This is the consequence of the small number of patients and the great differences between the methods applied in humans and animals studies, indeed no clear physiological conclusion or comparison can be done at the moment. Nevertheless the reported differences in discharge patterns between pain and non-pain patients should be taken in mind by neurophysiologists during the intraoperative microrecordings used to localize the posterior nucleus of the hypothalamus.

4.6. Conclusions

In this study I have reported quantitative analysis of the firing discharge of neurons located within the posterior hypothalamic nucleus in patients with pain and psychiatric pathologies. This represents the first attempt to characterize posterior hypothalamic neuronal activity in humans. I have demonstrated that neuronal activity should be expected when the microelectrode is within this nucleus. Behavioural states such as awake or sedated might influence the

neuronal activity. Similarly to what has been reported in animal models during wakefulness the PIH neuronal activity is lower than during sedated condition. Tactile and pin-prick stimulation did not evoke any discernable changes in the firing discharge, with the exception of one cell showing inhibition to contralateral stimulation of the ophthalmic branch. This is in contrast to what has been reported in rats. Similarly to what is described in rats and cats, all neurons were firing in tonic fashion with the exception of those recorded in the aggressive behaviour and epilepsy. In this case all neurons were firing rhythmically in burst mode. This latter result is not reported in animal models where is reported bursting discharge within the supramammillary and the medial mammillary nuclei but not within the PIH.

I suggest that PIH neurons have tonic and high frequency discharge during awake state, and tonic and low frequency discharge in sedated states. Moreover in epileptic states, PIH neurons are synchronized to the cortical theta activity, thus displaying rhythmic bursting activity. In the absence of data obtained from the posterior hypothalamus of normal humans I cannot exclude the possibility that these observations could be normal features of PIH neurons.

Nevertheless the small number of units along with the limited number of published reports of PIH neural activity in both humans and animals limited the strength of these conclusions. More data are needed in order to characterize PIH activity to help intraoperative neurophysiologists to map this area, thus increasing the probability to find the best spot to place the DBS leads. Moreover sampling spontaneous activity and the planning of more sophisticated experiments might help neuroscientists to unveil the pathophysiological role of the posterior hypothalamus in pain and psychiatric diseases

4.7. Future directions

In view of the emerging results from my analysis, there are several studies that would be worthwhile to perform in the future in order to better comprehending the role of the posterior nucleus of the hypothalamus in pain and aggressive behaviour.

I have found similarities between humans and animals PIH neuronal activity in regard to the behavioural states. A limitation of these observations is the small number of studied cells in human's PIH. Thus it is conceivable to suggest to sample more cells before any conclusion.

The evidence of increasing cerebral blood flow within the PIH during the TACs bouts (May *et al.*, 1998) suggests that there might be a concomitant increase in neuronal firing discharge. Thus could be of interest to induce a painful attack by the administration of nitroglycerin, which is shown to induce a cluster attack. On the other hand the anecdotal report (Leone *et al.*, 2003) that the administration of sumatriptans has stopped neuronal activity suggests to investigate and to quantify the supposed response of the firing discharge.

I have found differences between the various pathologies. It could be argued that there were differences in the sedation regimens between the two groups. The possibility to record neuronal activity from awake aggressive behaviour patients is not feasible due to the fact that these patients are not cooperative. A possibility is to record activity from psychiatric patients whom can bear an "awake anesthesia" neurosurgical procedure.

I have found bursting discharge pattern within the PIH of aggressive and epileptic patient. This is in contrast to what has been reported in rats, where is

reported tonic discharge pattern. It might be of potential interest to study the PIH activity in awake epileptic patients and to compare it with the sedated one. In addition in this patient intraoperative EEG activity is described to be low frequency with spike and wave discharge. The single-unit recordings displayed oscillatory activity locked in the theta band. It would be of interest to investigate the synchronization between the cortex and the PIH, employing a frequency-domain analysis, with the coherence technique. The analysis should be performed between the EEG activity recorded over scalp and the single-unit activity recorded in the PIH. In addition it would be of interest to investigate the role of ionic conductances in the generation of the bursting discharge.

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