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Heat-Excited and Heat-Inhibited Neurones in the Ventroposterior Nucleus of Thalamus of Anaesthetized Rats

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Summary

The activities of 39 single cells, located in the ventroposterior nucleus of the rat thalamus, were recorded from rats deeply anaesthetized with xylazine and ketamine. The activity of each neurone was recorded before and during noxious tail heating. In all, 17 neurones were excited, 11 were inhibited, and 11 were not affected by the noxious stimulation. The possible function of each type of response in the coding of nociceptive information is discussed.

Key words
Noxious heat – Ventrobasal thalamus – Ketamine-anaesthetized rats – Nociception

Introduction

The ventroposterior nucleus (VP) is an area of the thalamus which has been considered important in the perception of pain across several mammalian species, including the rat, the monkey and man (Price and Dubner 1977, Albe-Fessard *et al.* 1985, Besson and Chaouch 1987). One distinct area of the monkey ventroposterior thalamus, the ventroposterolateral nucleus (VPL), has been found to be involved in the perception and localization of somatosensory information, including pain. Willis (1987) demonstrated that the response of the STT-VPL neurones in the monkey respond to nociceptive information and additional studies have shown that the monkey VPL receives its noxious and non-noxious mechanical and thermal input directly from the dorsal horn *via* the anterolateral spinothalamic tract (STT) (Peschanski *et al.* 1983)

Studies in the rat have shown that in this species the neurones of the STT also project to the VP (Lund and Webster 1967). The VP thalamus of the rat, which is also referred to as the VB, can be distinguished into a medial and a lateral component: the ventroposteromedial (VPM) and the VPL respectively. The VPM contains neurones with receptive fields in the anterior parts of the body, i.e. the snout and the face (Bullit 1990, Emmers 1981). The receptive fields of the VPL neurones are located in the entire surface of the head, body and the tail (Bullit 1990, Emmers 1981). It has also been shown that several neurones of the rat VB respond exclusively to thermal and mechanical noxious stimuli (Guilbaud *et al.* 1980, Mitchel and Hellon 1977). The nociceptive nature of the VB neurones has also been confirmed in a series of immunoreactivity studies using *c-fos*. Bullit (1989, 1990) found that after noxious mechanical or thermal stimulation has been presented to a hind limb, neurones in the VPL and the VPM increased their *c-fos* immunoreactivity. However, only the response of the VPL neurones was statistically significant.

At least one study has shown that noxious heat stimulation of the tail resulted in the activation of many thalamic neurones, the majority of which were located in the VPL (Yen *et al.* 1989). The evidence suggests that the responses of the VPL and VPM to the noxious stimulus might differ slightly from each other depending on the site of the peripheral stimulation (Bullit 1989, 1990).

Previous studies recorded the activity of VB neurones which increase their rate of firing following noxious stimulation (Mitchel and Hellon 1977, Emmers 1981, Willis 1987, Bullit 1989, Yen *et al.* 1989). However, initial studies in our laboratory (Reinis *et al.* 1992, Tsoukatos *et al.* 1991a,b) have revealed that some neurones in the VPL and VPM were activated, whereas the others were inhibited following noxious thermal stimulation (NTS). These preliminary findings

led us to explore the possibility that there is a second group of VB nociceptive neurone inhibited by noxious stimulation. The present study was designed to study the differences between neurones that are either excited or inhibited by NTS.

Methods

Subjects, anaesthesia, recording parameters, and histology

The experiments were performed on 39 male Long-Evans rats weighing between 300–380 g. Following the injection of 100 mg/kg of ketamine (i.p.), and 10 mg/kg of xylazine (i.m.), a small craniotomy was performed above the VB and a recording electrode was lowered into the VB using a hydraulic microdrive. The coordinates for each electrode placement were between 2.8–3.8 mm posterior to the bregma, 2.1–3.2 mm lateral to the midline, and 4.6–7.2 mm ventral to dura (Paxinos and Watson 1986).

The recording method and the software used for the collection and analysis of the data were developed in our laboratory and have been described previously in detail (Tsoukatos *et al.* 1991b). Briefly, neuronal activity was recorded extracellularly using a microelectrode constructed from a tungsten wire etched to a point of less than 5 μm and insulated with an epoxy resin (impedance 1–3 MΩ, at 135 Hz). The raw signal from the electrode was preamplified, and passed through a Krohn-Hite filter (model 3550; low pass: 6000 Hz, high pass: 850–1200 Hz). The data were recorded on tape and also digitized using a microcomputer and an analog-to-digital converter (Tecmar Labmaster) at the rate of either 10 or 12 kHz and stored on disk for later analysis.

Once a single unit was identified, its activity was observed for approximately 20–30 min. All the experiments which required recording of the neuronal activity began after the observation period, thereby ensuring that the neuronal activity was stable and that the rate of firing remained constant in the spontaneous condition.

A deep level of anaesthesia was maintained throughout the experiment by additional injections of 10 mg ketamine every 30 min or sooner, if the corneal reflex was present.

At the completion of each experiment, a small electrolytic lesion in the area of recording was produced, and the animals were euthanized with an overdose of euthanal (100 mg/kg). The brains were extracted, stored in a formol-saline solution and were later frozen, sliced and stained with metachromatic thionine.

Identification and analysis of response types to stimulation

For each neurone, an equal number (between 5 and 12) of 10 s periods of neuronal activity were recorded, first during spontaneous firing, and then during repeated presentations of the noxious stimulus. For the purposes of our analysis, however, only the five sweeps recorded before the NTS and the responses to the first five NTSs were considered. NTS consisted of tail immersion in a hot water bath (55±1 °C) for 10 seconds every 2.5 min. The recording of activity during NTS coincided with the 10 s period marked by the onset and offset of the noxious stimulus.

The total number of spikes during each stimulus presentation was calculated for every single unit and this was used to establish the direction of change as a result of the stimulation. As a guideline, at least a 20 % change in either direction (i.e. positive or negative) was required in the total firing rates between spontaneous and stimulated conditions for a cell to be considered responsive to stimulation.

Peristimulus time histograms (PSTHs) were used in order to evaluate the nature and the duration of the response to stimulation. The activity of every cell, recorded before and after the stimulation, was divided into 100 ms bins and the total number of spikes in every bin was plotted relative to the onset of the noxious stimulus. The standard deviation (S.D.) of the means was calculated in each group.

Table 1
Type of response to NTS according to the location of each neurone in the VPM and VPL. The percentage of cells within each nucleus reflects the total number of cells in the same class, i.e. excited, inhibited, or not responding. Percentages of all cells in each category indicate the proportion out of the total number of responding cells.

	Type of response to noxious heat		
	Excitation	Inhibition	No Response
Site of electrode			
VPL	7 (41 %)	5 (45 %)	5 (45 %)
VPM	10 (59 %)	6 (55 %)	6 (55 %)
Total	17 (44 %)	11 (28 %)	11 (28 %)

Results

Histological reconstruction of the recording sites

All the cells recorded in this experiment were located in the VPM and the VPL, verified by the physiological recordings and histological analysis. The locations were plotted in Figure 1 according to the

stereotaxic atlas (Paxinos and Watson 1986). The classification of cell responses according to the type and recording location, VPM or VPL, is summarized in Table 1. There were no apparent clusters and it appeared that all the types of cells were equally distributed among the different nuclei searched.

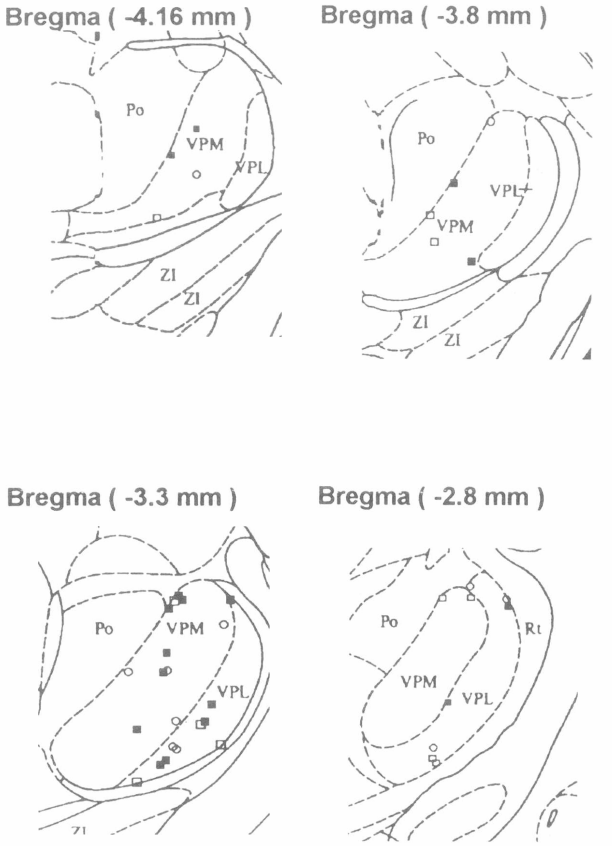

Fig. 1. *Recording sites in the VPM and VPL. The site of each recorded cell is shown according to the response to NTS (open circle: no response to NTS, open square: inhibition due to NTS, full square: excitation due to NTS). The distances from bregma correspond to the stereotaxic coordinates given by Paxinos and Watson (1986).*

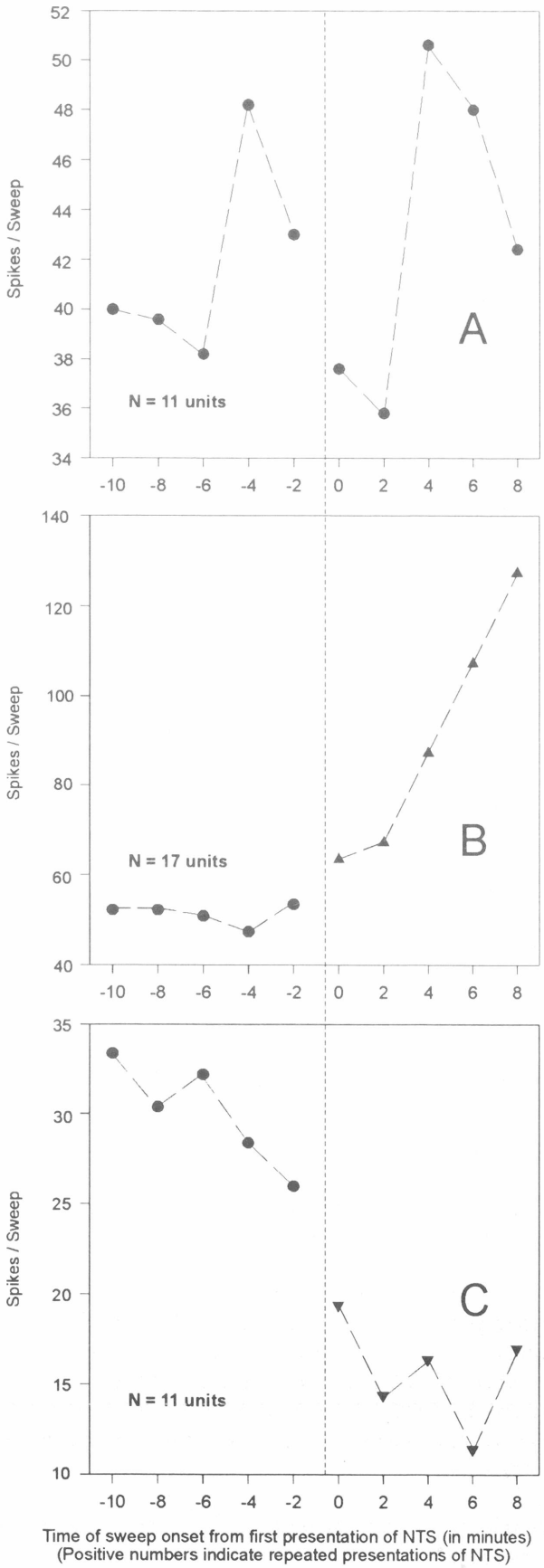

Fig. 2. *Types of response to NTS. Average of the total firing rate during a 5-second sweep in non-responsive (A), NTS-excited (B), and NTS-inhibited (C) neurones. Vertical line coincides with the presentation of the first NTS (at 0 s). Positive times reflect additional presentations of NTS. Negative times represent the time at which the sweep was taken prior to first NTS.*

Depending on the observed levels of activity following the onset of the NTS, each unit was classified as an excited unit (at least 20 % above spontaneous levels), or as an inhibited unit (at least 20 % under the spontaneous levels), or as a non-responsive unit (less than 20 % above or 20 % below spontaneous levels). The NTS-inhibited and NTS-excited units had stable spontaneous firing rates while the firing of the non-responsive units was more variable. Figure 2 depicts the average firing rates over five sweeps first in the spontaneous and later in the stimulated condition period for each of the three groups. In addition, the NTS-excited units were excited by more than 20 % and the NTS-inhibited units were inhibited below 20 % of their spontaneous levels every time the NTS was applied. The non-responsive units never surpassed the 20 % criterion during NTS. Figure 3 shows the relative change over or below the levels of spontaneous activity for all three classes of neurones.

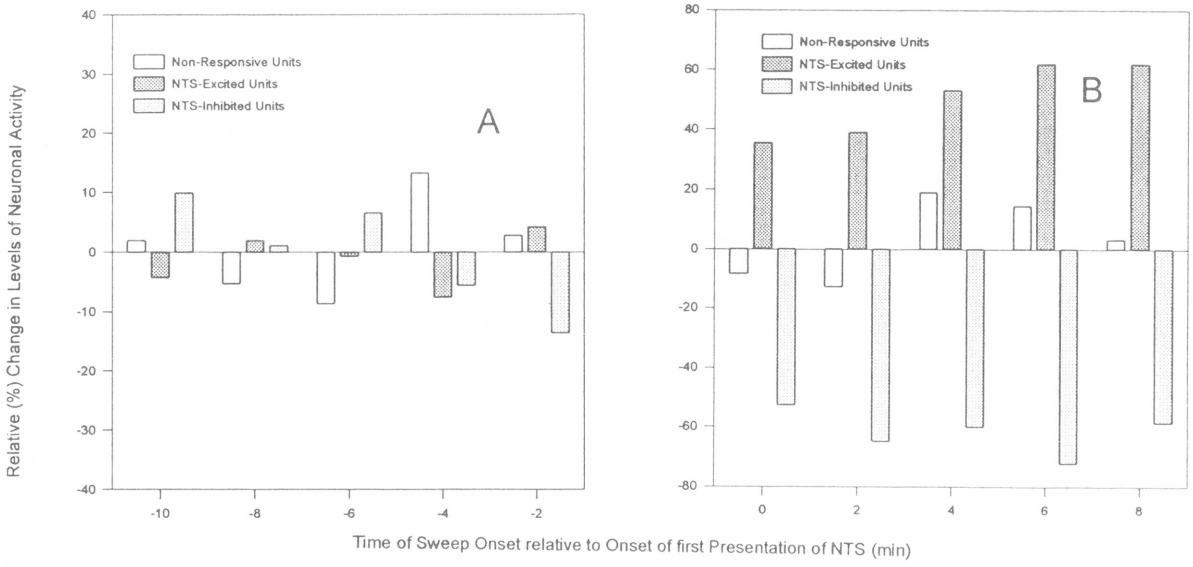

Fig. 3. *Changes in the spontaneous levels of activity during NTS. There is no significant change in the levels of activity during the spontaneous condition (A), but there are dramatic changes in the levels of activity for the NTS-excited and the NTS-inhibited units during NTS (B).*

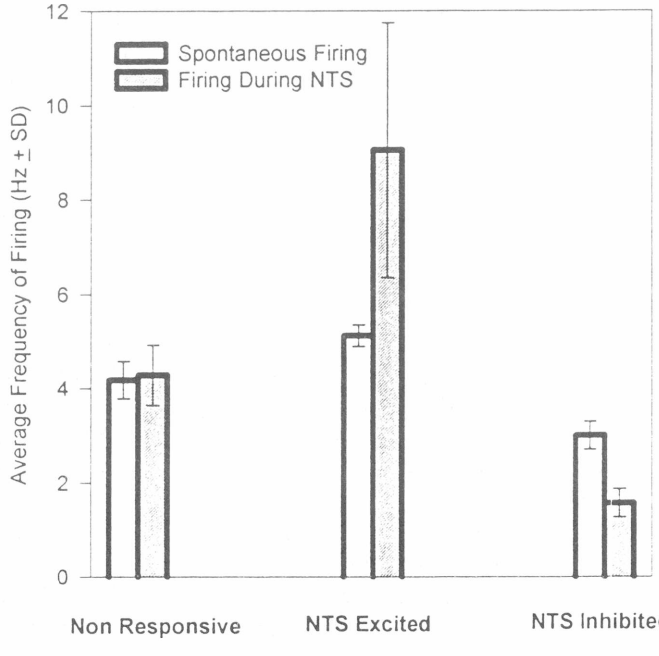

Fig. 4. *Spontaneous firing frequencies and changes during NTS. With the exception of non-responsive units the spontaneous frequency of firing either increased (NTS-excited) or decreased (NTS-inhibited).*

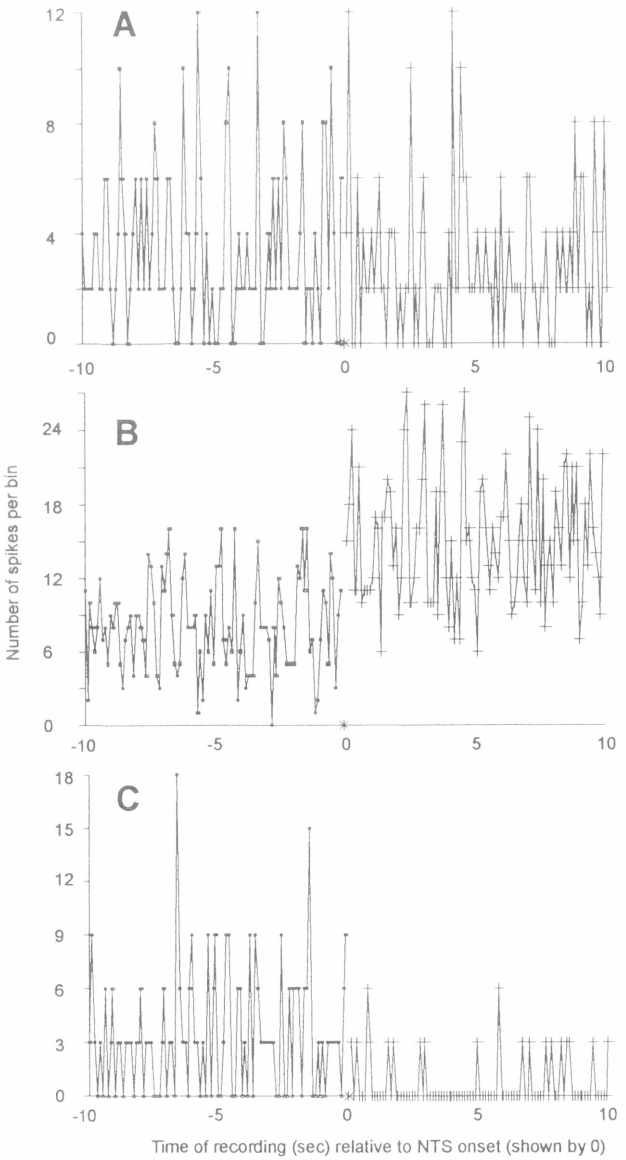

Fig. 5. *Peristimulus time histograms (PSTH) of the response to NTS. There were no changes in the firing characteristics of the non-responsive units following the presentation of the NTS at point 0 (A). The heat-excited (B) and heat-inhibited (C) units responded immediately to the presentation of the NTS and the response was present throughout the duration of the stimulus (10 s).*

Further analysis of the response to NTS revealed that the firing frequencies of the neurones during the spontaneous condition were related to the type of response to NTS, i.e., excitation, inhibition or no response (Fig. 4).

Seventeen cells were excited following the onset of NTS and exhibited a spontaneous rate of firing at 5.12±0.39 Hz which increased to 9.05±2.70 Hz during NTS. The PSTHs of the total firing rates of all NTS-excited units revealed that the activity of each unit increased immediately following the onset of NTS (Fig. 5a).

Eleven cells were inhibited following the onset of NTS and their rate of firing decreased from 3.0±0.6 Hz to 1.57±0.3 Hz. The PSTHs of the NTS-inhibited units showed that the activity of the inhibited units decreased in a dramatic fashion immediately following the onset of NTS (Fig. 5b). In most cases, the cells ceased their firing completely during NTS.

The remaining eleven neurones did not exhibit any significant change in their firing patterns, 4.18±0.39 Hz in the spontaneous and 4.28±0.64 Hz in the stimulated condition. Similarly the PSTHs of the NTS-non-responsive single units showed no significant differences in firing per bin between the spontaneous and the stimulated conditions (Fig. 5c).

Discussion

The purpose of this study was to examine the characteristics of the neuronal activity in the VB and to identify changes due to noxious thermal stimulation, while preserving the distinction between the VPL and the VPM. It was originally thought that the neurones responding to the NTS of the tail surface would be located in the VPL since their receptive fields are located in the second somaesthetic region. Instead, it was found that the neurones responding to NTS of the tail did not conform to the somatotopic distribution proposed by Emmers (1981), but were evenly distributed within the VPM and the VPL in a manner similar to the one described by Yen *et al.* (1989).

Most importantly, this experiment has shown that in the VB there are three types of responses to the NTS of the tail. For the first time, we report the existence of VB neurones which are directly inhibited by NTS. The existence of heat-inhibited neurones suggests a rather elaborate involvement of thalamic neurones in the perception of pain and this has been confirmed for other thalamic nuclei, like the parafascicular nucleus (Benabid *et al.* 1983), but not for the VP. The lack of this finding in the VP may be related to the neurone selection criteria outlined below.

Most commonly, only single units which increase their activity following painful stimulation are recorded while other responses tend to be ignored. In the present study, the selection criteria differed. The activity from each neurone was recorded as long as it was stable over a period of 20 min. The response of the neurone to noxious stimulation was then used to assign the cell to one of the three classes.

While it is certain that the heat-excited neurones signal that there is noxious stimulation, the role of the heat-inhibited neurones is not clear. Since the heat-inhibited response was observed immediately following the noxious stimulation, it is possible that these are interneurones which affect other nociceptive

neurones in the area. It is possible, for instance, that the activation and inhibition of the VB neurones may trigger inhibition of neurones in the parafascicular nucleus (Benabid *et al.* 1983). Further research may also show that the inhibition of the VP neurones may interrupt nociceptive transmission to other thalamic structures and initiate antinociception.

The use of ketamine as an anaesthetic did not appear to influence the responses of the thalamic neurones we studied, although it has been known for some time that ketamine produces some degree of analgesia. It is imperative, however, to clarify that any analgesic effects produced by ketamine are limited since it acts postsynaptically and it modulates only the evoked activity of the secondary neurones which depend on NMDA transmission (Dickenson 1994). It is also obvious that since the effects of ketamine do not involve the initial inputs to the postsynaptic neurones, ketamine acts only to reduce the wind-up postsynaptically (Dickenson 1994). Therefore, while the action of ketamine may result in the reduction of some inputs from NMDA channels, it does not influence the activity of any other pathways, or the primary afferents which continue to transmit information to the thalamus.

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