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T. Bartsch, P. J. Goadsby

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T. Bartsch and P. J. Goadsby

Headache Group, Institute of Neurology, National Hospital for Neurology and Neurosurgery, London, UK

Correspondence to: Professor P. J. Goadsby, Headache Group, Institute of Neurology, Queen Square, London WC1N 3BG, UK
E-mail: peterg@ion.ucl.ac.uk

Summary

Pain referral and spread in headache patients may be attributed to a sensitization of central nociceptive neurons with an increased excitability to afferent input. We investigated if noxious dural stimulation evokes sensitization of second-order neurons that leads to an increased responsiveness to stimulation of cervical afferents. Recordings were made from 29 nociceptive neurons in the C_2 dorsal horn of the rat that received convergent synaptic input from trigeminal and cervical afferents. Trigeminal afferents of the supratentorial dura mater were activated by mustard oil (MO) and the responses of second-order neurons to stimulation of the greater occipital nerve (GON) were studied before and after dural stimulation. Projection sites to the contralateral thalamus were determined by antidromic stimulation. After dural application with MO, mechanical thresholds of the dura significantly decreased ($P < 0.05$) and an enlargement of the trigeminal and cervical cutaneous mechanoreceptive fields was observed in 71% of neurons. The responses to noxious mechanical stimulation of deep paraspinal muscles increased after MO application ($P < 0.001$). Similarly, an increase in the excitability to electrical stimulation of the GON was observed in C-fibre responses ($P < 0.001$). These results suggest that stimulation of nociceptive afferent C-fibres of the dura mater leads to a sensitization of second-order neurons receiving cervical input. This mechanism might be involved in the referral of pain from trigeminal to cervical structures and might contribute to the clinical phenomena of cervical hypersensitivity in migraine and cluster headache. Understanding this interaction is likely to be pivotal in characterizing the physiology of treatment with manipulations involving cervical input, such as GON injection.

Keywords: greater occipital nerve; dura mater; central sensitization; pain referral; headache

Abbreviations: GON = greater occipital nerve; MO = mustard oil; NS = nociceptive-specific; WDR = wide dynamic range

Introduction

The clinical changes seen in primary headache syndromes, such as increased cutaneous sensitivity, hyperalgesia and allodynia in the trigeminal territory, are very suggestive of an altered trigeminal nociceptive system (Burstein *et al*., 2000*a*, *b*; Katsarava *et al*., 2002; Kaube *et al*., 2002). As the distribution of pain in those patients is not always confined to the front of the head, facilitation of central neurons to dural stimulation may explain, in part, the clinical phenomena of spread and referral of pain with a hypersensitivity of deep somatic cervical structures, such as paraspinal muscles, during acute headache (Selby and Lance, 1960; Drummond, 1987; Langemark and Olesen, 1987; Anthony, 1992; Bogduk, 1997; Goadsby *et al*., 2002). However, a prerequisite for this mechanism would be a convergence of the afferent inflow from the meningies (Kerr, 1961; Kerr and Olafson, 1961; Goadsby *et al*., 1997) and the upper cervical roots onto the same second-order neuron in the trigeminocervical complex (Goadsby, 2001). The central sensitization of nociceptive second-order neurons can be induced by strong nociceptive inputs and is reflected in a reduction of the activation threshold, an increased responsiveness to afferent stimulation and an enlargement of receptive fields or the emergence of new receptive fields (Wall and Woolf, 1984; Woolf and King, 1990; McMahon *et al*., 1993; Schaible and Grubb, 1993).

It is now well accepted that small-diameter afferents in the trigeminal nerve innervate the supratentorial dura mater and

cranial vessels and that this innervation mediates the nociceptive inflow from the meningies to the brain (Hoskin *et al*., 1996; Strassman *et al*., 1996; Bove and Moskowitz, 1997). This innervation is considered to be the peripheral substrate of head pain in primary headache syndromes, such as migraine or cluster headache (Goadsby, 2001). Primary nociceptive afferents from the meninges terminate within the medullary dorsal horn of the caudal trigeminal nucleus (Dostrovosky *et al*., 1991; Strassman *et al*., 1994; Burstein *et al*., 1998; Schepelmann *et al*., 1999; Ebersberger *et al*., 2001) and in the upper ($C_{1/2}$) spinal segments (Goadsby and Zagami, 1991; Kaube *et al*., 1993; Strassman *et al*., 1994), where they also receive synaptic input from skin and muscle afferents in the upper cervical roots (Pfaller and Arvidsson, 1988; Neuhuber and Zenker, 1989). Suboccipital structures, such as deep paraspinal muscles, are mainly innervated by the greater occipital nerve (GON) that is a branch of the C_2 spinal root (Scheurer *et al*., 1983). Recently, we have described a population of neurons in the C_2 dorsal horn that receive convergent input from the supratentorial dura mater and the GON (Bartsch and Goadsby, 2002).

Central afferent convergence and sensitization of afferent second-order neurons may underlie the spread of pain and referral from the supratentorial dura mater to areas innervated by cervical afferents, such as muscle and joints. These mechanisms would be consistent with the 'convergence-projection' theory of referred pain whereby pain originating from an affected tissue is perceived as originating from a distant receptive field (Ruch, 1965; Arendt-Nielsen *et al*., 2000).

In this study, we wished to determine if neurons receiving convergent trigeminal input from the dura mater and cervical input from the GON could develop central sensitization to noxious stimulation as described for other nociceptive neurons within the trigeminal and spinal system (Hu *et al*., 1992; Yu *et al*., 1993; Burstein *et al*., 1998). The responses of second-order neurons to afferent stimulation of the GON were studied before and after C-fibre afferent stimulation of the dura mater. We studied the changes of cutaneous mechanoreceptive fields, the responses to mechanical stimulation of deep paraspinal muscles and the responses to electrical stimulation of the GON, as well as projections to the thalamus.

Material and methods

General procedure

Experiments were conducted on Sprague–Dawley rats (300–400 g) that initially were anaesthetized with pentobarbitone sodium (Sagatal®, Rhone Merieux, Harlow, Essex, UK; 65 mg/kg intaperitoneally). Anaesthesia was maintained by bolus injections of α-D-gluco-chloralose (α-chloralose, Serva, 1% in Tyrode's solution, 10–20 mg/kg) through a catheter placed in the femoral vein. A sufficient depth of anaesthesia was judged from the absence of the corneal blink reflex and withdrawal reflexes in the unparalysed state, and, during muscular paralysis, from the absence of gross fluctuations of blood pressure and heart rate. Arterial blood pressure was monitored continuously through the cannulated femoral artery. The animals were paralysed with pancuronium bromide (Pavulon®, Organon, Cambridge, UK, 1 mg/kg initially, maintenance with 0.4 mg/kg) and artificially ventilated using O_2-enriched room air (Ugo Basile, Comerio, VA, Italy). End-tidal CO_2 was monitored and kept between 3.5 and 4.5%. The ECG was monitored continuously. Rectal temperature was kept constant at 37°C by means of a servo-controlled heating blanket.

To expose the stimulation and recording sites, the head of the animals was fixed in a stereotaxic frame and a midline incision was made. The dura mater and the middle meningeal artery were exposed by performing a parietal craniotomy and were covered with mineral oil. The muscles of the dorsal neck were separated carefully in the midline and an ipsilateral hemilaminectomy of C_1 was performed. The atlanto-occipital membrane and the dura mater were incised to expose the brainstem and the C_2 spinal cord segment. The pia mater was left intact. The distal part of the GON was exposed before its termination adjacent to the auricle and covered with warm paraffin oil in a pool made from skin flaps. All experiments were carried out under a project licence issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986.

Stimulation and recording

A stimulation electrode was placed on the dura mater, and electrical square-wave stimuli (0.5 –1 Hz) of 0.5–2 ms duration were applied. The GON was mounted on a pair of hook electrodes and stimulated (0.5 Hz, 2 ms, 5–30 V). Extracellular recordings were made from neurons in the spinal dorsal horn of C_2 using tungsten microelectrodes (WPI, Stevenage, Hertfordshire, UK; impedance 2 MΩ, tip diameter 1 μm). Electrodes were lowered into the spinal cord with a microstepper in 5–10 μm steps. Nerve signals were amplified, bandpass filtered and displayed on an oscilloscope. Original signals were stored on a digital tape recorder (PCM-R300, Bio-Logic, Claix, France). Signals were fed into a window discriminator connected through an interface (CED Power 1401plus, Cambridge Electronic Design, Cambridge, UK) to an IBM-compatible computer. Post- and peri-stimulus time histograms of neural activity were displayed and analysed using SPIKE 2.01 (CED).

Characterization of neurons

Neurons with convergent input from the dura mater and GON were identified as the recording electrode was advanced into the dorsal horn of C_2 and while electrical stimuli were applied to the dura mater. When a dura-sensitive neuron was found, it was tested for convergent A- and C-fibre input by short-lasting electrical GON stimulation. The distance from the

dural stimulation site to the trigeminal ganglion (10–12 mm) and from the ganglion to the C_2 segment (15–17 mm), as well as from the GON stimulation site to the central recording site (38–40 mm), was measured and the conduction velocities were calculated. According to the latencies to stimulation, neurons were classified as A-fibres (>1.5 m/s) or C-fibres (<1.5 m/s).

The receptive field of each neuron was tested systematically using a range of different stimuli. The cutaneous facial and cervical receptive field, including the cornea, was assessed in all three trigeminal innervation territories and upper cervical roots, respectively. Additionally, input from suboccipital neck muscles and dura mater was also tested. The mechanoreceptive field was mapped by applying non-noxious and noxious stimuli. The two-dimensional features of the cutaneous receptive field were transferred to a 1 : 1 drawing of the rat's head and neck. Non-noxious stimuli were applied to the receptive field by gently brushing, softly stroking and applying light pressure with a blunt probe. Noxious mechanical stimuli consisted of pinching with forceps or heavy pressure that was painful when applied to humans. According to the cutaneous receptive field properties, neurons were classified as low-threshold mechanoreceptive neurons, which responded only to innocuous stimulation, wide-dynamic range (WDR) neurons, which responded to both non-noxious and noxious stimuli, or nociceptive-specific (NS) neurons that responded only to noxious input. No receptive fields outside the trigeminocervical innervation were found. Dural receptive fields were tested qualitatively using a fine probe, and quantitatively as the dural mechanical threshold was assessed using a set of calibrated von Frey hairs (Stoelting Instruments Inc., Wood Dale, IL, USA). The von Frey hairs were applied at the most sensitive site of the dural receptive field in ascending order. The mechanosensitivity from suboccipital paraspinal muscles (M. semispinalis capitis, M. rectus capitis posterior) was tested for 10 s using a calibrated probe which exerted a force of 3 Newton that has been reported to be in the noxious range (Hoheisel and Mense, 1990; Yu *et al*., 1991; Hoheisel *et al*., 1993).

Experimental protocol

The responses of nociceptive convergent neurons to electrical stimulation of the GON, to mechanical stimulation of suboccipital paraspinal muscles and dura mater, as well as changes in the receptive fields were tested before and after chemical stimulation of the supratentorial dura. Dural afferents were stimulated with the C-fibre activator mustard oil (MO; allyl isothiocyanate; Sigma-Aldrich Company Ltd, Gillingham, Dorset, UK; 10%, in paraffin oil) by applying a cotton swab soaked with MO into the centre of the dural receptive field for 4–5 min (Woolf and Wall, 1986; Handwerker *et al*., 1991). The extension of the receptive fields and the dural mechanical thresholds were tested every 20 min. The responses to mechanical stimulation of suboccipital muscles and the responses to electrical stimulation of the GON were tested every 10 min for the first hour and then every 20 min. Electrical GON stimulation consisted of trains of 20 stimuli (0.5–1 Hz) starting at least 30 min prior to any conditioning stimulus. Responses to electrical stimulation were analysed using post-stimulus histograms separated for A-fibre and, if present, C-fibre responses. To compensate for changes in background spontaneous activity, an interval of ongoing activity was recorded before each stimulation period that was then subtracted from the stimulation interval. Only one neuron in each animal was tested with the application of MO. Spontaneous activity in neurons was determined from time periods of 5 min under control conditions.

Recording and projection sites

At the conclusion of the experimental protocol, the projection sites of the neurons were determined by antidromically stimulating the contralateral thalamus with a stimulation electrode that was moved through the midbrain (10 Hz, 0.2 ms) from –2.56 to –4.8 mm from bregma. Antidromically evoked spikes were defined by a constant latency, high-frequency following and a collision with spontaneously occurring or orthodromically induced spikes (Lipski, 1981; Fields *et al*., 1995). The recording site within the spinal cord was marked with an electrolytic lesion by passing current through the recording electrode. The tissue was then removed, fixed in 1% potassium ferrocyanide in 10% formaldehyde, cut into 40 μm frozen sections, collected on glass slides and stained for cresyl violet. Lesion sites were examined under the light microscope and transferred to a standard cross-section.

Statistical analysis

For statistical analysis, responses to electrical stimulation were normalized and expressed as a percentage of the mean preconditioning baseline response. Raw data were used for the analysis of spontaneous activity and the responses to afferent muscle stimulation. Analysis of variance (ANOVA) for repeated measurements was used to determine the time course of neural responses before and after interventions. Statistical significance was set at $P < 0.05$. In repeated measures ANOVAs, Greenhouse–Geisser corrections were used if assumptions of sphericity were violated. Where applicable, the Bonferroni correction was applied for multiple comparisons. Analysis of von Frey measurements was performed using the non-parametric Wilcoxon signed-rank test. Data are expressed as mean ± SEM or mean ± SD, as appropriate, for a number of observations. Statistical analysis was carried out using SPSS (10.0, SPSS Inc, Chicago, IL, USA).

Results

General properties

Recordings were made from 29 neurons in the C_2 dorsal horn that received convergent synaptic input from trigeminal and cervical primary afferents. Neurons showed an ongoing mean activity of 7.1 ± 1.9 Hz (mean ± SEM). Fifteen neurons (52%) showed no or low initial spontaneous activity (0–3 Hz), whereas in six neurons (21 %) spontaneous activity was >10 Hz (Fig. 1).

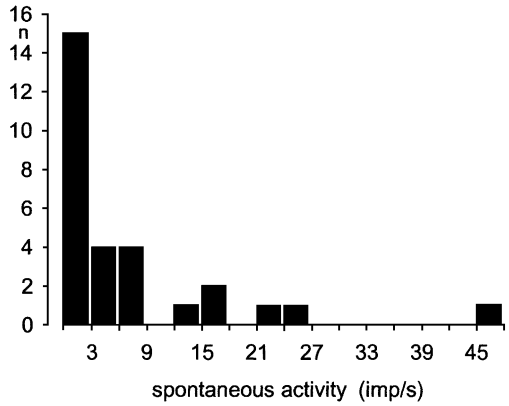


Fig. 1 Distribution of the rate of spontaneous activity (classes of three impulses/s) in convergent C_2 dorsal horn neurons (n = 29).

The lesion sites within the C_2 dorsal horn indicating the recording site could be identified in 24 animals and were found at a mean depth of 751 ± 148 μm (mean ± SD). The sites corresponded to the laminae V, VI and VII of the C_2 dorsal horn (Fig. 2A). In 13 out of 17 animals, the site in the contralateral thalamus from which the dorsal horn neurons were antidromically activated could be identified (Figs 2B and 3A–E).

Electrical stimulation of the dura mater elicited a short-latency response at 5–18 ms; the calculated conduction velocities of the afferent fibres were in the Aδ-fibre range. With increasing stimulation intensity, neurons showed additional long-latency responses between 30 and 100 ms consistent with C-fibre activation. Similarly, electrical stimulation of the GON elicited responses in all neurons in the Aδ- and C-fibre range (see example in Fig. 7B). The responses generated by stimulation of the GON at supramaximal C-fibre

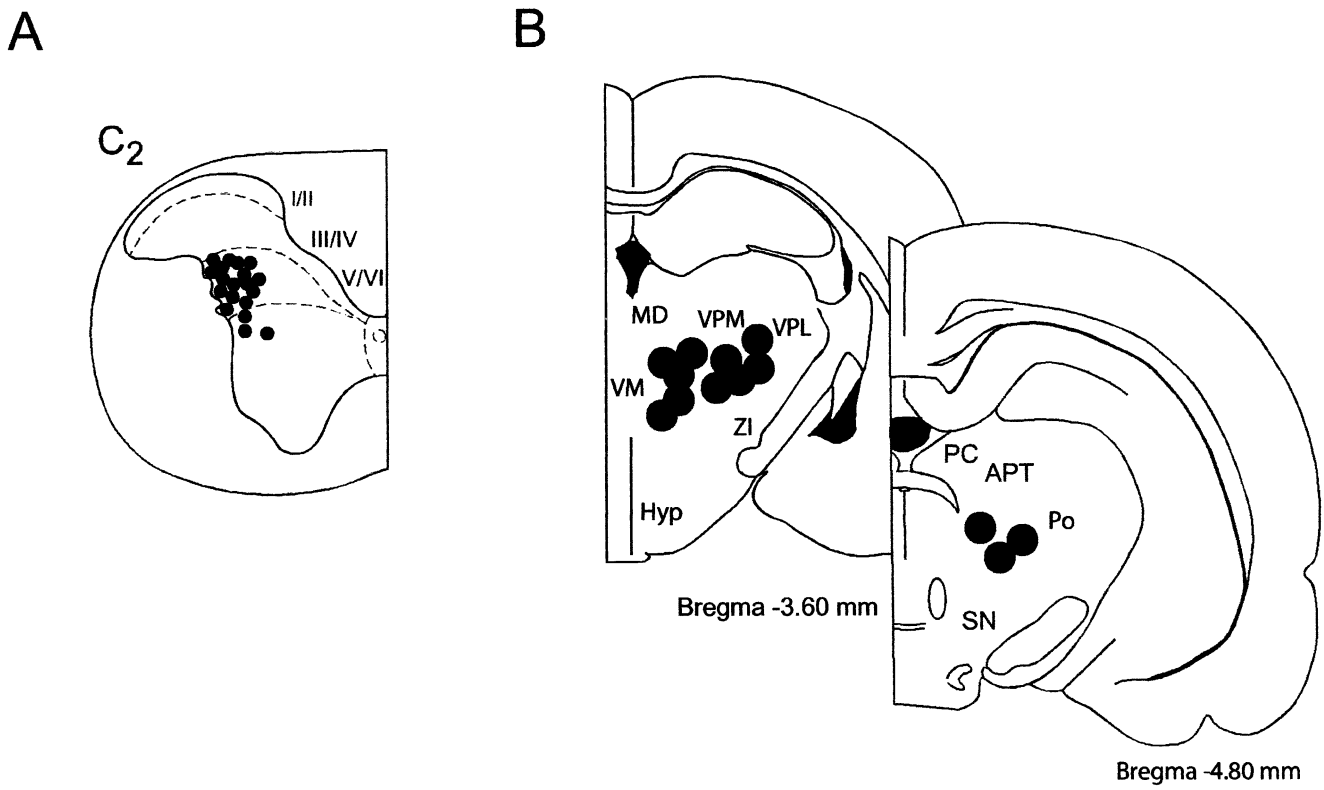


Fig. 2 (**A**) Summary of the locations of C_2 dorsal horn lesions indicating the recording sites of 24 nociceptive neurons receiving convergent synaptic input from the dura mater and the GON. The locations of the neurons that were retrieved (filled circles) were plotted on a representative cross-section of the C_2 spinal cord segment (Molander and Grant, 1995). Recording sites were confined to the laminae V/VI. (**B**) Projection sites of convergent nociceptive trigeminocervical neurons. Location of the lesions (filled circles) indicating the sites in the diencephalon from which the spinal neurons could be activated antidromically (n = 13). Locations are plotted on ideal cross-sections (Paxinos and Watson, 1998). APT = anterior pretectal nucleus; Hyp = hypothalamus; MD = thalamic mediodorsal nucleus; PC = posterior commissure; PO = posterior thalamic nuclear group; SN = substantia nigra; VM = thalamic ventromedial nucleus; VPL = thalamic ventroposterior lateral nucleus; VPM = thalamic ventroposterior medial nucleus; ZI = zona incerta.

strength elicited a wind-up phenomenon in the long-latency response (Fig. 7B). In contrast, a wind-up phenomenon in the long-latency responses was not observed in response to stimulation of the dura mater.

Receptive fields

On the basis of their response properties to cutaneous stimulation, the neurons were either classified as WDR (n = 25) or NS neurons (n = 4). All neurons had facial cutaneous receptive fields, mostly restricted to the first division of the trigeminal nerve, including cornea (n = 13). In some experiments, the receptive field included the second (n = 14) and third trigeminal division (n = 4). The ophthalmic branch of the trigeminal division proved to be most sensitive to afferent stimulation if the receptive field included more than one trigeminal division. The cutaneous receptive fields were also located caudally in the ophthalmic division and included the C_2 dermatome extending from the occipital skin to the auricle (Fig. 4). Additionally, these neurons showed mechanosensitive input from deep suboccipital paraspinal muscles (M. semispinalis capitis, M. rectus capitis posterior; Figs 3A and 6B). All neurons showed a small mechanosensitive dural receptive field (diameter 1–3 mm) that was confined to the vicinity of the middle meningeal artery or one of its branches (Fig. 3A).

MO application onto dura mater

Stimulation of dural small-diameter afferents with MO produced an immediate increase in ongoing activity up to 43.5 ± 32.8 Hz (mean ± SEM) within 5 min of application [F(1.9,18) = 20.6; n = 18; $P < 0.001$]. Within 20 min of application, activity gradually recovered to values that were not significantly different from baseline activity and controls ($P > 0.05$; Fig. 5A). The application of vehicle (mineral oil) had no effect on the activity of convergent nociceptive neurons ($P > 0.05$; n = 8; Fig. 5B). The mechanical von Frey threshold of the dural receptive field was tested in 18 neurons. Overall mean threshold was 1.03 ± 0.48 g (mean ± SEM). The von Frey threshold before and after MO application was tested in 13 neurons. After MO application, von Frey thresholds were decreased in 11 neurons and increased or unchanged in one neuron each. Overall, the mechanical threshold of the dura mater significantly decreased from 1.05 ± 0.6 g to 0.17 ± 0.1 g (mean ± SEM; $P < 0.05$; Wilcoxon test) within 30 min of MO application (Fig. 5C).

Receptive field changes

After chemical irritation of the dura mater with MO, an enlargement of the cutaneous mechanosensitive receptive field was observed in 12 neurons. The enlargement included one or more divisions of the trigeminal nerve and the cervical innervation territory of the C_2 and C_3 dermatomes (Fig. 4). The receptive field did not change in five neurons. The expansion of the cutaneous receptive fields developed within 30 min of stimulation of the dura mater with MO. Application of vehicle (mineral oil) onto the dura had no effect on the size of the cutaneous receptive fields (n = 6).

Responses to noxious mechanical stimulation of cervical muscles

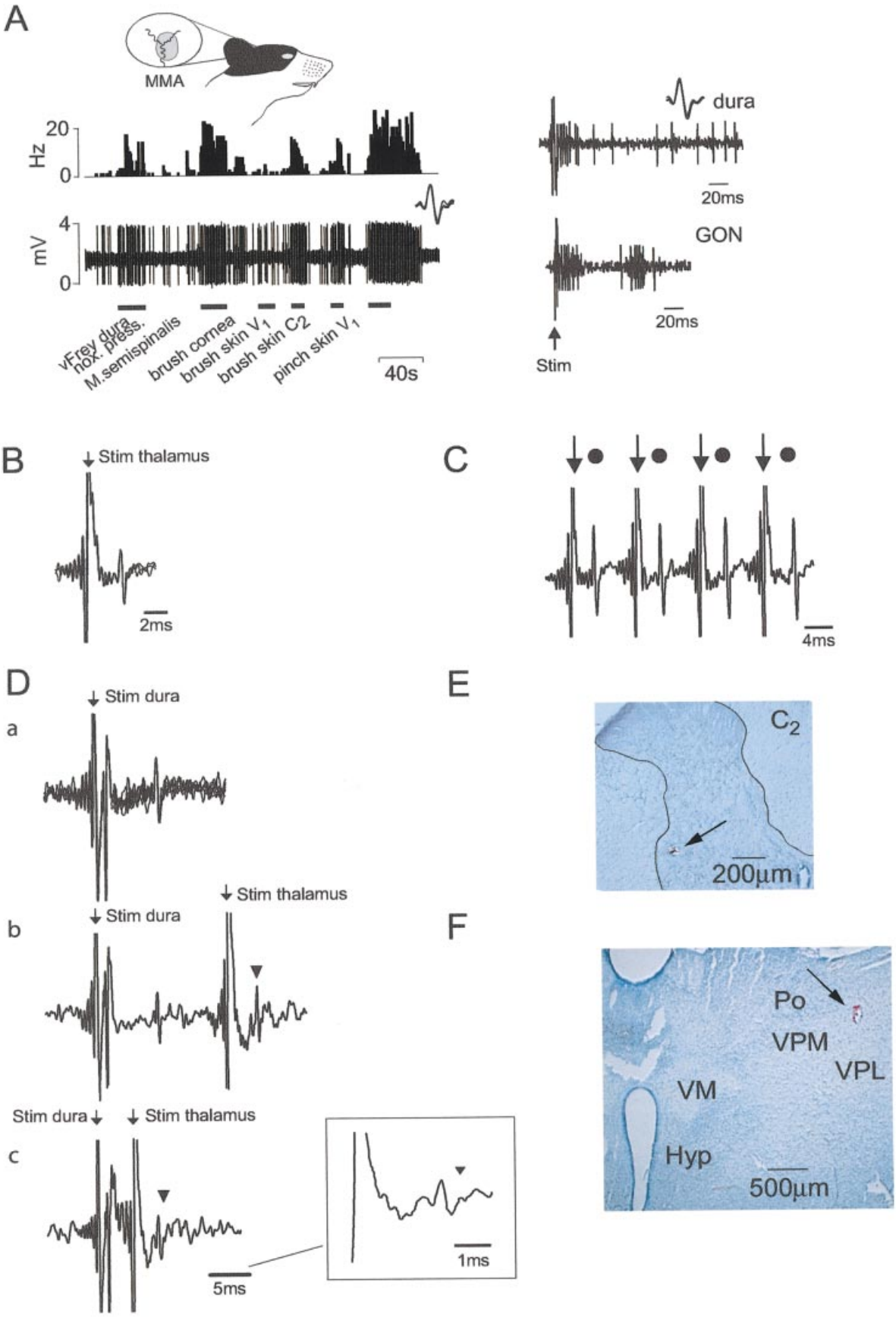
The responses of convergent neurons to noxious pressure applied to deep paraspinal muscles (M. semispinalis capitis, M. rectus capitis posterior) were tested before and after dural MO application. The neurons responded to innocuous mechanical stimulation and showed maximal discharge rates to noxious stimulation. The responses to mechanical stimulation of the deep paraspinal muscles after MO application significantly increased over time and peaked within 60 min [F(1.8,8) = 10.4; n = 8; $P < 0.01$; Fig. 6A]. A brief afterdischarge that outlasted the noxious stimulus was observed in some neurons (Fig. 6B). Application of mineral oil onto the dura had no significant effect on the responses to noxious pressure applied to the suboccipital paraspinal muscles ($P > 0.05$; n = 5; Fig. 6A).

Responses to electrical stimulation of the GON

In 14 convergent neurons, the responses to supramaximal electrical stimulation of Aδ- and C-fibres in the GON was studied with trains of single pulses before and after application of MO onto the dura mater (Fig. 7). The C-fibre responses to electrical stimulation of the GON were significantly increased 20 min after MO application [F(2.6,14) = 11.5; n = 14; $P < 0.001$], peaked ~60 min after MO application and remained elevated until the end of the observation period. The responses to Aδ-fibre stimulation remained unchanged ($P > 0.05$) during the observation period (Fig. 7A). In five neurons, the responses to C-fibre stimulation were transiently decreased within the first 20 min of MO application.

Discussion

In this study, we describe a population of nociceptive neurons in the deep layers of the C_2 spinal dorsal horn that received afferent convergent input from the supratentorial dura mater, innervated by the trigeminal nerve, as well as from cervical skin and muscle that are innervated by the GON. Stimulation of dural afferent C-fibres increased the background activity, extended the size of cutaneous trigeminal and cervical receptive fields, and decreased the thresholds to mechanical dural stimulation. The responses to electrical stimulation of the GON and to mechanical stimulation of the deep paraspinal muscles were also increased. These findings suggest that dural stimulation may lead to a central sensitization of nociceptive convergent second-order neurons with an increased responsiveness to stimulation of cervical



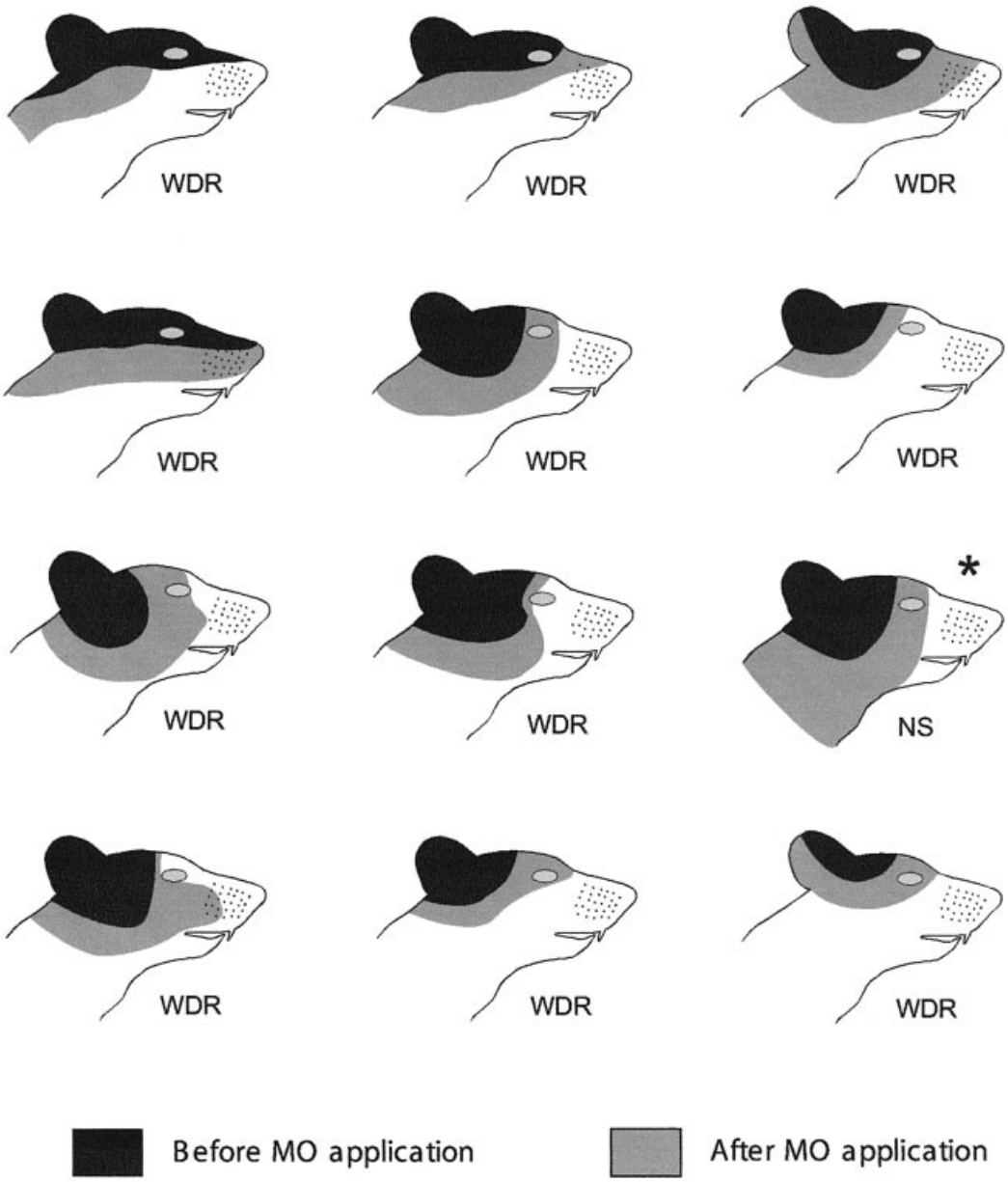


Fig. 4 (**A**) Example of a nociceptive convergent neuron in the C_2 dorsal horn responding with an increase of activity to MO application onto the supratentorial dura mater. (**B**) MO application on to the dura (n = 18, filled circles) elicited a rapid increase in ongoing activity that gradually settled down to baseline activity. *$P < 0.05$ (ANOVA). Application of vehicle (n = 8, open circles) did not change ongoing activity as measured over 90 min. Data are presented as mean ± SEM. (**C**) Dural mechanical thresholds assessed by von Frey hair measurements before and after MO application showing a decrease of the thresholds except in two neurons.

Fig. 3 (**A**) Illustration of the neural responses of a WDR neuron to natural stimulation of the cutaneous and deep receptive field. Inset: the cutaneous facial and dural receptive field and the responses to electrical stimulation of the dural mater and the GON. (**B–D**) Electrophysiological traces demonstrating responses of the nociceptive neuron in the C_2 dorsal horn to antidromic stimulation of the contralateral thalamus. The neuron displayed a constant latency (**B**), the ability to follow high-frequency stimulation (**C**) (downward arrow, antidromic stimulation; filled circle, activated spike) and showed collision (**D**, c) with an orthodromic action potential generated by electrical stimulation of the dura mater (**D**, a and b) (in c, a dura evoked spike blocked the occurrence of an antidromically evoked spike (inverted triangle). Inset: the collision on an extended time scale. (**E**) Cross-section at the C_2 level showing the recording site (arrow) in the deep layers of the C_2 dorsal horn (**F**) and the projection site in the posterior thalamus (arrow) from which the neuron could be antidromically activated (**E**). Abbreviations as for Fig. 2.

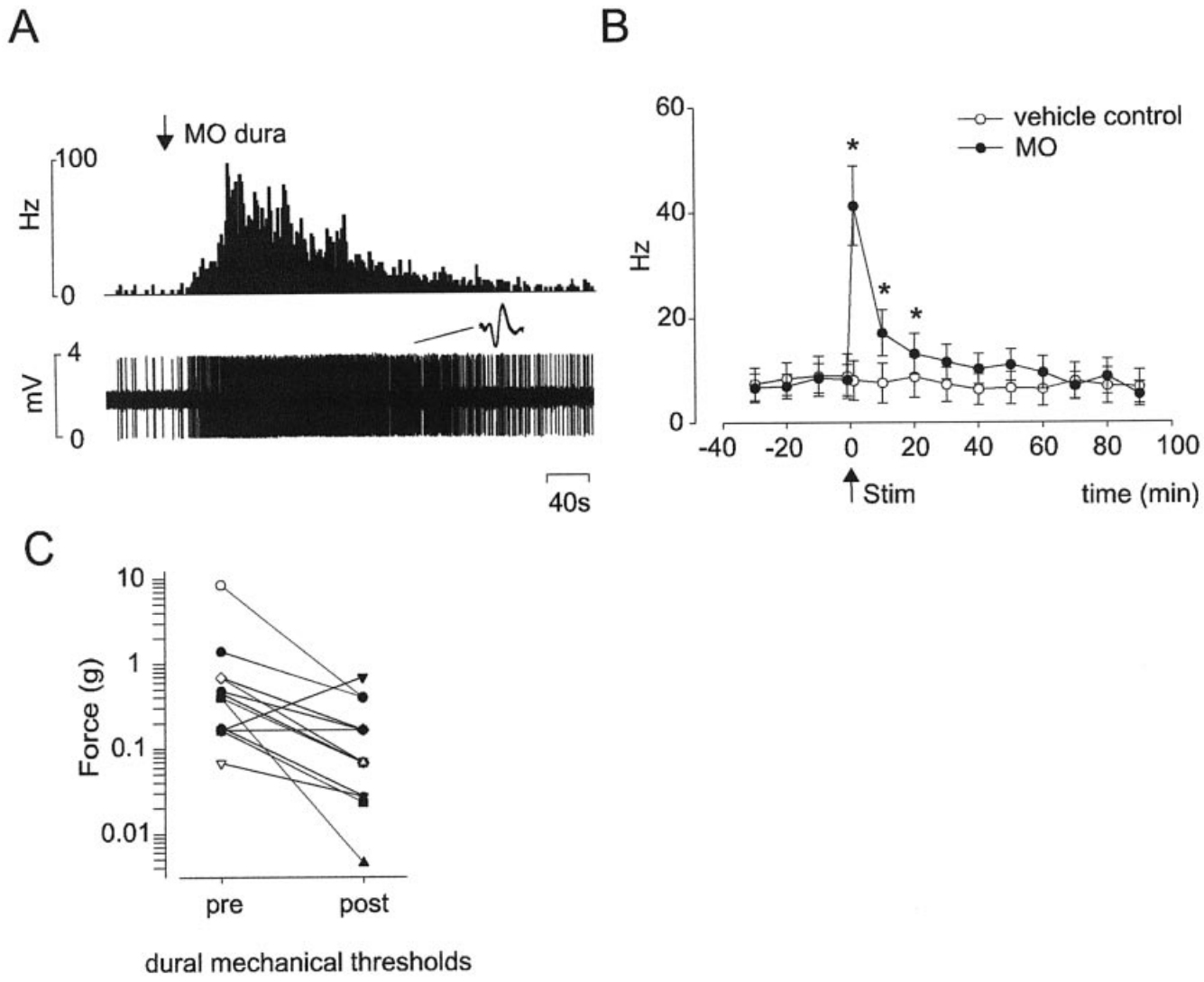


Fig. 5 Summary of the changes in the size of the cutaneous receptive fields in 12 animals after stimulation of the dura mater with MO. The mechanosensitive receptive fields before (black) and after (grey) dural stimulation represented responses to brush (WDR neurons) or to noxious pinch (NS neurons). With the exception of one neuron that was a NS neuron, all neurons were classified as WDR neurons. *Expansion of the receptive field included not only the C_2/C_3 dermatomes but also the ipsilateral forelimb and the forepaw.

afferents. Clinically, this mechanism may contribute to trigeminocervical hypersensitivity in headache patients. This mechanism may also be involved in pain referral from trigeminal to cervical structures and does not necessarily involve a peripheral pathology in the cervical innervation territory (Bogduk, 1997).

The locations of the recording sites of neurons responding to convergent input from the dura mater and the GON were confined to the laminae V/VI of the C_2 dorsal horn, which is consistent with other studies analysing responses of convergent neurons to stimulation of dura mater and dural vessels (Davis and Dostrovsky, 1988*b*; Burstein *et al*., 1998; Schepelmann *et al*., 1999). The location of the recorded neurons corresponds to the dorsal horn area that receives projections from the ophthalmic division of the trigeminal nerve (Strassman *et al*., 1994), which constitutes the primary source of afferents from the supratentorial dura mater (Mayberg *et al*., 1984; Andres *et al*., 1987; Burstein *et al*., 1998; Schepelmann *et al*., 1999; Ebersberger *et al*., 2001). Furthermore, the receptive fields included cervical skin in the C_2/C_3 dermatomes and deep paraspinal muscles innervated by the GON. Primary afferents from both cervical skin and muscles have been shown to terminate in the deep layers of the C_1–C_3 spinal dorsal horn (Scheurer *et al*., 1983; Bakker *et al*., 1984; Pfister and Zenker, 1984; Neuhuber and Zenker, 1989). Since the second-order neurons receive convergent synaptic input from anatomically distinct groups of primary afferents, the observed sensitization is most probably generated heterosynaptically (Thompson *et al*., 1993).

The current concept of central sensitization considers an increased barrage from primary nociceptive afferents onto second-order neurons as crucial in the development of a

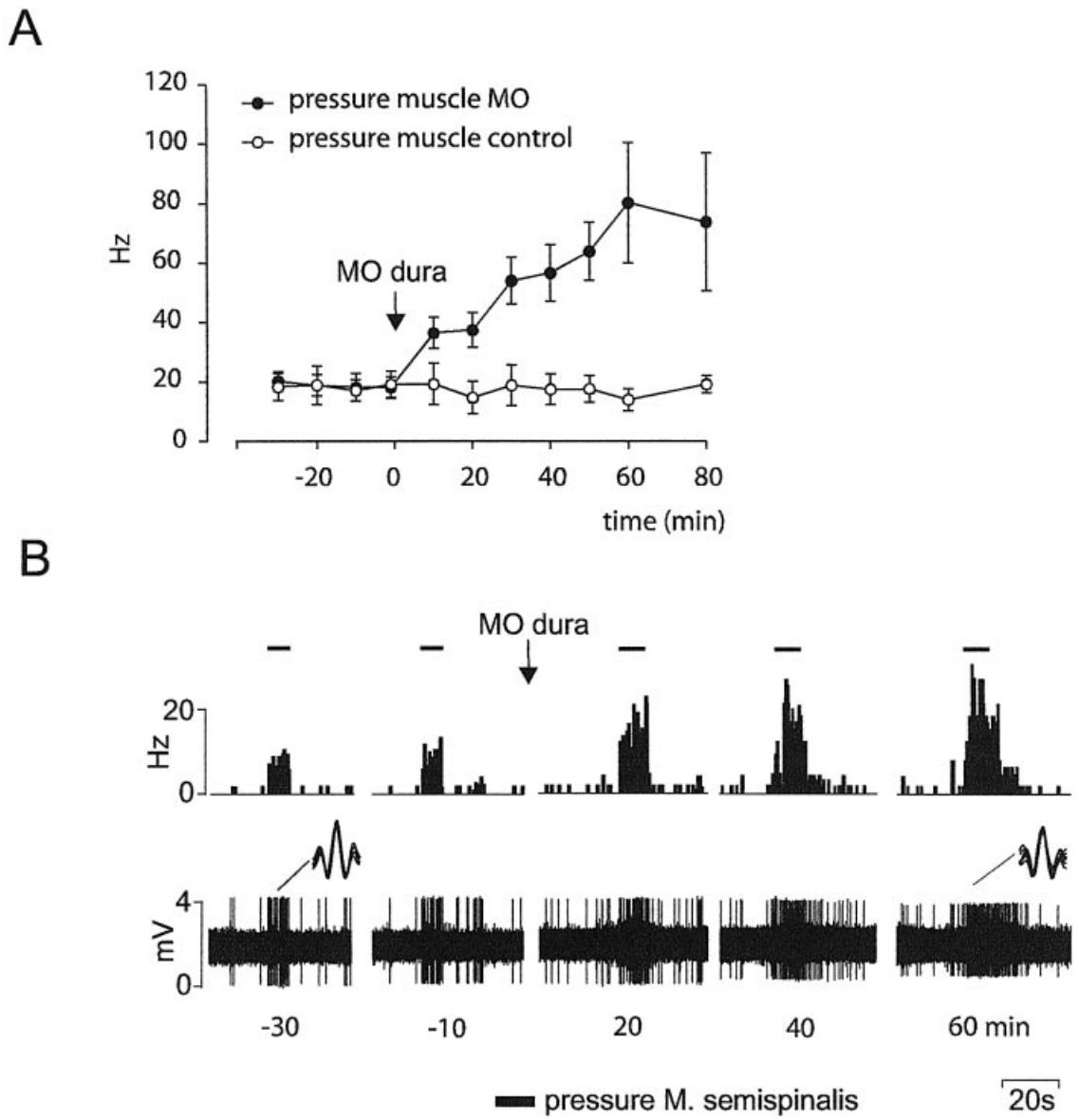


Fig. 6 (**A**) Responses of convergent nociceptive neurons to mechanical stimulation of deep paraspinal muscles before and after stimulation of the dura mater with MO ($n = 8$). The responses to mechanical stimulation increased gradually after MO application onto the dura mater and peaked within 60 min (filled circles) ($P < 0.01$; ANOVA). Dural application of vehicle had no effect on neural responses to mechanical stimulation (open circles) ($P > 0.05$; ANOVA). Data are presented as mean ± SEM. (**B**) Representative example showing increased responses to mechanical stimulation of the M. semispinalis capitis. The neural responses 60 min after MO application show a brief afterdischarge to mechanical stimulation. Note that the spike amplitude became progressively smaller over time but retained its principal shape.

transient or long-lasting central hyperexcitability with the effect of an increased responsiveness to afferent stimulation (Woolf, 1983; McMahon *et al*., 1993; Schaible and Grubb, 1993). The clinical correlates of this central hypersensitivity include the development of spontaneous pain, hyperalgesia and allodynia. Nociceptive second-order neurons receive convergent afferent input from different target organs such as skin, muscles and viscera (Foreman, 2000), and an increased sensitivity may extend to these convergent inputs (Yu *et al*., 1993; Cervero and Laird, 1999). Interestingly, a frequency-dependent increase in neural excitability (wind-up) was observed in the long-latency responses to GON stimulation, but not to dural stimulation. This might indicate further differences between somatic and visceral nociceptive systems since spinal nociceptive neurons typically show wind-up to somatic afferent C-fibre stimulation (Herrero *et al*., 2000), but spinal neurons with visceral input do not (Cervero and Laird, 1999). Although wind-up is regarded as a display of central sensitization, it seems not to be a prerequisite for eliciting central sensitization in viscero-afferent neurons (Herrero *et al*., 2000), such as in dura-responsive neurons.

Application of an 'inflammatory soup' onto the dura mater can induce a central sensitization of trigeminal second-order neurons in the caudal trigeminal nucleus with a subsequent increased responsiveness to dural and cutaneous facial stimulation (Burstein *et al*., 1998). Furthermore, stimulation of cervical skin and deep paraspinal muscles innervated by the GON increased the excitability of afferent dural input in convergent nociceptive neurons (Bartsch and Goadsby, 2002). These findings, together with our new observations, underline the potential of dura-sensitive neurons in the trigeminocervical complex to undergo a central sensitization with an increased excitability to extradural afferent stimulation. This convergence and sensitization may be involved in the clinical phenomenon of spread and referral of pain whereby signals originating from an affected tissue are perceived as originating from a distant receptive field (Mackenzie, 1909; Ruch, 1965). These mechanisms, together with differences in cutaneous and muscle input (Bartsch and Goadsby, 2002), may be reflected in the clinical changes in migraine patients who frequently complain of neck discomfort in the premonitory phase (Giffin *et al*., 2003) or during their attacks (Goadsby *et al*., 2002).

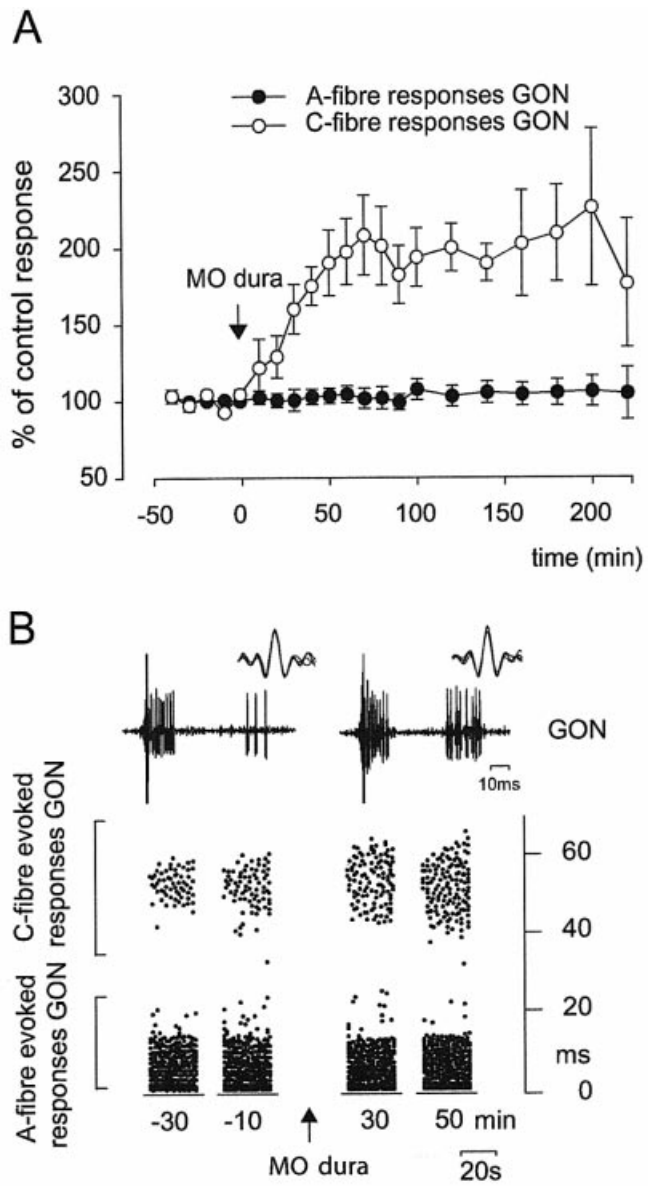


Fig. 7 (**A**) Changes in excitability of convergent neurons to electrical stimulation of the GON before and after MO application onto the dura mater ($n = 14$). After dural stimulation, the C-fibre responses (open circles) to electrical stimulation of the GON gradually increased and peaked at ~1 h post-application ($P < 0.001$; ANOVA), whereas the Aδ-fibre responses remained unchanged (filled circles). Data are presented as mean ± SEM. (**B**) Example of neural GON responses before and after MO dural application. Raster dot display (each dot represents one evoked neuronal spike) of neural responses (Aδ- and C-fibre components) to electrical GON stimulation before (at time points –30 min and –10 min) and after MO application (at time points 30 min and 50 min) showing an increase of excitability in the C-fibre component (40–60 ms latency). Note the wind-up in the long-latency responses following repeated stimulation (0.7 Hz) as the C-fibre evoked responses progressively increase. Inset: original traces of the neural responses to GON stimulation before and after MO stimulation of the dura.

Nerves innervating visceral organs contain a relatively high proportion of small-diameter afferents, especially C-fibres (Cervero, 1987) that are activated by MO (Woolf and King, 1990; Handwerker *et al*., 1991). MO has been shown to induce a central sensitization in trigeminal (Hu *et al*., 1992; Yu *et al*., 1993) and spinal neurons (Woolf and King, 1990; Koltzenburg *et al*., 1994). Since the majority of dura-sensitive second-order neurons respond to local application of capsaicin (Schepelmann *et al*., 1999) and MO, it seems that C-fibre afferents constitute the main nociceptive input from the meningies, at least in the rat.

The time course of the development of the central sensitization in convergent neurons is consistent with other studies that have investigated the mechanisms of central sensitization after afferent stimulation with MO (Woolf and King, 1990; Hu *et al*., 1992, 1995; McMahon *et al*., 1993; Mense, 1993; Yu *et al*., 1993; Woolf *et al*., 1994; Nebe *et al*., 1998). Human data show that spread and referral of pain may develop within a few minutes after noxious stimulation (Wolff, 1948; Wirth and van Buren, 1971; Arendt-Nielsen *et al*., 2000; Piovesan *et al*., 2001).

In this study, we cannot completely rule out that the surgical intervention *per se* might have induced some of the observed changes in the second-order neurons. In particular, we cannot rule out that repetitive mechanical stimulation of cervical muscles itself may result in secondary changes. In view of the time course of the effects after dural application of MO, the stability of baseline and control responses and the low rate of ongoing activity, this seems unlikely (Yu *et al*., 1991).

The present results confirm projections of convergent nociceptive neurons in the deep dorsal horn to different subnuclei of the contralateral thalamus. Other studies have shown a projection of trigeminothalamic tract neurons to widely separated nuclei within the thalamus including the thalamic ventroposterior complex, the posterior nuclear

group and the medial thalamus (Hu *et al*., 1981; Davis and Dostrovsky, 1988*a*; Goadsby and Zagami, 1990; Zagami and Lambert, 1990; Dostrovosky *et al*., 1991; Yoshida *et al*., 1991; Yu *et al*., 1993; Burstein *et al*., 1998). The projection to the thalamus might suggest the possibility that a further sensitization may take place in supraspinal third-order neurons, e.g. in the thalamus (Guilbaud *et al*., 1989). This may account for the clinical observations during migraine attacks in which the hypersensitivity spreads to regions that do not actually belong to the receptive field of the second-order neuron (Lance and Goadsby, 1998; Burstein *et al*., 2000*a*, *b*).

Activation of dural nociceptors with subsequent induction of a central sensitization also evokes responses in spinal motoneurons. EMG activity and neural activity in motoneurons are widely used as models to study changes in central excitability after stimulation of nociceptive afferents (Wall and Woolf, 1984; Hu *et al*., 1993). Dural stimulation with MO induces an increase in EMG activity in dorsal paraspinal muscles (Hu *et al*., 1995). This is in accordance with clinical and experimental data showing changes in the EMG of neck muscles or muscle hypersensitivity in headache patients, and supports our observation of an increased central excitability (Wolff, 1948; Selby and Lance, 1960; Bakal and Kaganov, 1977; Drummond, 1987; Langemark and Olesen, 1987). Sensitization of central nociceptive neurons and motoneurons might also contribute to the muscle stiffness and muscle hyperalgesia during acute secondary headache syndromes generated by haemorrhage or inflammation, such as in meningitis, where these phenomena represent a crucial diagnostic sign (Silberstein *et al*., 2002).

In conclusion, our observations show a central sensitization of second-order neurons to dural stimulation that may account for the pain referral to cervical structures and cervical hypersensitivity in many headache patients. The data demonstrate that the trigeminocervical complex may be regarded as a functional continuum in terms of processing nociceptive input from the head. Moreover, our observations underscore the importance of not assuming that neck pain results from cervical structural pathology, and initiating inappropriate spinal manipulative therapy in lieu of a more careful clinical history for primary headache problems.

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References

Andres KH, During Mvon, Muszynski K, Schmidt RF. Nerve fibres and their terminals in the dura mater encephali of the rat. Anat Embryol (Berl) 1987; 175: 289–301.

Anthony M. Headache and the greater occipital nerve. Clin Neurol Neurosurg 1992; 94: 297–301.

Arendt-Nielsen L, Laursen RJ, Drewes AM. Referred pain as an indicator for neural plasticity. Prog Brain Res 2000; 129: 343–56.

Bakal DA, Kaganov JA. Muscle contraction and migraine headache: psychophysiologic comparison. Headache 1977; 17: 208–15.

Bakker DA, Richmond FJ, Abrahams VC. Central projections from cat suboccipital muscles: a study using transganglionic transport of horseradish peroxidase. J Comp Neurol 1984; 228: 409–21.

Bartsch T, Goadsby PJ. Stimulation of the greater occipital nerve induces increased central excitability of dural afferent input. Brain 2002; 125: 1496–509.

Bogduk N. Headache and the neck. In: Goadsby PJ, Silberstein SD, editors. Headache. Boston: Butterworth Heinemann; 1997. p. 369–81.

Bove GM, Moskowitz MA. Primary afferent neurons innervating guinea pig dura. J Neurophysiol 1997; 77: 299–308.

Burstein R, Yamamura H, Malick A, Strassman AM. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. J Neurophysiol 1998; 79: 964–82.

Burstein R, Cutrer MF, Yarnitsky D. The development of cutaneous allodynia during a migraine attack. Brain 2000a; 123: 1703–9.

Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. An association between migraine and cutaneous allodynia. Ann Neurol 2000b; 47: 614–24.

Cervero F. Fine afferent fibres from viscera and visceral pain: anatomy and physiology of viscero-somatic convergence. In: Schmidt RF, Schaible HG, Vahle-Hinz C, editors. Fine afferent nerve fibres and pain. Weinheim: VCH; 1987. p. 321–33.

Cervero F, Laird JM. Visceral pain. Lancet 1999; 353: 2145–2148.

Davis KD, Dostrovsky JO. Properties of feline thalamic neurons activated by stimulation of the middle meningeal artery and sagittal sinus. Brain Res 1988a; 454: 89–100.

Davis KD, Dostrovsky JO. Responses of feline trigeminal spinal tract nucleus neurons to stimulation of the middle meningeal artery and sagittal sinus. J Neurophysiol 1988b; 59: 648–66.

Dostrovosky JO, Davis KD, Kawakita K. Central mechanisms of vascular headache. Can J Physiol Pharmacol 1991; 69: 652–8.

Drummond PD. Scalp tenderness and sensitivity to pain in migraine and tension headache. Headache 1987; 27: 45–50.

Ebersberger A, Schaible H-G, Averbeck B, Richter F. Is there a correlation between spreading depression, neurogenic inflammation, and nociception that might cause migraine headache? Ann Neurol 2001; 49: 7–13.

Fields HL, Malick A, Burstein R. Dorsal horn projection targets of ON and OFF cells in the rostral ventromedial medulla. J Neurophysiol 1995; 74: 1742–59.

Foreman RD. Integration of viscerosomatic sensory input at the spinal level. Prog Brain Res 2000; 122: 209–21.

Giffin NJ, Ruggiero L, Lipton RB, Silberstein S, Tvedskov JF, Olesen J, et al. Premonitory symptoms in migraine: an electronic diary study. Neurology 2003; 60: 935–40.

Goadsby PJ. The pathophysiology of headache. In: Silberstein SD, Lipton RB, Solomon S, editors. Wolff's headache and other head pain. Oxford: Oxford University Press; 2001. p. 57–72.

Goadsby PJ, Zagami AS. Thalamic processing of craniovascular pain in the cat: a 2-deoxyglucose study [abstract]. Soc Neurosci Abstr 1990; 16: 1144.

Goadsby PJ, Zagami AS. Stimulation of the superior sagittal sinus increases metabolic activity and blood flow in certain regions of the brainstem and upper cervical spinal cord of the cat. Brain 1991; 114: 1001–11.

Goadsby PJ, Hoskin KL, Knight YE. Stimulation of the greater occipital nerve increases metabolic activity in the trigeminal nucleus caudalis and cervical dorsal horn of the cat. Pain 1997; 73: 23–8.

Goadsby PJ, Lipton RB, Ferrari MD. Migraine—current understanding and treatment. N Engl J Med 2002; 346: 257–70.

Guilbaud G, Benoist JM, Eschalier A, Gautron M, Kayser V. Evidence for peripheral serotonergic mechanisms in the early sensitization after carrageenin-induced inflammation: electrophysiological studies in the ventrobasal complex of the rat thalamus using a potent specific antagonist of peripheral 5-HT receptors. Brain Res 1989; 502: 187–97.

Handwerker HO, Forster C, Kirchhoff C. Discharge patterns of human C-fibers induced by itching and burning stimuli. J Neurophysiol 1991; 66: 307–15.

Herrero JF, Laird JM, Lopez-Garcia JA. Wind-up of spinal cord neurones and pain sensation: much ado about something? Prog Neurobiol 2000; 61: 169–203.

Hoheisel U, Mense S. Response behaviour of cat dorsal horn neurones receiving input from skeletal muscle and other deep somatic tissues. J Physiol 1990; 426: 265–80.

Hoheisel U, Mense S, Simons DG, Yu XM. Appearance of new receptive fields in rat dorsal horn neurons following noxious stimulation of skeletal muscle: a model for referral of muscle pain? Neurosci Lett 1993; 153: 9–12.

Hoskin KL, Kaube H, Goadsby PJ. Central activation of the trigeminovascular pathway in the cat is inhibited by dihydroergotamine. A c-*Fos* and electrophysiological study. Brain 1996; 119: 249–56.

Hu JW, Dostrovsky JO, Sessle BJ. Functional properties of neurons in cat trigeminal subnucleus caudalis (medullary dorsal horn). I. Responses to oral–facial noxious and nonnoxious stimuli and projections to thalamus and subnucleus oralis. J Neurophysiol 1981; 45: 173–91.

Hu JW, Sessle BJ, Raboisson P, Dallel R, Woda A. Stimulation of craniofacial muscle afferents induces prolonged facilitatory effects in trigeminal nociceptive brain-stem neurones. Pain 1992; 48: 53–60.

Hu JW, Yu XM, Vernon H, Sessle BJ. Excitatory effects on neck and jaw muscle activity of inflammatory irritant applied to cervical paraspinal tissues. Pain 1993; 55: 243–50.

Hu JW, Vernon H, Tatourian I. Changes in neck electromyography associated with meningeal noxious stimulation. J Manipulative Physiol Ther 1995; 18: 577–581.

Katsarava Z, Lehnerdt G, Duda B, Ellrich J, Diener H-C, Kaube H. Sensitization of trigeminal nociception specific for migraine but not pain of sinusitis. Neurology 2002; 59: 1450–1453.

Kaube H, Keay KA, Hoskin KL, Bandler R, Goadsby PJ. Expression of c-*Fos*-like immunoreactivity in the caudal medulla and upper cervical cord following stimulation of the superior sagittal sinus in the cat. Brain Res 1993; 629: 95–102.

Kaube H, Katsarava Z, Przywara S, Drepper J, Ellrich J, Diener H-C. Acute migraine headache. Possible sensitization of neurons in the spinal trigeminal nucleus? Neurology 2002; 58: 1234–8.

Kerr FWL, Olafson RA. Trigeminal and cervical volleys. Arch Neurol 1961; 5: 69–76.

Kerr RW. A mechanism to account for frontal headache in cases of posterior fossa tumours. J Neurosurg 1961; 18: 605–9.

Koltzenburg M, Torebjork HE, Wahren LK. Nociceptor modulated central sensitization causes mechanical hyperalgesia in acute chemogenic and chronic neuropathic pain. Brain 1994; 117: 579–91.

Lance JW, Goadsby PJ. Mechanism and management of headache. 6th edn. Oxford: Butterworth-Heinemann; 1998.

Langemark M, Olesen J. Pericranial tenderness in tension headache. A blind, controlled study. Cephalalgia 1987; 7: 249–55.

Lipski J. Antidromic activation of neurones as an analytic tool in the study of the central nervous system. J Neurosci Methods 1981; 4: 1–32.

Mackenzie J. Symptoms and their interpretation. London: Shaw and Sons; 1909.

Mayberg MR, Zervas NT, Moskowitz MA. Trigeminal projections to supratentorial pial and dural blood vessels in cats demonstrated by horseradish peroxidase histochemistry. J Comp Neurol 1984; 223: 46–56.

McMahon SB, Lewin GR, Wall PD. Central hyperexcitability triggered by noxious inputs. Curr Opin Neurobiol 1993; 3: 602–10.

Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993; 54: 241–89.

Molander C, Grant G. Spinal cord cytoarchitecture. In: Paxinos G, editor. The rat nervous system. 2nd edn. San Diego: Academic Press; 1995. p. 39–45.

Nebe J, Vanegas H, Schaible H-G. Spinal application of omega-conotoxin GVIA, an N-type calcium channel antagonist, attenuates enhancement of dorsal spinal neuronal responses caused by intra-articular injection of mustard oil in the rat. Exp Brain Res 1998; 120: 61–9.

Neuhuber WL, Zenker W. Central distribution of cervical primary afferents in the rat, with emphasis on proprioceptive projections to vestibular, perihypoglossal, and upper thoracic spinal nuclei. J Comp Neurol 1989; 280: 231–53.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th edn. San Diego: Academic Press; 1998.

Pfaller K, Arvidsson J. Central distribution of trigeminal and upper cervical primary afferents in the rat studied by anterograde transport of horseradish peroxidase conjugated to wheat germ agglutinin. J Comp Neurol 1988; 268: 91–108.

Pfister J, Zenker W. The splenius capitis muscle of the rat, architecture and histochemistry, afferent and efferent innervation as compared with that of the quadriceps muscle. Anat Embryol (Berl) 1984; 169: 79–89.

Piovesan EJ, Kowacs PA, Tatsui CE, Lange MC, Ribas LC, Werneck LC. Referred pain after painful stimulation of the greater occipital nerve in humans: evidence of convergence of cervical afferences on trigeminal nuclei. Cephalalgia 2001; 21: 107–9.

Ruch TC. Pathophysiology of pain. In: Ruch TC, Patton HD, editors. Physiology and biophysics. 19th edn. Philadelphia: Saunders; 1965. p. 345–63.

Schaible H-G, Grubb BD. Afferent and spinal mechanisms of joint pain. Pain 1993; 55: 45–54.

Schepelmann K, Ebersberger A, Pawlak M, Oppmann M, Messlinger K. Response properties of trigeminal brain stem neurons with input from dura mater encephali in the rat. Neuroscience 1999; 90: 543–54.

Scheurer S, Gottschall J, Groh V. Afferent projections of the rat major occipital nerve studied by transganglionic transport of HRP. Anat Embryol (Berl) 1983; 1983: 425–38.

Selby G, Lance JW. Observations on 500 cases of migraine and allied vascular headache. J Neurol Neurosurg Psychiatry 1960; 23: 23–32.

Silberstein SD, Lipton RB, Goadsby PJ. Headache in clinical practice. 2nd edn. London: Martin Dunitz; 2002.

Strassman AM, Mineta Y, Vos BP. Distribution of fos-like immunoreactivity in the medullary and upper cervical dorsal horn produced by stimulation of dural blood vessels in the rat. J Neurosci 1994; 14: 3725–35.

Strassman AM, Raymond SA, Burstein R. Sensitization of meningeal sensory neurons and the origin of headaches. Nature 1996; 384: 560–4.

Thompson SW, Woolf CJ, Sivilotti LG. Small-caliber afferent inputs produce a heterosynaptic facilitation of the synaptic responses evoked by primary afferent A-fibers in the neonatal rat spinal cord in vitro. J Neurophysiol 1993; 69: 2116–28.

Wall PD, Woolf CJ. Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. J Physiol 1984; 356: 443–58.

Wirth FP, van Buren JM. Referral of pain from dural stimulation in man. J Neurosurg 1971; 34: 630–42.

Wolff HG. Headache and other head pain. New York: Oxford University Press; 1948.

Woolf CJ. Evidence for a central component of post-injury pain hypersensitvity. Nature 1983; 306: 686–88.

Woolf CJ, King AE. Dynamic alterations in the cutaneous mechanoreceptive fields of dorsal horn neurons in the rat spinal cord. J Neurosci 1990; 10: 2717–26.

Woolf CJ, Wall PD. Relative effectiveness of C primary afferent fibers of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. J Neurosci 1986; 6: 1433–42.

Woolf CJ, Shortland P, Sivilotti LG. Sensitization of high mechanothreshold superficial dorsal horn and flexor motor neurones following chemosensitive primary afferent activation. Pain 1994; 58: 141–55.

Yoshida A, Dostrovsky JO, Sessle BJ, Chiang CY. Trigeminal projections to the nucleus submedius of the thalamus in the rat. J Comp Neurol 1991; 307: 609–25.

Yu XM, Hua M, Mense S. The effects of intracerebroventricular injection of naloxone, phentolamine and methysergide on the transmission of nociceptive signals in rat dorsal horn neurons with convergent cutaneous–deep input. Neuroscience 1991; 44: 715–23.

Yu XM, Sessle BJ, Hu JW. Differential effects of cutaneous and deep application of inflammatory irritant on mechanoreceptive field properties of trigeminal brain stem nociceptive neurons. J Neurophysiol 1993; 70: 1704–7.

Zagami AS, Lambert GA. Stimulation of cranial vessels excites nociceptive neurones in several thalamic nuclei of the cat. Exp Brain Res 1990; 81: 552–66.

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