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Stimulation of the greater occipital nerve induces increased central excitability of dural afferent input

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Summary

Patients with primary headaches often report pain that involves not only the front of the head, innervated by the first (ophthalmic) division of the trigeminal nerve, but also the back of the head, innervated by the greater occipital nerve (GON) that is a branch of the C_2 spinal root. The aim of this work was to study the physiology of trigeminocervical input in a model of cranial nociception by describing a population of nociceptive neurones that receive convergent input from the supratentorial dura and the GON. Rats were anaesthetized with pentobarbital, paralysed and ventilated. The parietal dura above the middle meningeal artery was stimulated through a closed cranial window. The GON was exposed in the neck and stimulated. Recordings were made from 67 neurones (52 wide dynamic range neurones/15 nociceptive-specific-neurones) in the C_2 spinal dorsal horn, which responded to stimulation of the dura and the GON. Most neurones showed receptive fields corresponding to the first trigeminal division as well as input from C_2 skin and muscle. Neurones were recorded in superficial and deep layers of the dorsal horn. All neurones tested received input from the ipsilateral and contralateral GON (n = 5). The responses to dural stimulation were analysed before and after stimulation of the GON. Supramaximal electrical stimulation of the GON (20 s to 5 min) enhanced afferent dural input in 8/20 neurones. Application of the C-fibre activator mustard oil (MO) to the cutaneous receptive field or suboccipital muscles innervated by the GON induced an increased excitability of dural responses in 8/12 and 9/10 neurones, respectively. Stimulation of muscle afferents had a significant longer facilitatory effect than cutaneous stimulation ($P < 0.05$). These results show that a considerable population of neurones show convergent input from both dura as well as cervical cutaneous and muscle territories, which supports the view of a functional continuum between the caudal trigeminal nucleus and upper cervical segments involved in cranial nociception. The facilitatory effect of GON stimulation on dural stimulation suggests a central mechanism at the second order neurone level. This mechanism may be important in pain referral from cervical structures to the head and therefore have implications for most forms of primary headache.

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

Abbreviations: GON = greater occipital nerve; IMP = impulse; MO = mustard oil

Introduction

The afferent input from pain-producing cranial structures like the dura mater and the cranial vessels is considered to be the substrate of pain in primary headaches, such as migraine or cluster headache (Goadsby *et al*., 2002). Headache patients often report pain that involves the front of the head, innervated by the first (ophthalmic) division of the trigeminal nerve. However, the pain frequently exceeds trigeminal innervation territories as pain from the back of the head, innervated by the greater occipital nerve (GON) is also described (Anthony, 1992). Furthermore, it has been shown that stimulation of structures in the neck that are innervated by the upper cervical roots, such as by posterior fossa tumours (Kerr, 1961), stimulation of infratentorial dura mater (Wolff, 1948), direct stimulation of cervical roots (Hunter and Mayfield, 1949; Kerr, 1961) and subcutaneous tissue innervated by the GON (Piovesan *et al*., 2001), may be perceived as frontal headpain (Gowers, 1888; Bogduk, 1997). Similarly, direct stimulation of the supratentorial dura mater leads to pain mostly referred to the first (ophthalmic) division of the trigeminal nerve (Wolff, 1948). The most likely mechanism for these observations is that the afferent inflow from the meninges and from the upper cervical roots converge onto the same central neurone (Goadsby, 2001). This convergence is thought to be the basis for the phenom-

enon of referred pain whereby pain originating from deep somatic tissue is perceived as originating from a distant receptive field (Ruch, 1965).

Primary nociceptive afferents from the meninges terminate within the medullary dorsal horn of the caudal trigeminal nucleus (Burstein *et al*., 1998; Schepelmann *et al*., 1999). Recent findings, however, have shown that afferent trigeminal terminations extend to the C_2 spinal segment (Kaube *et al*., 1993; Strassman *et al*., 1994). This is the same segmental distribution of primary afferents from the upper cervical roots (Pfaller and Arvidsson, 1988). Structures in the back of the head are mainly innervated by the GON that is a branch of the C_2 spinal root (Scheurer *et al*., 1983). Although an anatomical overlap of trigeminal and cervical afferents throughout the trigemino cervical complex from the level of the caudal trigeminal nucleus to at least the C_2 segment has been suggested (Kerr, 1972; Pfaller and Arvidsson, 1988), a direct functional coupling between meningeal afferents and cervical afferents in the spinal dorsal horn has not been described (Goadsby *et al*., 1997).

After stimulation of nociceptive afferents, especially C-fibres, convergent neurones in the spinal cord and caudal trigeminal nucleus are subject to neuroplastic changes that are reflected in an increase in central neurone excitability (Woolf and King, 1990; Schaible and Grubb, 1993; Sandkuhler *et al*., 2000). These changes have also been shown to take place in the trigeminal system (Hu *et al*., 1992; Burstein *et al*., 1998; Schepelmann *et al*., 1999); however, it remains unclear if neurones receiving convergent trigeminal and cervical input display similar neuroplastic properties as described for convergent neurones within the trigeminal and spinal system.

Therefore, we wished to describe the properties of a population of nociceptive neurones in the spinal dorsal horn of the C_2 segment of the rat, which receives convergent input from the supratentorial dura as well as from the GON, and to study the interaction of both inputs after activation of C-fibre afferents in the GON.

Materials and methods

General procedure

Experiments were conducted on Sprague–Dawley rats (230–400 g), which were initially anaesthetized with pentobarbitone sodium (Sagatal® 65 mg/kg i.p.). Anaesthesia was maintained by bolus injections of pentobarbitone sodium (dissolved in Tyrode's solution 1 : 3, 10 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 (Pavulon®, Organon, UK, 1 mg/kg initially followed by maintenance with 0.4 mg/kg when necessary) and artificially ventilated using O_2-enriched room air (Ugo Basile, Italy). End-tidal CO_2 was monitored (Capstar-100; CWE Inc., USA) and maintained between 3.5 and 4.5%. ECG was monitored continuously (CWE Inc., PA, USA). Rectal temperature was kept constant at 37°C by means of a servo-controlled heating blanket. The eyes were covered with an ointment to prevent drying of the cornea. At the end of the experiments, animals were killed under deep anaesthesia by intravenous injection of a lethal dose of pentobarbital.

For exposing the stimulation and recording sites, the heads of the animals were fixed in a stereotactic frame and a midline incision was made. The parietal dura mater adjacent to the middle meningeal artery was stimulated through a closed cranial bone window. Therefore, the parietal bone was exposed and the bone was thinned using a dental drill under liquid cooling until the middle meningeal artery became clearly visible (Cumberbatch *et al*., 1999). Care was taken to leave the bone membrane intact, which was covered with warm mineral oil.

The muscles of the dorsal neck were carefully separated 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 brain stem 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 in accordance with UK Home Office guidelines.

Stimulation and recording

A pair of bipolar electrodes was placed on the bone window and electrical square-wave stimuli (0.5 Hz) of 0.5–2 ms duration up to 30 V, corresponding to a current of 0.2–5 mA, 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 neurones in the spinal dorsal horn of C_2 using tungsten microelectrodes (WPI, FL, USA; 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, band pass 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 to an A/D interface (CED Power 1401*plus*; Cambridge Electronic Design, Cambridge, UK) and then to an IBM-compatible computer. Spike discrimination was controlled by means of an electronic delay unit. Post- and peri-stimulus–time histograms of neural activity were displayed and analysed using SPIKE 2.01 (Cambridge Electronic Design).

Characterization of neurones

To identify neurones with convergent input from the dura mater and GON, the recording electrode was advanced into

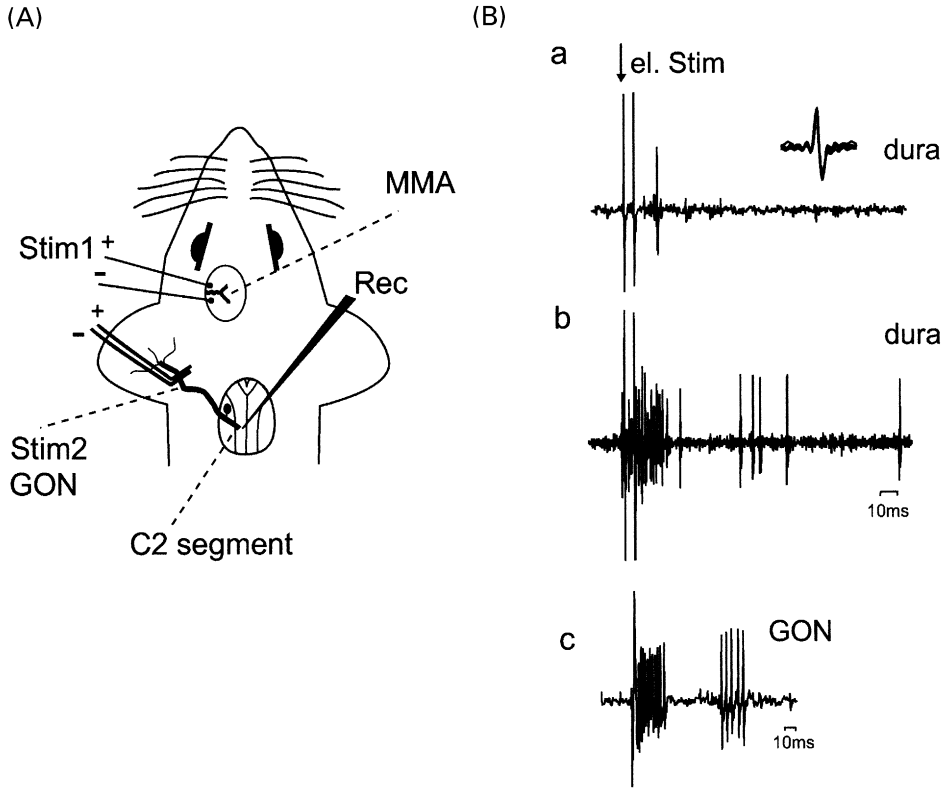

Fig. 1 Schematic drawing of the experimental set-up, showing the location of the stimulation and recording electrodes. (**A**) The supratentorial parietal dura was stimulated adjacent to the middle meningeal artery (Stim 1) whereas the GON was stimulated distally close to its peripheral terminations close to the auricle (Stim 2). The recording electrode was lowered into the dorsal horn of the C_2 spinal segment (Rec). MMA = middle meningeal artery. (**B**, a) Original trace showing stimulation of the dura mater (double stimulus) elicited an early latency response (15 ms) within the A-fibre range. The inset shows the superimposition of five traces for spike identification. (b) Increasing the stimulation parameters recruits further A-fibre as well as C-fibre latency responses. (c) Stimulation of the GON shows A-fibre and C-fibre latency responses.

the dorsal horn of C_2 while electrical stimuli were applied to the parietal dura. When a dura-sensitive neurone was found it was tested for convergent GON A- and C-fibre input by short-lasting electrical GON stimulation. The search stimuli were then paused for 10 min to allow the neurones to recover. For identification of electrical stimulation, only units with constant latency, consistent spike amplitude and waveform were included, and these criteria were regularly checked throughout the recording time (Fig. 1). By means of measurements of the distance from 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), the conduction velocities were calculated. According to the latencies to stimulation, neurones were classified as A-fibres (>1.5 m/s) or C-fibres (<1.5 m/s). One millisecond was added to dural latency values due to the central synaptic delay, the delay in activation of the peripheral axons and the narrowing of afferents in the trigeminal spinal tract. Post-mortem studies verified the course and innervation territories of the GON from its cutaneous terminations, via its branches to suboccipital paraspinal muscles to the C_2 dorsal root ganglion.

The cutaneous and deep receptive field of each neurone was assessed using a wide 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 neck muscles was also tested. The receptive field was mapped by applying non-noxious and noxious stimuli. The two-dimensional features of the receptive field was transferred to a 1 : 1 drawing of the rat's head and neck. Non-noxious stimuli were applied by gently brushing, softly stroking and applying light pressure with a blunt probe to the cutaneous receptive field. Noxious mechanical stimuli consisted of pinching with forceps or heavy pressure that was painful when applied to humans. To avoid alterations in spontaneous activity the application of prolonged noxious stimuli was restricted. Since the dura mater was stimulated through a cranial bone window, dural receptive fields were not tested. Furthermore, to avoid further sensitization of cutaneous afferents by repeated assessment of

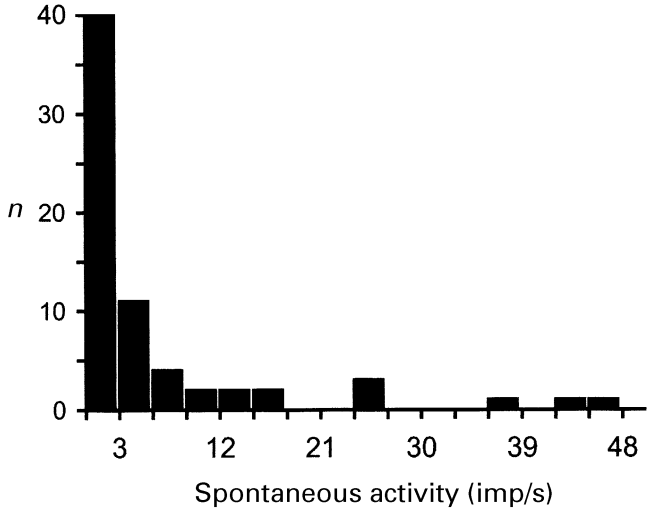

Fig. 2 Distribution of the rate of spontaneous activity in convergent C_2 dorsal horn neurones.

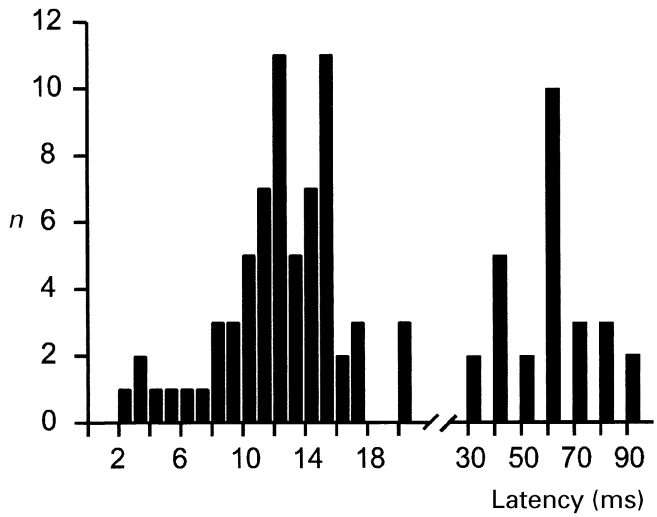

Fig. 3 Distribution of the latencies to electrical stimulation of the dura mater in nociceptive convergent neurones in the trigeminocervical complex. Latencies are separated for A-fibre (in 1-ms intervals; $n = 67$) and C-fibre latencies (in 10-ms intervals; $n = 27$). Note that in 27 neurones, A- and C-fibre latencies were present.

cutaneous receptive fields, and since it is known that receptive fields can be subjected to spontaneous variations, we refrained from analysing facial receptive fields before and after MO application (Dubuisson *et al*., 1979; McMahon and Wall, 1984; Hoheisel *et al*., 1993). According to the cutaneous receptive field properties, neurones were classified as low-threshold mechanoreceptive neurones that responded only to innocuous stimulation, wide-dynamic range neurones, which responded to non-noxious and noxious stimuli, and nociceptive-specific-neurones, which responded primarily to noxious input (Yu *et al*., 1993). Only trigeminocervical receptive fields were identified.

Experimental protocol

The responses to electrical dural stimulation were analysed before and after stimulation of the GON. Electrical stimulation of the dura mater was used as a monitor of central excitability changes (Wall and Woolf, 1984; Hu *et al*., 1992; Woolf *et al*., 1994). For assessing the baseline responses to dural stimulation, trains of 20 stimuli were delivered in 10 min intervals starting at least 30 min prior to any conditioning stimulus. As a conditioning stimulus the GON was stimulated electrically supramaximally to activate C-fibre afferents (1 Hz, 20 s to 5 min) or stimulated by MO application, a C-fibre activator (Woolf and Wall, 1986). MO (Sigma, UK; allyl isothiocyanate, 20% in paraffin oil) was either injected (30 µl) into GON-innervated muscles (m. semispinalis capitis, m. rectus capitis posterior minor and major), or applied to the centre of the cutaneous receptive field of the GON by a cotton swab. After application of the conditioning stimulus, dural stimulation was performed in 10 min intervals for the first 60 min, and then in 20 min intervals. After MO application to the skin and muscle, dural stimulation was also performed after 5 min. MO application was clearly associated with signs of inflammation, such as flare and oedema. Only one neurone in each animal was tested with the application of a conditioning stimulus. Controls were performed by injecting vehicle (mineral oil) only into the semispinalis muscle in neurones with a muscle receptive field.

Spontaneous activity in neurones was determined from time periods of 1 min under control conditions. 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 spontaneous activity, an interval of ongoing activity was recorded before each train of dural stimulation, which was then subtracted from the stimulation interval. Results are expressed as mean $\pm$ SD, if not otherwise stated. A change of $\geqslant$30% in neural activity was considered as a response (Nagler *et al*., 1973). Statistical analyses were carried out with the use of the Student's *t*-test ($P < 0.05$ was considered significant). Data were normalized for graphical representation by expressing each test interval as a percentage of the mean preconditioning baseline response. The duration of the excitability increase after the application of the conditioning stimulus was calculated as the time taken for the number of spikes elicited by the test stimulus to return to within mean $\pm$ 2 SD of the baseline, or until the end of the observation period if it did not return.

Histology

At the conclusion of recordings, the recording site within the spinal cord dorsal horn was determined by: (i) electrolytic lesions by passing current through the recording electrode; (ii) in some experiments the exact location of the electrode

penetration was noted, a tungsten microelectrode was inserted at that site to the same depth, and an electrolytic lesion was made; and (iii) recording sites were estimated by plotting the *x*- (distance from midline) and *y*-coordinates (relative depth from surface) as read from the micropositioner on an ideal spinal cord cross-section, considering the differences in animal weight and hence brain weight. The tissue was removed, fixed in 1% potassium ferrocyanide in 10% formaldehyde, cut into 40 μm frozen sections, collected on glass slides and stained with cresyl violet. Lesion sites were examined under the light microscope and transferred to an ideal cross-section of the C_2 spinal cord segment (Fig. 4) (Molander and Grant, 1995).

Results

Identification of cells

Recordings were made from 67 neurones in the C_2 dorsal horn that received convergent input from the supratentorial parietal dura mater and the GON. Most neurones showed an ongoing activity with a mean of 6.7 impulses/s (imp/s) (range 0.01–48 imp/s). Some neurones [40/67 (60%)] showed no or low spontaneous activity (0–3 imp/s), whereas in 12/67 (18%) neurones spontaneous activity was >10 imp/s (Fig. 2).

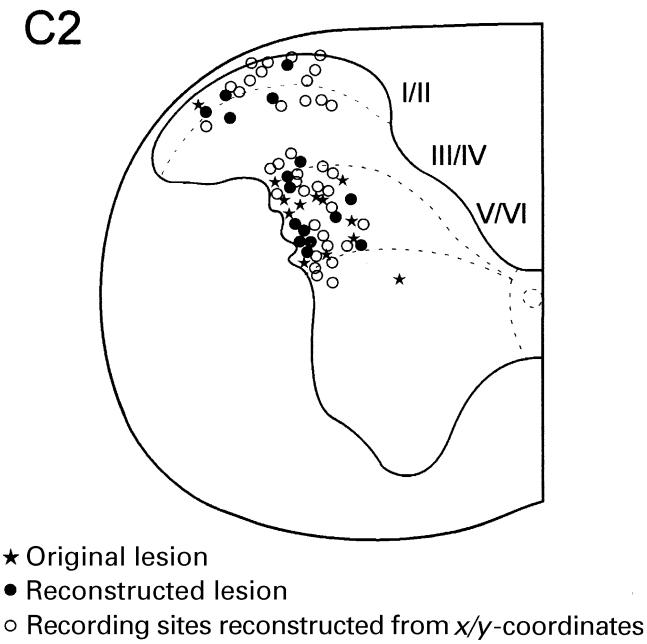

Fig. 4 Summary of the locations of the recording sites of 67 nociceptive convergent neurones receiving input from the dura and the GON. Locations of all neurones are plotted on an ideal cross-section of the C_2 spinal cord segment. The different locations were determined by electrolytic lesions of the original electrode (n = 13; filled stars), reconstructed lesions by reinserting the electrode (n = 16; filled circles) or by reconstructing the recording sites according to the *x*- (distance from midline) and *y*-coordinates (relative depth from surface) as read from the micropositioner (n = 38; open circles).

Electrical stimulation of the dura elicited in all neurones a short latency response with a mean latency of 12 ± 3.7 ms (Figs 1 and 3). The calculated conduction velocities were in the Aδ-fibre range. In 27 neurones an additional long latency response between 30 and 100 ms (58 ± 17 ms) could be elicited and was within the C-fibre range. These long latency responses showed a higher threshold to electrical stimulation. If there was a C-fibre component present, the experimental protocol was performed with the dural stimulation being in C-fibre strength.

Electrical stimulation of the GON in A-fibre strength elicited a short latency response in all neurones studied with a latency of between 1 and 20 ms. Increasing the stimulation parameters produced an additional response with a longer latency between 40 and 80 ms. Frequently (n = 25), a wind-up phenomenon was observed in the long latency response of the GON during electrical stimulation in C-fibre strength (Figs 5 and 9).

Location of neurones

Convergent neurones were found in two clusters within the C_2 spinal cord segment (Fig. 4). One cluster (n = 21) was found at a mean depth of 199 ± 104 μm, which corresponded to the outer laminae I and II, although also included the borderzone to lamina III. The majority of neurones (70%), including 73% of all neurones that showed sensitization, were found in deep layers corresponding to laminae V and VI, including the borderzone to lamina IV of the spinal dorsal horn at a mean depth of 721 ± 166 μm (n = 46).

Receptive fields

All neurones analysed within the C_2 segment could be classified as either wide-dynamic range neurones (n = 52) or nociceptive-specific neurones (n = 15) on the basis of their cutaneous receptive field properties. No unit could be classified as a low-threshold mechanoreceptive neurone. All neurones showed cutaneous receptive fields in the face, mostly restricted to the first division of the trigeminal nerve (n = 59) including cornea (n = 40). In some experiments the receptive field included the second (n = 21) and third trigeminal division (n = 13). However, if the receptive field included more than one trigeminal division the ophthalmic division proved to be most sensitive to afferent stimulation. The trigeminal receptive fields were caudally located close to the border to the C_2 dermatome (Figs 5–7). All cells showed a cutaneous receptive field corresponding to the C_2 dermatome extending from the occipital skin to the auricle as well as additional deep input from suboccipital paraspinal muscles (m. semispinalis capitis, m. rectus capitis posterior minor and major; n = 45) (Fig. 6). Of all cells studied (n = 139), 48% showed convergence of both dural and GON input, while 8% showed exclusive dural input and 44% showed exclusive GON input. Neurones showing only input from either dura or GON were not studied further.

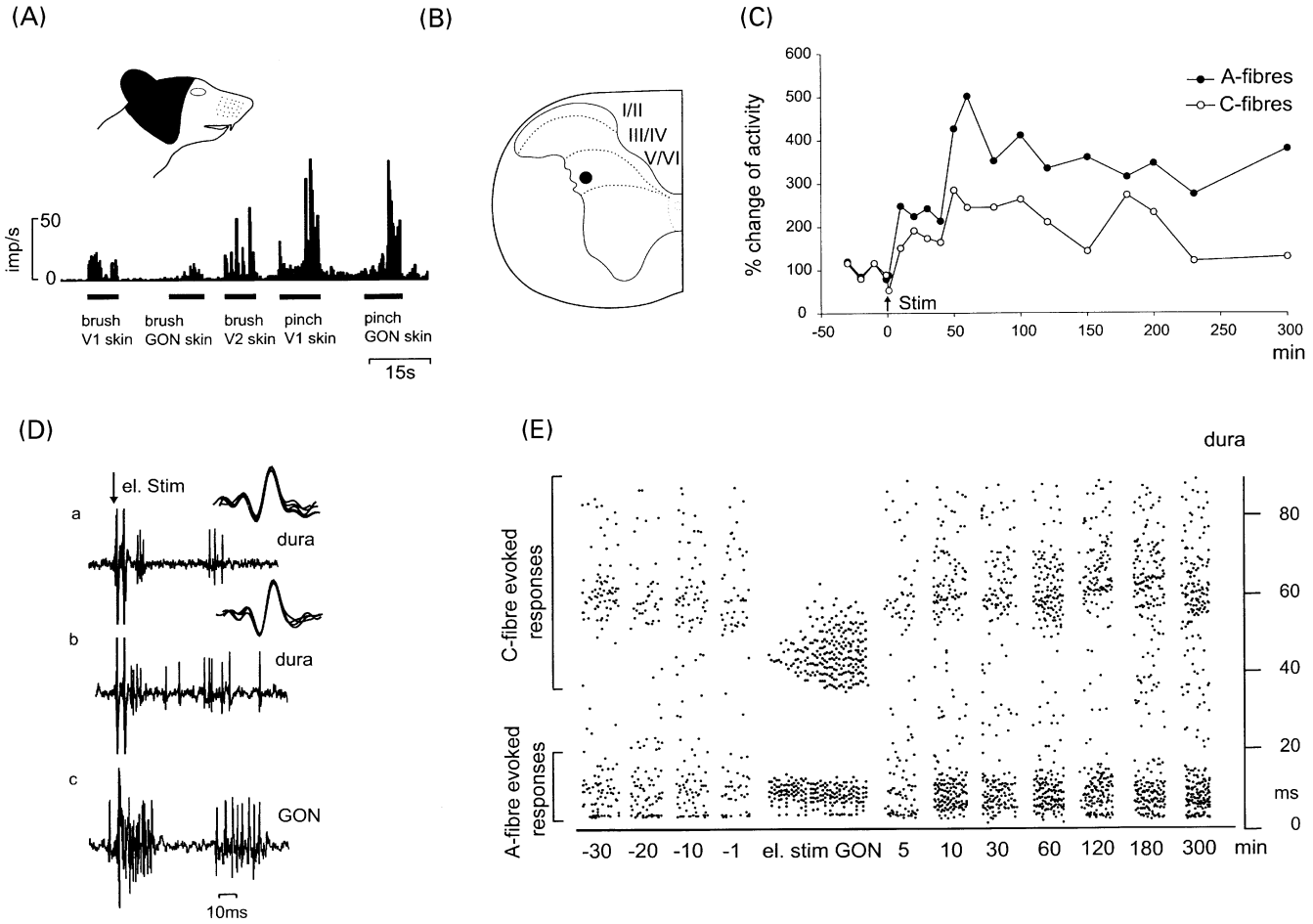

Fig. 5 Neural responses of a convergent wide-dynamic range neurone to electrical stimulation of the GON as a conditioning stimulus. (**A**) Illustration of the neural responses to natural stimulation of the cutaneous receptive field (black). (**B**) Location of the recording site within the deep layers of the C_2 spinal dorsal horn. (**C**) Electrical stimulation of the GON over 3 min in C-fibre strength elicited an increased excitability of dural input up to 300 min in the A- (filled circle) and C-fibre (open circle) component. (**D**) Original trace of the neural responses to dural stimulation before (a) and after MO stimulation (b) and GON stimulation (c). The insets show the superimposition of five traces of the C-fibre component. (**E**) Raster dot display (each dot represents one evoked neuronal spike) of neural responses (A- and C-fibre components) to dural stimulation before and after electrical GON stimulation. Only the first 40 electrical GON stimuli are shown. Note that GON stimulation elicited a wind-up phenomenon in the C-fibre component within the first 20 stimuli.

Effect of electrical stimulation of GON on responses to stimulation of dura mater

The GON in its distal part close to its termination near the auricle is a mixed sensory nerve containing both skin and muscle afferents (Scheurer *et al.*, 1983). To activate C-fibres the GON was stimulated supramaximally for between 20 s and 5 min (n = 20). After GON stimulation, eight neurones showed an increased excitability to dural stimulation up to 207 ± 52% (mean ± SD; peak baseline change) (Table 1; Fig. 5) for the A-fibre component (n = 6) and 201 ± 7% for the C-fibre component (n = 3). The increase of excitability after electrical GON stimulation lasted 92 ± 111 min for the A-fibre component (n = 6), whereas it was 113 ± 126 min for the C-fibre component (n = 3). In one neurone the increase in excitability lasted throughout the whole observation period of 300 min (Fig. 5). In two neurones, sensitization was restricted to the C-fibre component, while in four neurones an initial decrease in the excitability was observed within the first 10 min after GON stimulation.

Effect of stimulation of muscle afferents in the GON on responses to dura mater stimulation

Since electrical GON stimulation activates both cutaneous and deep somatic afferents from muscles, we selectively activated cutaneous and muscle afferents by applying the C-fibre activator MO to the cutaneous (n = 12) and muscle receptive field (n = 10) of the GON. MO injection into the m. semispinalis capitis and m. rectus capitis posterior produced an immediate increase in spontaneous activity up to 79 ± 24 imp/s (mean ±

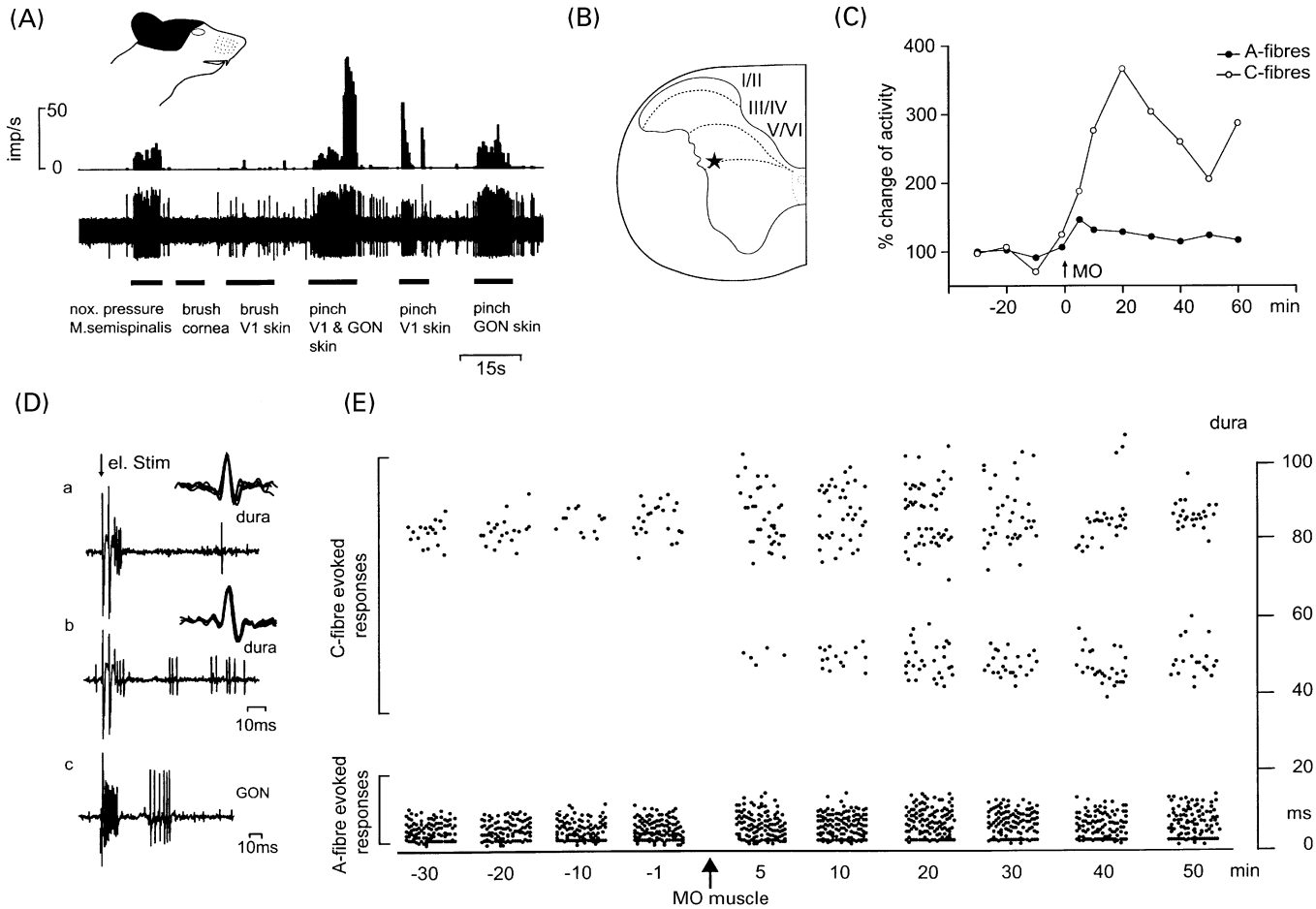

Fig. 6 Neural responses of a convergent neurone to MO injection into the m. semispinalis capitis as a conditioning stimulus. (**A**) Neural responses to natural stimulation of the cutaneous receptive field (black). Note the response to noxious pressure to the m. semispinalis. (**B**) The location of the recording site was retrieved within the deep layers of the C_2 dorsal horn. (**C**) Stimulation of muscle afferents in the GON produced an increased excitability to dural stimulation up to 60 min in the A- (filled circle) and C-fibre (open circle) component. (**D**) Original trace of the neural responses to dural stimulation before (a) and after MO stimulation (b) and GON stimulation (c). The insets show the superimposition of five traces of the C-fibre component. (**E**) Raster dot display (each dot represents one evoked neuronal spike) of neural responses (A- and C-fibre components) to dural stimulation before and after MO application, showing an increase in excitability with a novel recruitment of a C-fibre response in the latency range between 40 and 60 ms (see also **D**).

SEM) within 5 min. Within 20 min activity gradually settled down to values that were not significantly different from baseline activity (Fig. 8). Spontaneous activity was not altered by intramuscular application of vehicle (Fig. 8). After stimulation of muscle afferents by MO, the responses to dural stimulation were increased to 244 ± 116% (mean ± SD; peak baseline change) (Table 1; Fig. 6) for the A-fibre component (n = 9) and 360 ± 6% for the C-fibre component (n = 2). The increase in excitability gradually rose within the first 30 min after MO application and lasted 51 ± 21 min for the A-fibre component (n = 9) and 48 ± 8 min for the C-fibre component (n = 2). In two experiments an initial decrease in the excitability was observed within the first 5 min after MO application. Stimulation of muscle afferents showed a significantly longer facilitatory effect compared with stimulation of cutaneous afferents ($P < 0.05$).

Dural responses to stimulation of cutaneous afferents in the GON

MO application to the cutaneous receptive field produced an immediate increase in spontaneous activity up to 80 ± 31 imp/s (mean ± SEM) within 5 min and gradually settled (Fig. 8). There was no difference in the time course and peak spontaneous activity between stimulation of muscle and skin afferents (Fig. 8). After stimulation of cutaneous afferents in the GON, responses to dural stimulation were increased to 176 ±41% (mean ± SD; peak baseline change) (Table 1; Fig. 7) for the A-fibre component (n = 8) and 597 ± 234% for the C-fibre component (n = 4). Similar to muscle afferent stimulation, the increase in excitability peaked within the first 30 min after cutaneous stimulation and lasted 18 ± 12 min for the A-fibre component (n = 8) and 14 ± 4 min for the C-fibre component (n = 4). In three neurones an initial decrease of the excitability was observed within the first 10

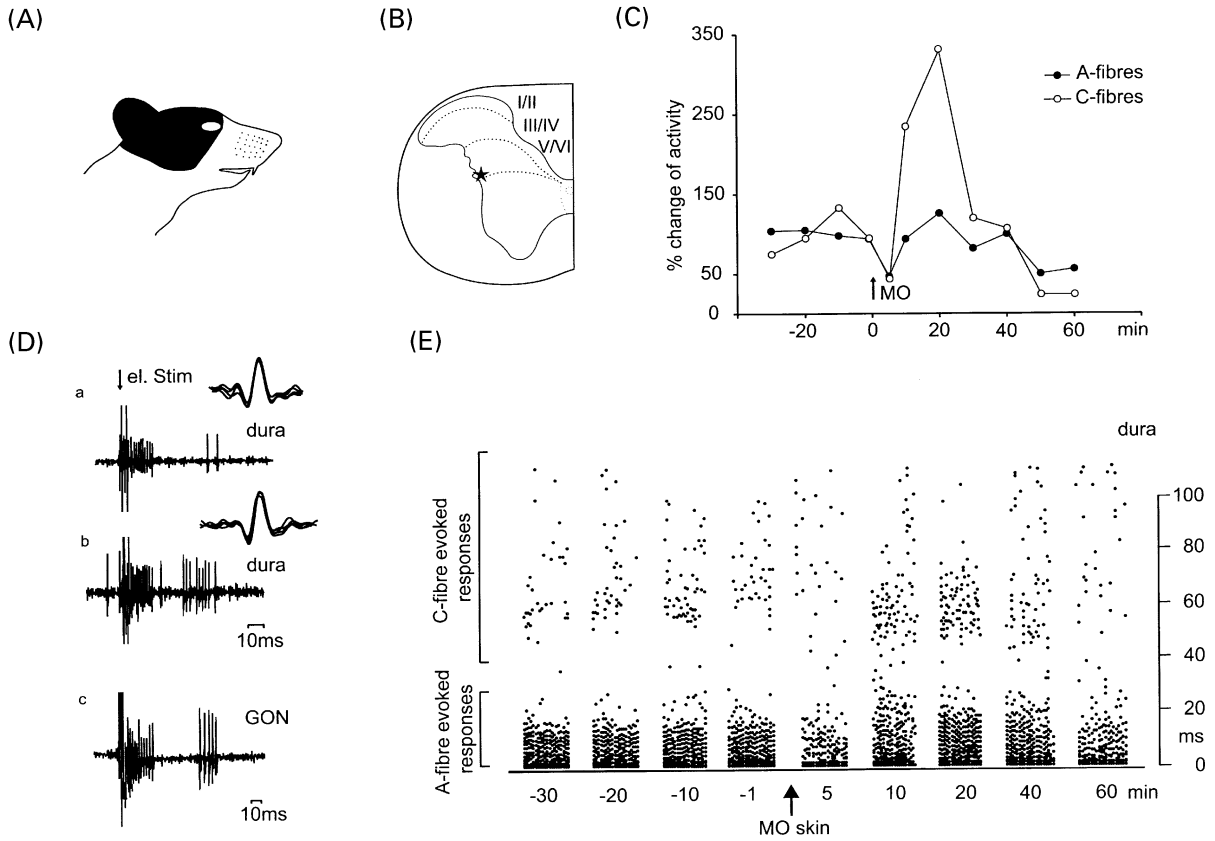

Fig. 7 Neural responses of a convergent neurone to MO application to the cutaneous receptive field of the GON. (**A**) Cutaneous receptive field (black), which included the first and second trigeminal division and the C_2 dermatome. (**B**) The location of the recording site was retrieved within the deep layers of the C_2 dorsal horn. (**C**) Stimulation of cutaneous afferents in the GON produced an increased excitability to dural stimulation in the A- (filled circle) and C-fibre (open circle) component, peaking within 30 min of MO application. (**D**) Original trace of the neural responses to dural stimulation before (a) and after MO stimulation (b) and GON stimulation (c). The insets show the superimposition of five traces of the C-fibre component. (**E**) Raster dot display of neural responses (A- and C-fibre components) to dural stimulation before and after cutaneous MO application. Note an initial inhibition of the dural responses 5 min after MO application.

min after MO application. A synopsis of the changes after GON stimulation is presented in Table 1.

Stimulation of ipsi- and contralateral GONs

In five animals it was tested if neurones that receive input from ipsilateral dura and GON also respond to contralateral GON stimulation. In all neurones tested (n = 5), bilateral GON stimulation showed that dura-sensitive convergent neurones receive input from the ipsilateral as well as from the contralateral GON (Fig. 9).

Discussion

In this study we wished to characterize nociceptive neurones in the C_2 spinal dorsal horn of the rat that receive convergent afferent input from the meninges and from the GON. These neurones showed input from GON innervated skin and muscles, and additional convergent input from the facial skin. Stimulation of GON muscle and cutaneous C-fibre afferents with MO increased the excitability of the afferent meningeal input. The physiology of these neurones is fundamental to understanding perhaps the most common referral pattern for primary headache syndromes between trigeminal and cervical regions. These data underpin the phenotypes of a large number of patients seen in clinical practice and emphasize the importance of cervical input in headache.

Localization and characterization of neurones

The verification of the recording sites by electrolytic lesions, by reinserting an electrode and by making calculations from the *x*-/*y*-coordinates of the micropositioner, led to comparable results and showed that convergent neurones were found in two clusters in the superficial and deep C_2 dorsal horn. The majority of neurones that showed increased dural responses after GON stimulation were found in the deeper layers of the dorsal horn, as described typically for convergent nociceptive

Table 1 *Summary of all neurones that showed increased excitability after stimulation of the GON*

No	Type	RF	SA (imp/s)	Laminar location	Dural input	Stimulation	Peak baseline change (%)	Duration of increase (min)
1	WDR	$V_{1–3}$,C_2	5.6	d	Aδ	MO muscle	308	55
2	WDR	V_1,C_2	1.8	s	Aδ	MO muscle	530	70
3	WDR	$V_{1–3}$,C_2	2.2	s	Aδ	MO muscle	201	70
4	WDR	V_1,C_2	7.1	d	Aδ	MO muscle	295	80
5	WDR	$V_{1–3}$,C_2	1.6	d	Aδ	MO muscle	167	40
6	NS	V_1,C_2	<1	s	Aδ	MO muscle	246	15
7	NS	V_1,C_2	<1	d	Aδ	MO muscle	165	25
8	NS	V_1,C_2	<1	d	Aδ + C	MO muscle	146 (366)	40 (55)
9	NS	V_1,C_2	3.6	d	Aδ + C	MO muscle	142 (354)	60 (40)
10	WDR	$V_{1–2}$,C_2	3.3	d	Aδ	MO skin	225	20
11	NS	V_1,C_2	<1	s	Aδ	MO skin	155 (879)	15 (15)
12	NS	V_1,C_2	<1	d	Aδ + C	MO skin	168	45
13	WDR	V_1,C_2	29	d	Aδ + C	MO skin	227	15
14	NS	V_1,C_2	16	d	Aδ + C	MO skin	225	25
15	WDR	V_1,$C_{2–3}$	17	d	Aδ + C	MO skin	130 (403)	5 (10)
16	WDR	$V_{1–3}$,$C_{2–3}$	19	d	Aδ + C	MO skin	130 (331)	10 (20)
17	WDR	V_1,C_2	19	d	Aδ + C	MO skin	144 (777)	10 (10)
18	WDR	V_1,C_2	2.8	d	Aδ + C	GON el	159	5
19	WDR	V_1,C_2	<1	d	Aδ	GON el	170	45
20	WDR	V_1,C_2	2.3	d	Aδ	GON el	139	10
21	WDR	V_1,C_2	4.9	s	Aδ	GON el	(208)	(40)
22	WDR	$V_{1–3}$,C_2	<1	d	Aδ + C	GON el	248 (191)	290 (290)
23	WDR	V_1,C_2	2	d	Aδ + C	GON el	(205)	(10)
24	WDR	$V_{1–3}$,$C_{2–3}$	39	d	Aδ	GON el	253	199
25	WDR	$V_{1–3}$,C_2	28	s	Aδ	GON el	275	5

WDR = wide dynamic range neurones; NS = nociceptive specific neurones; RF = receptive fields; SA = spontaneous activity. All neurones received A- and C-fibre input to electrical GON stimulation. Receptive fields are expressed as cutaneous trigeminal ($V_{1–3}$) and cutaneous and muscle cervical ($C_{2–3}$) receptive fields. The distribution within the C_2 dorsal horn is expressed as superficial (s; laminae I–II + borderzone III) and deep (d; laminae V–VI + borderzone IV). The type of GON stimulation is expressed as stimulation of cutaneous (MO skin), muscle (MO muscle) afferents and electrical stimulation (GON el). Changes are expressed as percentage peak change from baseline within the first 30 min after conditioning stimulation. C-fibre values are set in parentheses.

neurones (Davis and Dostrovsky, 1988; Hu *et al*., 1992; Burstein *et al*., 1998; Schepelmann *et al*., 1999). However, some convergent neurones were also found in superficial layers of the dorsal horn (Strassman *et al*., 1994). The locations corresponded to the area that receives projections from the ophthalmic division of the trigeminal nerve (Strassman *et al*., 1994), which is the primary source of afferents from the supratentorial dura (Mayberg *et al*., 1984; Andres *et al*., 1987). Primary afferents from C_2-innervated neck muscles have been shown to terminate in the deep layers of the dorsal horn (Bakker *et al*., 1984; Pfister and Zenker, 1984; Neuhuber and Zenker, 1989), where cutaneous afferents in the GON terminate (Scheurer *et al*., 1983). All neurones in this study showed latencies in the Aδ-fibre range to dural stimulation, while one-third of neurones also displayed latencies in the C-fibre range, which is in accordance with other studies in the rat (Strassman *et al*., 1996; Burstein *et al*., 1998).

Receptive fields and convergence

Dural responsive neurones typically showed convergent input from the facial skin centered around the ophthalmic division of the trigeminal nerve, including the cornea (Strassman *et al*., 1994; Ebersberger *et al*., 1997; Burstein *et al*., 1998; Schepelmann *et al*., 1999). As a reflection of the convergent input, neurones showed cutaneous and muscle receptive fields corresponding to the GON-innervated territory of C_2. Similar nociceptive neurones with convergent superficial and deep input have been described (Yu *et al*., 1993). Published data suggest an overlap between trigeminal and upper cervical afferents throughout the trigeminocervical complex from the caudal trigeminal nucleus to the upper cervical segments (Kerr and Olafson, 1961; Kerr, 1972; Abrahams *et al*., 1979; Sessle *et al*., 1986; Pfaller and Arvidsson, 1988), although only minimal direct electrophysiological evidence for coupling of meningeal and cervical afferents exist (Angus-Leppan *et al*., 1997). Nociceptive trigeminal afferents from the meninges terminate in the medullary dorsal horn but also extend to the spinal segments of C_3 in the rat (Strassman *et al*., 1994), cat (Kaube *et al*., 1993) and monkey (Goadsby and Hoskin, 1997). On the other side, afferents in the GON also terminate from the C_2 segment up to the medullary dorsal horn (Scheurer *et al*., 1983). After stimulation of the GON, metabolic activity increased in the trigeminal nucleus caudalis and the C_1 and C_2 dorsal horn, which is the same

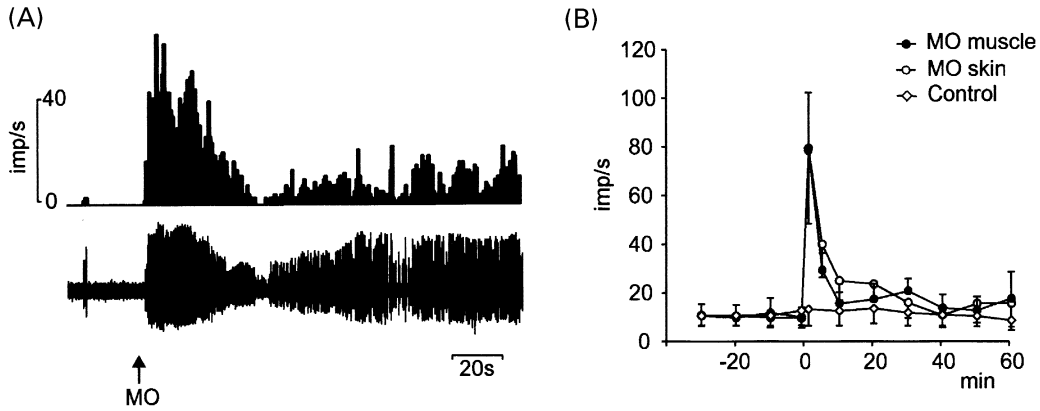

Fig. 8 (**A**) Neural responses to MO application. Peristimulus time histogram and original trace of a nociceptive neurone (same neurone as in Fig. 6) found to be responsive to MO injection into the m. semispinalis capitis, which led to an immediate and profound increase in spontaneous activity with a biphasic time course consisting of an initial peak of activity and a second tonic discharge. (**B**) Time course of the spontaneous activity in nociceptive convergent neurones receiving input to dural and GON stimulation. MO was injected into the semispinalis muscle (n = 10; filled circles) and applied to the cutaneous receptive field of the GON (n = 12; open circles). Control injection of vehicle into the semispinalis muscle (n = 5; open diamonds) measured over 60 min showed no change in ongoing activity. Activity peaked within 5 min after MO application and then gradually settled down. Results are expressed as mean ± SEM.

region that shows activation on trigeminal stimulation (Goadsby *et al*., 1997).

All convergent neurones tested in this study received ipsilateral as well as contralateral input from the GON. Similar contralateral projections have been shown following labelling of the trigeminal and cervical dorsal root ganglia (Jacquin *et al*., 1982; Pfaller and Arvidsson, 1988) as well as after facial afferent (Ellrich *et al*., 1999) or GON stimulation (Goadsby *et al*., 1997). The bilateral activation following unilateral stimulation may correspond to the sensation of dull and poorly localized quality of pain spread from deep somatic afferents (Linderoth and Brodin, 1994) in the trigeminocervical region.

Responses to GON stimulation and central sensitization

In this study we showed that meningeal and GON afferents make a functional connection, as stimulation of C-fibre afferents in the GON produced sensitization of the afferent meningeal input. Since the convergence of the meningeal and cervical input is most likely not due to collaterals of peripheral sensory nerves, but to central convergence, the second order neurone in the spinal cord is the most likely site of this heterosynaptic sensitization (Thompson *et al*., 1993). Stimulation of nociceptive afferents, especially C-fibres, induces central changes, such as a reduction of the threshold for activation, an increase in spontaneous firing and an increase in central neurone excitability (Hu *et al*., 1992; Schaible and Grubb, 1993; Woolf *et al*., 1994). In particular, stimulation of muscle afferents is more effective in changing neural excitability than cutaneous input. Stimulation of muscle C-fibre afferents produces greater facilitatory effects on the flexion reflex than cutaneous C-fibres (Wall and Woolf, 1984). Muscle afferents selectively develop spontaneous activity after peripheral nerve lesions (Michaelis *et al*., 2000). There is a difference in neuropeptide content between muscle and cutaneous afferents (O'Brien *et al*., 1989), and muscle pain is referred to other deep somatic tissues and generally not to the skin (Mense, 1993). Inflammation of ventral neck muscles with MO leads to an enlargement of deep and cutaneous receptive fields, in contrast to application to cutaneous receptive fields, which leads only to a cutaneous receptive field expansion (Yu *et al*., 1993). Additionally, considering the prolonged changes evoked by neck muscle stimulation, the number of muscle afferents may play an important role since unmyelinated, small-diameter afferents and free nerve endings predominate in neck muscles (Richmond *et al*., 1976; Abrahams and Richmond, 1988).

The time course of the facilitatory changes in convergent neurones is consistent with other studies analysing central facilitatory mechanisms after afferent stimulation by MO (Hu *et al*., 1992; Yu *et al*., 1993; Woolf *et al*., 1994; Park *et al*., 2001). Interestingly, even brief stimuli are sufficient to evoke changes in excitability that outlast the stimulus (Cook *et al*., 1987). In some of the neurones an initial and transient decrease of the excitability after GON stimulation was observed. It has been shown that nociceptive stimulation of the GON can alter the release of calcitonin gene-related peptide (Vincent *et al*., 1992), an effect that is probably modulated via opioidergic mechanisms (Collin *et al*., 1993; Williamson *et al*., 2001) and that might account for these changes in excitability. In common with convergent neurones in the deep layers of the spinal dorsal horn, the phenomenon of wind-up, which consists of a progressive increase in C-fibre-evoked responses following repeated stimulation (Mendell and Wall, 1965; Dickenson, 1995), was often observed in the long-latency responses of convergent neurones to electrical GON stimulation. Since wind-up is generally regarded as a display of central hyperexcitability (Woolf, 1996; Herrero

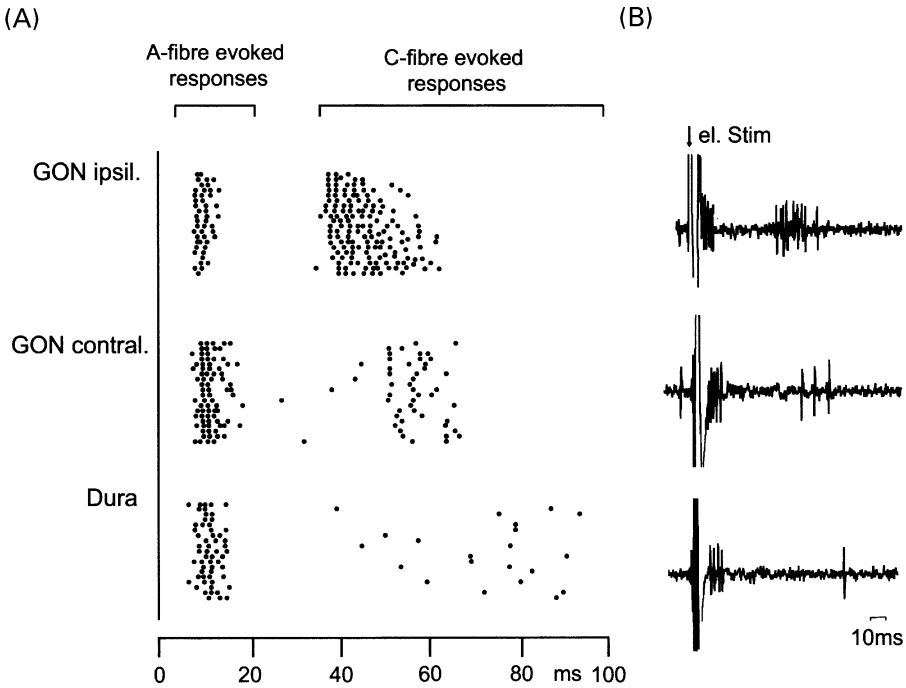

Fig. 9 Example of a nociceptive convergent neurone receiving input from the dura mater as well as from the ipsi- and contralateral GON. (**A**) Raster dot display (each dot represents one evoked neuronal spike) showing trains of 20 stimuli and the evoked responses separated after A- and C-fibre latencies. Note that ipsilateral GON stimulation demonstrates a wind-up phenomenon. (**B**) Original traces to electrical stimulation.

et al., 2000), this further supports our findings that GON stimulation is capable of inducing central excitability changes.

Referred pain

Clinical observations show that pain in primary headache syndrome is often perceived in the front of the head as well as in the neck (Wolff, 1948). Furthermore, structures in the neck that are innervated by the upper cervical roots are an important source of head pain (Bogduk and Marsland, 1988; Anthony, 1992; Bogduk, 1997). Stimulation of cervical roots and stimulation of structures innervated by upper cervical roots in humans, such as the dura mater, vessels and tumours of the posterior fossa, leads to pain referred to the front of the head (Ray and Wolff, 1940; Hunter and Mayfield, 1949; Feinstein *et al*., 1954; Kerr, 1961; Wirth and van Buren, 1971; Cremer *et al*., 1995; Pollmann *et al*., 1997; Hutchinson *et al*., 2000; Piovesan *et al*., 2001). Some headache forms benefit from a blockade of the GON (Bovim and Sand, 1992). The spatial convergence of trigeminal and cervical afferents may account for the holocephalic perception of pain that involves the front of the head, innervated by the first division of the trigeminal nerve and the back of the head, innervated by the GON (Goadsby *et al*., 1997), providing a mechanism for pain referred to trigeminal territories. Since stimulation of supratentorial dura in humans evokes solely painful sensations, regardless of the stimuli applied, the afferent inflow from the meninges may be facilitated and perceived as painful (Ray and Wolff, 1940). This does not exclude the possibility that spatial convergence takes place more supraspinally in structures such as the thalamus (Burstein *et al*., 2000). Finally, these mechanisms comply with the widely accepted explanation of referred pain as the 'convergence projection' theory (Ruch, 1965).

Conclusion

These data provide clear evidence of functional coupling between nociceptive meningeal afferents and cervical afferents in the GON at the second-order neurone level. The spatial convergence of nociceptive information has implications for the pain perception in headache syndromes, as the trigeminocervical complex may be regarded as a functional continuum involved in cranial nociception. The facilitatory effect of GON stimulation on the meningeal input may be involved in the referral of pain originating from structures innervated by the upper cervical roots to trigeminal innervation territories. An understanding of the physiology and pharmacology of this important interaction has fundamental implications for most forms of primary headache, providing a physiological basis for a very common clinical phenomenon.

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