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Stimulation of the middle meningeal artery leads to Fos expression in the trigeminocervical nucleus: a comparative study of monkey and cat

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ABSTRACT

The pain of a migraine attack is often described as unilateral, with a throbbing or pulsating quality. The middle meningeal artery (MMA) is the largest artery supplying the dura mater, is paired, and pain-producing in humans. This artery, or its branches, and other large intracranial extracerebral vessels have been implicated in the pathophysiology of migraine by theories suggesting neurogenic inflammation or cranial vasodilatation, or both, as explanations for the pain of migraine. Having previously studied in detail the distribution of the second order neurons that are involved in the transmission of nociceptive signals from intracranial venous sinuses, we sought to compare the distribution of second order neurons from a pain-producing intracranial artery in both monkey and cat. By electrically stimulating the middle meningeal artery in these species and using immunohistochemical detection of the proto-oncogene Fos as a marker of neuronal activation, we have mapped the sites of the central trigeminal neurons which may be involved in transmission of nociception from intracranial extracerebral arteries. Ten cats and 3 monkeys were anaesthetised with α-chloralose and the middle meningeal artery was isolated following a temporal craniotomy. The animals were maintained under stable anaesthesia for 24 h to allow Fos expression due to the initial surgery to dissipate. Following the rest period, the vessel was carefully lifted onto hook electrodes, and then left alone in control animals (cat n = 3), or stimulated (cat n = 6, monkey n = 3). Stimulation of the left middle meningeal artery evoked Fos expression in the trigeminocervical nucleus, consisting of the dorsal horn of the caudal medulla and upper 2 divisions of the cervical spinal cord, on both the ipsilateral and contralateral sides. Cats had larger amounts of Fos expressed on the ipsilateral than on the contralateral side. Fos expression in the caudal nucleus tractus solitarius and its caudal extension in lamina X of the spinal cord was seen bilaterally in response to middle meningeal artery stimulation. This study demonstrates a comparable anatomical distribution of Fos activation between cat and monkey and, when compared with previous studies, between this arterial structure and the superior sagittal sinus. These data add to the overall picture of the trigeminovascular innervation of the intracranial pain-producing vessels showing marked anatomical overlap which is consistent with the often poorly localised pain of migraine.

Key words: Headache; migraine; *Macaca nemestrina*; trigeminal system.

INTRODUCTION

The role of the dura mater and more importantly the dural vasculature in migraine is at the crux of an ongoing debate concerning the peripheral sources for pain in migraine (Goadsby, 1997). In this context the distribution of trigeminal neurons activated by stimulation of large dural arteries, such as the middle meningeal artery, provides important information concerning the central processing of signals from these pain-producing structures. Having characterised the distribution of the large venous sinuses in cat

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(Kaube et al. 1993) and monkey (Goadsby & Hoskin, 1997), it is of interest to consider the extent to which the nociceptive innervation of arterial and venous structures and their dural covering overlap, given the very variable patterns of pain seen in migraine and cluster headache (Lance & Goadsby, 1998). Moreover, it is important to consider evidence for species differences given that much of what is known about primary headache is derived from experiments in nonprimates.

The dura mater provides the dual roles of a supporting structure and a protective covering for the brain. Anatomical evidence has shown that the dura mater is very vascular with a rich capillary bed (Kerber & Newton, 1973; Roland et al. 1987). The vessels of the dura mater are densely innervated by sympathetic (Keller et al. 1989) and sensory (Steiger & Meakin, 1984) nerves. The primary afferent fibres arising from the pain-producing intracranial structures travel predominantly in the ophthalmic division of the trigeminal nerve (Feindel et al. 1960), traverse the trigeminal ganglion and synapse in the caudal medulla and upper & divisions of the cervical spinal cord in rat (Arbab et al. 1986; Strassman et al. 1994), cat (Kaube et al. 1993; Hoskin et al. 1996*a*, *b*) and monkey (Goadsby & Hoskin, 1997). It is accepted that visceral afferents converge onto neurons in the trigeminal nucleus caudalis (Hu et al. 1981), which is often termed the medullary dorsal horn due to its structural similarities to the spinal cord dorsal horn (Gobel et al. 1977).

Noxious information from the dura mater is conveyed to the central nervous system by unmyelinated (C fibres) and small myelinated ($A\delta$) fibres that form a plexus around the vessels of the dura mater (Keller & Marfurt, 1991). Nociceptive afferents have cell bodies in the trigeminal ganglion and to a lesser extent the upper cervical dorsal root ganglia (C_1–C_3) in monkey (Penfield & McNaughton, 1940; Ruskell & Simons, 1987), cat (Steiger et al. 1982; Keller et al. 1985) and rat (Arbab et al. 1986). Tracing studies have shown that fibres innervating cerebral vessels arise from within the trigeminal ganglion and travel predominantly in the first division of the trigeminal nerve (Mayberg et al. 1984). Following application of horseradish peroxidase (HRP) to the middle meningeal artery, Mayberg et al. (1984) found cells predominantly in the ophthalmic division of trigeminal ganglia, with only a small number of cells found in the second and third divisions.

Electrophysiological studies have shown that electrical stimulation of the middle meningeal artery causes cell firing in the trigeminal nucleus caudalis (Davis & Dostrovsky, 1986) and in the C_2 spinal cord (Lambert et al. 1991). These cells were activated by noxious stimulation of the facial skin, observations which complement the findings of Wolff (1963) and Penfield & McNaughton (1940). In one study trigeminal ganglion cells have been found *not* to have divergent collaterals linking intracranial structures, such as the dura mater and middle meningeal artery, with the extracranial anterolateral forehead (Borges & Moskowitz, 1983). However, the existence of trigeminal cells having divergent axon collaterals that innervate 2 different intracranial structures has been documented in another study (O'Connor & van der Kooy, 1986) and given the clinical observations seem likely to exist in humans.

In this study we have used an immunohistochemical technique to detect the proto-oncogene Fos, the expression of which is rapid, with the number of cells activated being proportional to the level of the stimulus. In these studies we wished to observe the expression of Fos corresponding to the documented projection of trigeminal fibres from the middle meningeal artery on one side to the trigeminocervical complex and thus explore the extent to which arterial and venous structures have comparable projections. Further, by studying both monkey and cat, data comparing species could be collected given that primary neurovascular headaches are of particular interest as a clinical problem. Some of the results have previously been published in abstract form (Zagami et al. 1997).

MATERIALS AND METHODS

Anaesthesia and surgery

Cats (n = 9) weighing 2.5–3.5 kg and monkeys (*Macaca nemestrina*) (n = 3) weighing 8–14 kg were used. Animals were fasted before experimentation. Monkeys were transported to the laboratory under ketamine (10 mg/kg, i.m.i.) sedation. Animals were initially anaesthetised in the laboratory with α-chloralose (Calbiochem or Sigma) 60 mg/kg administered by intraperitoneal injection. The head and groin area were shaved in preparation for surgery. During surgical procedures halothane (0.5–3%) was administered via an endotracheal tube. The femoral artery and vein were cannulated for continuous monitoring of the blood pressure and heart rate, and for administration of additional anaesthetic and maintenance fluids, respectively. The response of cardiovascular parameters and the pupillary reaction to

noxious pinching of the forepaw in the cat and hand or foot of the monkey, were used to determine the need for supplementary anaesthesia. We have previously shown that this regimen provides stable anaesthesia for this type of study (Goadsby & Hoskin, 1997; Kaube et al. 1993; Storer et al. 1997). The animals were intubated endotracheally and ventilated with 40% oxygen. Body temperature and expired CO_2 were monitored and maintained within physiological parameters. Animals were mounted into a stereotaxic frame and cranial surgery was performed (see below). On completion of surgery, halothane was discontinued. Arterial blood was taken at intervals to measure blood gases and animals were continuously monitored in a quiet room for 24 h. All primate procedures were reviewed and approved by an Institutional Ethics Review Panel at the University of New South Wales and the cat studies were carried out under a project licence issued by the UK Home Office under the Animals (Scientific Procedures) Act, 1986.

To determine the length of time required for Fos elicited during surgery to dissipate we have followed the study of Menetrey et al. (1989) who reported that intracellular Fos due to a simple skin incision was almost nonexistent following a 16 h rest period and have examined the question systematically in cat in other experiments. We have found that a 24 h rest period gives an excellent signal-to-noise ratio for Fos expression (Hoskin & Goadsby, 1999). Fluid (either saline or 4% glucose in saline) was given intravenously at a rate of 3–5 ml/kg/h. Anaesthesia was maintained at a constant depth with intravenous α-chloralose (dissolved either in water or in a β-cyclodextrin solution; Storer et al. 1997), given at 2 h intervals (10–20 mg/kg). Blood pressure and heart rate were stable and within the physiological range for all animals throughout the experiments.

Middle meningeal artery stimulation

The middle meningeal artery is a branch of the external carotid artery ranging in internal diameter from 400–800 µm. To access the vessel the temporal muscle was retracted and a small craniotomy (less than 1 cm diameter) was performed above and slightly anterior to the external auditory canal. The middle meningeal artery was identified and the dura mater was cut parallel to it. In some cases branches arising from the vessel were cauterised distally to prevent bleeding. To activate trigeminal primary afferents the middle meningeal artery was suspended from bipolar hook electrodes, 24 h after the initial surgery and was stimulated at 0.3 Hz, for a period of 2 h in the cat and 1 h in the monkey (S88 stimulator, 150 V, 250 µs duration, driving an SIU5A stimulus isolation unit; Grass Instruments, Quincy, Ma, USA). This stimulation is sufficient to active terminals of nociceptive primary afferents (Bullitt et al. 1992). Controls were performed by suspending the vessel over the hook electrodes with no current applied in the cat but, for ethical reasons, and given that we had undertaken control studies contemporaneously for another similar series of active stimulation studies (Goadsby & Hoskin, 1997), we only performed active stimulation studies in the monkeys.

Perfusion

One hour after stimulation was ceased the animals were perfused transcardially with 1.5 l of 0.9% saline (containing 1000 IU heparin and 1% sodium nitrite) followed by 2 l of paraformaldehyde 4% in 0.1 M phosphate buffer (pH 7.2–7.4) and then 1 l of 30% sucrose solution in phosphate buffer. The brain and cervical spinal cord were removed and stored in 50% sucrose with 0.05% sodium azide. Coronal sections (40 µm) of the caudal medulla and C_1–C_3 spinal cord were cut on a freezing microtome or cryostat with every 5th section saved for processing.

Immunohistochemical detection of Fos

Two different Fos antibodies have been used: a commercially available rabbit polyclonal antibody to Fos protein (Oncogene Science, USA; Genesis, UK) and a locally produced research antibody *fos-x* (Gerrard Evan, Imperial Cancer Research). Thorough testing was performed to ensure the reproducibility and comparability of Fos labelling with each antibody. Free-floating sections were incubated at 4 °C for 3–7 d in a 1:1000 dilution of one of the above antibodies. Fos-like immunoreactivity was visualised using standard avidin-biotin peroxidase immunohistochemical techniques as previously reported (Goadsby & Hoskin, 1997; Kaube et al. 1993). Sections were mounted onto slides and coverslipped.

Sections were subsequently examined under a light microscope (×20 objective). The DAB reaction product was seen as a black precipitate due to the presence of nickel ammonium sulphate in the reaction bath. At the light microscopy level Fos positive cells appeared as dark round or ovoid structures with variable degrees of staining intensity. Cells were only considered positive if they conformed to the criteria

established by Hammond et al. (1992), i.e. the cell nucleus must be distinguishable from the background throughout a range of 20, 10 and 5 times magnification. Cells were plotted onto schematic sections of the caudal medulla and upper cervical spinal cord modified from the atlases of Rexed (1954) and Berman (1968) for the cat and of Burton & Loewy (1976) and Apkarian & Hodge (1989) for the primate work.

Plotting and statistics

The contralateral ventral horn was scored prioto sectioning to enable orientation of the cord when counting. The cells were plotted by hand onto schematic transverse sections of the trigeminal nucleus caudalis (2 mm caudal to the obex) and C_1 and C_2 spinal cord. A map of cell activation in this region was compiled and the distribution of Fos-positive cells was determined semiquantitatively by summating the number of cells in the 10 sections for each individual level giving a total of 30 sections for each trigeminocervical complex. The data are reported as the median with interquartile (25 %, 75 %) range and were tested using the Mann-Whitney test (Siegel, 1956) with a significance level of $P < 0.05$.

RESULTS

All animals included in this study were maintained within normal physiological limits for the duration of the experiment (Table 1).

Distribution of Fos expression

Lamina I and outer laminae II

Control—Cat. In control animals (n = 3) the suspension of the middle meningeal artery over the hook electrode without the application of any current, with the preceding surgery, was sufficient to elicit significant amounts of Fos expression in the superficial laminae of the medullary and upper cervical spinal cord dorsal horns (30 sections) ipsilaterally, with a median of 66 (range 52–81) cells, and with 34 (range 28–47) cells on the contralateral side.

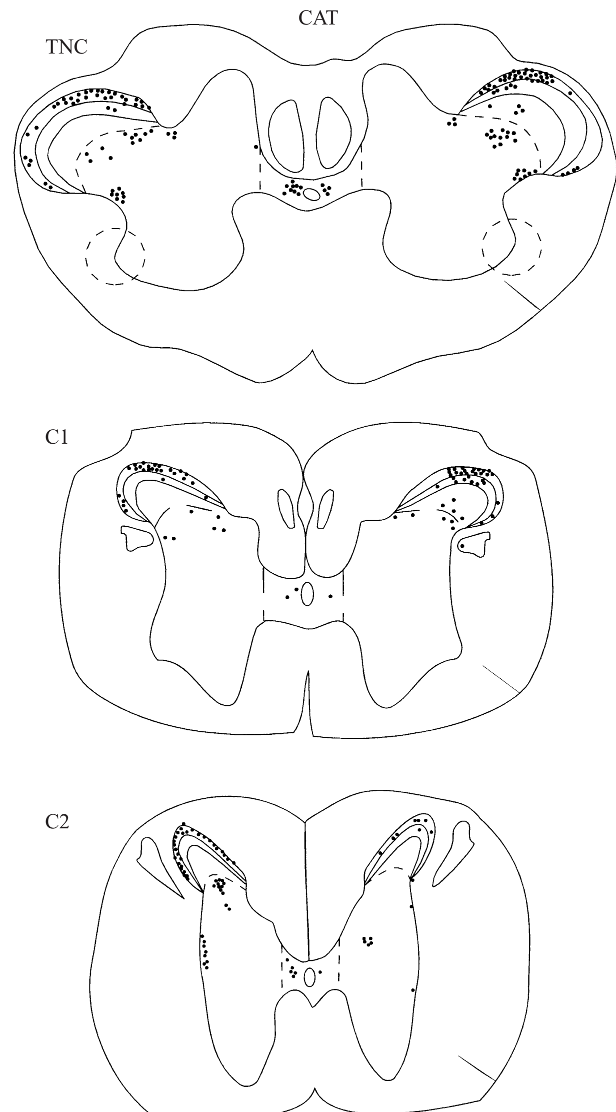

Fig. 1. Representative sections from the cervicomedullary junction, C_1 and C_2 of the cat after electrical stimulation of the middle meningeal artery. Data are plotted for Fos-positive cells ipsilateral (unscored) and contralateral (scored) to the side of stimulation. There is a robust increase in Fos expression in the trigeminocervical complex after middle meningeal artery stimulation which is seen bilaterally.

Stimulation–cat. Stimulation for 2 h (n = 6) elicited a median of 86 (66–138) Fos-positive cells in the ipsilateral superficial laminae (Fig. 1) compared with 57 (45–133) on the contralateral side. Considering the trigeminocervical complex as a whole, Fos activation

Table 1. *Mean data taken from all cats and monkeys included in these studies*

	Weight (kg)	pH	pCO_2 (mmHg)	pO_2 (mmHg)
Cat (n = 9)	3.0 ± 0.6	7.22 ± 0.06	26.1 ± 10.9	218 ± 111.9
Monkey (n = 3)	8.0 ± 4.0	7.48 ± 0.08	31 ± 12	256 ± 122

Table 2. *Total number of Fos-positive cells in lamina I and outer lamina II of the caudal medulla and upper cervical spinal cord, and caudal nucleus tractus solitarius and lamina X, following electrical stimulation of the middle meningeal artery in the cat and monkey*

	Control cat	Cat	Monkey
Laminae I/IIo			
Ipsilateral	66 (52–81)	86 (66–138)	135 (124–152)
Contralateral	34 (28–47)	57 (45–133)	142 (126–160)
Laminae X			
Ipsilateral	22 (17–26)	17 (15–29)	12 (7–27)
Contralateral	20 (13–26)	33 (23–35)	11 (7–28)

* Cells were taken from 30 sections of the trigeminocervical nucleus and to each from the trigeminal nucleus caudalis and C_1 and C_2 spinal cord. Data are presented as a median value with the interquartile ranges.

was greater than in control animals ($P < 0.05$; Table 2). Considering the levels individually the difference from control animals was significant for the C_2 level at 26 (17–28) cells ipsilaterally, and 10 (8–14), contralaterally ($P < 0.05$; Fig. 2).

Stimulation—monkey. In laminae I/IIo of the ipsilateral and contralateral trigeminal nucleus caudalis (TNC) and C_1 and C_2 cervical spinal cord electrical stimulation produced a median of 135 (range 124–152) and 142 (126–160) Fos-positive cells, respectively, for the entire trigeminocervical complex (n = 3, Table 2; Fig. 3).

Lamina X

Fos labelling was also present bilaterally in the caudal nucleus tractus solitarius and its caudal extension, lamina X of the upper cervical spinal cord. In the control group there was a median of 14 (12–19) cells ipsilaterally and 30 (21–35) cells, contralaterally. For the stimulated cats 21 (17–36) cells on the ipsilateral side where observed while on the contralateral side a median of 31 (22–36) cells were seen (Table 2).

DISCUSSION

These data demonstrate that stimulation of the middle meningeal artery results in the activation of neurons in the trigeminocervical complex, consisting of the trigeminal nucleus caudalis and its caudal extension, the dorsal horn of the upper segments of the cervical spinal cord. Neuronal activation was also demonstrated in the caudal nucleus tractus solitarius and its caudal extension, lamina X of the upper cervical spinal cord. Fos labelling was observed on both the

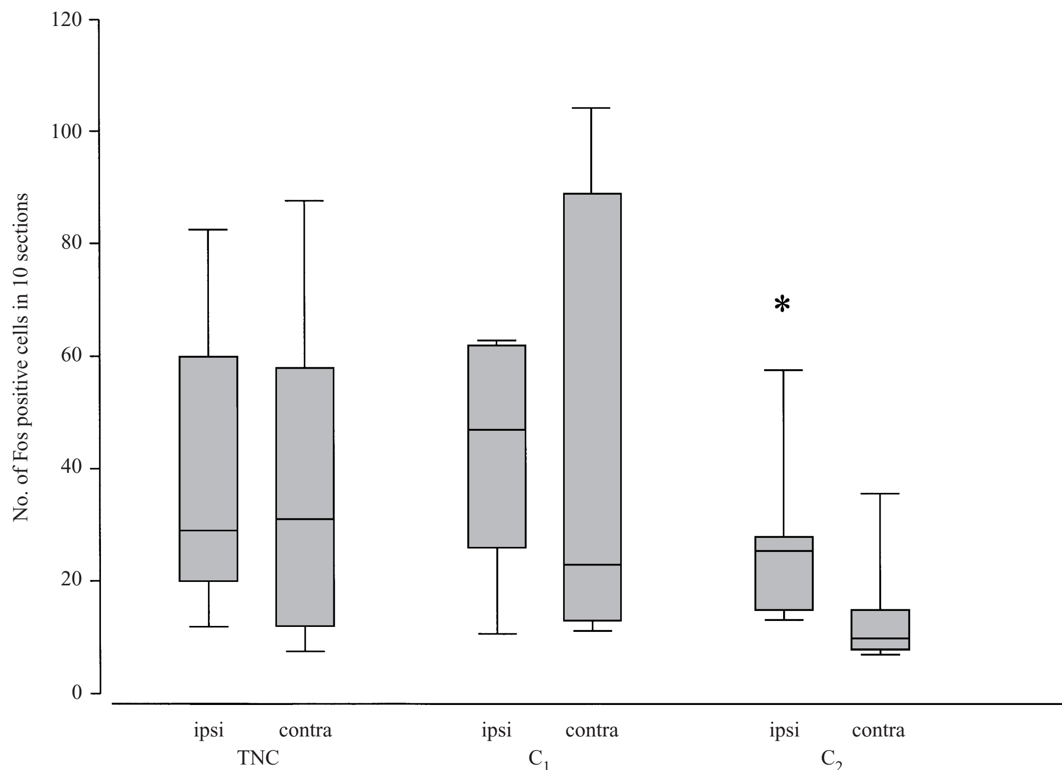

Fig. 2. Box plots summarising the population effect of stimulation of the middle meningeal artery on Fos expression in the trigeminocervical complex, consisting of the trigeminal nucleus caudalis and C_1 and C_2 dorsal horn laminae I/IIo. There is a significant difference for the ipsilateral and contralateral sides at the level of C_2 and the trigeminocervical complex as a whole (see text).

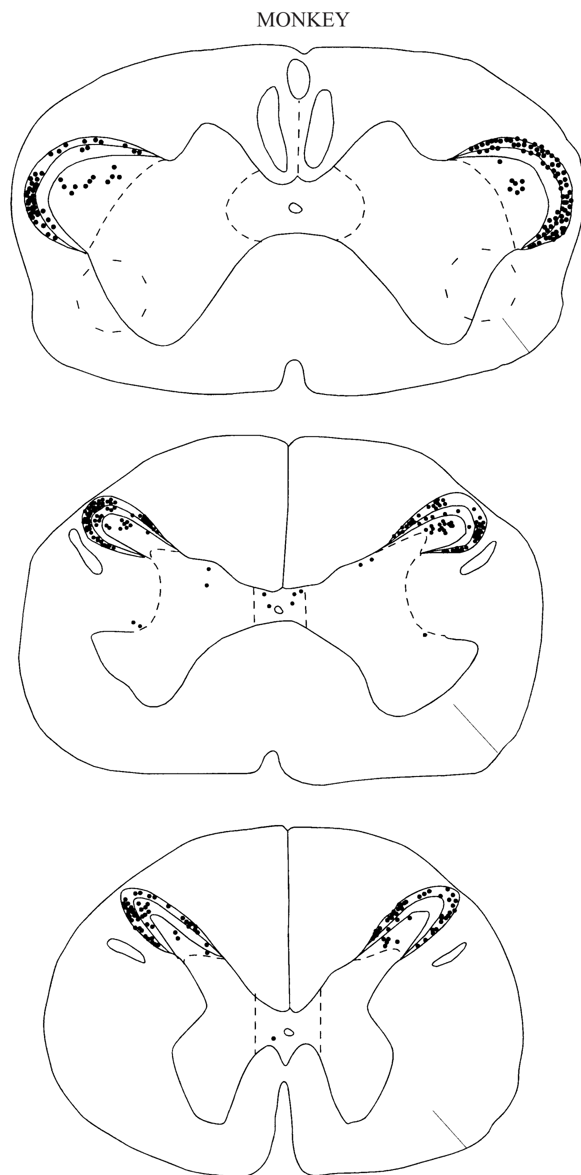

Fig. 3. Representative sections from the cervicomedullary junction, C_1 and C_2 of the monkey, *Macaca nemestrina*, after electrical stimulation of the middle meningeal artery. Data are plotted for Fos-positive cells ipsilateral and contralateral to the side of stimulation. There is a robust increase in Fos expression in the trigeminocervical complex after middle meningeal artery stimulation in a distribution similar to that seen in the cat (Fig. 1).

ipsilateral and the contralateral sides, although this must be interpreted cautiously in light of the irritative effect of lifting the vessel in the control animals. The areas activated are comparable to previous Fos studies as the central ramification site of trigeminal afferents from the pain-producing superior sagittal sinus in the cat (Kaube et al. 1993) and monkey (Goadsby & Hoskin, 1997), and it is in these areas (lamina I, outer lamina II) of the spinal cord that peripheral nociceptive fibres terminate (Bullitt, 1991). Furthermore, these data are consistent with electrophysiological studies reporting that cells linked to electrical stimulation of the middle meningeal artery can be recorded in the caudal medulla (Davis & Dostrovsky, 1986; Strassman et al. 1986) and upper cervical spinal cord (Lambert et al. 1991) of the cat. In addition, the nucleus tractus solitarius, when stimulated, causes retching and emesis (Fukuda & Koga, 1991), which is remarkable since vomiting and nausea are so often associated with migraine (Silberstein et al. 1998).

The middle meningeal artery is surrounded by a periarterial network of nerves with frequent nerve endings (Fang, 1961). As with other intracranial pain-producing structures, the middle meningeal artery receives its innervation from the trigeminal ganglion. Retrograde tracing studies have revealed that these fibres arise predominantly in the ophthalmic division of the trigeminal ganglion (Mayberg et al. 1981), with a considerable proportion also arising in the maxillary branch (O'Connor & van der Kooy, 1986). The trigeminal ganglion predominantly innervates structures on the ipsilateral side, except those structures close to the midline, the dura matter adjacent to the superior sagittal sinus (Mayberg et al. 1981), and deep structures, the anterior cerebral artery and rostral part of the anterior basilar artery (Arbab et al. 1986).

The trigeminocervical complex: a bilateral projection

It has been many decades since Olszewski & Baxter (1954) proposed that the caudal medulla was the natural rostral extension of the upper cervical spinal cord with similar anatomical and physiological roles. Trigeminal afferents project to the trigeminal ganglion ipsilaterally from their intracranial or extracranial site of origin, to synapse somatotopically, the ophthalmic division dorsolaterally and the mandibular division ventromedially, with the maxillary division lying between. This distribution has been determined using both anatomical and physiological techniques. Anatomical degeneration, cutting the proximal trigeminal root or lesioning parts of the trigeminal ganglion, and tracing studies have reported that the trigeminal primary afferents project predominately ipsilaterally to the trigeminocervical complex, as we have defined it. The development of the Fos technique (Hunt et al. 1987) has made it possible to further map this region by localising Fos expression following cortical spreading depression (Moskowitz et al. 1993) or KCl injection (Ingvardsen et al. 1997), irritation of the dura by injecting blood into the subarachnoid space (Nozaki et al. 1992), chemical (Strassman et al. 1994), mechanical (Hoskin et al. 1996*b*) and electrical stimulation of the dural vessels (Kaube et al. 1993; Goadsby & Hoskin, 1997).

Centrally, anatomical degeneration (Clarke & Bowsher, 1962; Torvik, 1967; Westrum et al. 1976; Gobel et al. 1977; Dubbeldam & Karten, 1978) and tracing studies (Grant & Arvidsson, 1975; Marfurt, 1981; Pfaller & Arvidsson, 1988) have shown that trigeminal afferents project ipsilaterally with a small component crossing the midline to synapse in the contralateral dorsal horn. The most likely site for fibres to cross the midline is the region starting at the caudal level of the pyramidal decussation to the C_2 level of the spinal cord (Torvik, 1956; Clarke & Bowsher, 1962; Jacquin et al. 1982).

Electrophysiological evidence is somewhat contradictory with some authors reporting that there is a contralateral projection to the trigeminal nucleus caudalis (Wall & Taub, 1962; Darian-Smith et al. 1963*a, b*) and some who did not observe a bilateral projection (Nord, 1967; Kerr et al. 1968; Kruger et al. 1977). Our study can only make limited inferences about side to side issues since we have found the signal-to-noise ratio for middle meningeal stimulation less optimal than with superior sagittal sinus stimulation (Kaube et al. 1993; Goadsby & Hoskin, 1997). It is likely this is due to a combination of the smaller size of the vessel, the fact that leaving soaked cotton wool balls on the vessel was to some extent irritative, and the extent of the temporal muscle exposure and craniotomy. The distribution of Fos is, however, very consistent between species and when compared with sagittal sinus stimulation (Kaube et al. 1993; Goadsby & Hoskin, 1997) with this comparative result being the primary question of the study. In addition some inferences can be made by comparison with unstimulated control animals which show symmetric minimal Fos expression (Kaube et al. 1993; Goadsby & Hoskin, 1997).

The caudal nucleus tractus solitarius and its caudal extension lamina X

Trigeminal afferents are also seen to terminate in the caudal nucleus tractus solitarius and its caudal extension, lamina X in the upper cervical spinal cord (Torvik, 1956; Nomura et al. 1984; Pfaller & Arvidsson, 1988). This region also contains peptides implicated in nociceptive transmission, namely calcitonin gene related peptide (CGRP) and substance P(SP), in quantities comparable to those found in laminae I/IIo (Gibson et al. 1981). The Fos expression in lamina X in the current study was similar to that seen following stimulation of the superior sagittal sinus (Kaube et al. 1993) and was located in equivalent amounts bilaterally. The presence of fibres crossing in the caudal part of the nucleus commissuralis has been reported (Torvik, 1956; Arbab et al. 1988) and suggests that sensory impulses are conveyed bilaterally to this structure.

The results reported here may be of some clinical significance. Studies by Wolff (1963) on locally anaesthetised conscious patients demonstrated that distension of the middle meningeal artery, by placing the tip of the forceps inside the lumen and stretching the vessel between them, produced pain in the homolateral temporal region. The pain was described as deep and aching in quality and was often accompanied by nausea (Ray & Wolff, 1940). Application of the local anaesthetic procaine hydrochloride to the middle meningeal artery usually rendered the whole temporoparietal region of the dura insensitive (Penfield & McNaughton, 1940). Similarly, stimulation by endovascular dilatation of other unilateral intracranial blood vessels, such as the middle cerebral artery, causes localised frontotemporal pain on the ipsilateral side of the head (Nichols et al. 1990; Martins et al. 1993). Bilateral perception of pain can be seen from posterior fossa structures (Wolff, 1963), which may also refer pain to the frontal region (Nichols et al. 1993).

The trigeminal nerve not only transmits sensory signals centrally for pain perception but also releases neurotransmitters peripherally, such as CGRP and SP, which are involved in the regulation of local blood flow (Edvinsson et al. 1993) and in inflammatory processes (Moskowitz & Cutrer, 1993). The perikarya of the trigeminal ganglia provide the intracranial vasculature with these vasoactive peptides in the rat (Yamamoto et al. 1983; Wanaka et al. 1986) and cat (Edvinsson et al. 1981; Uddman et al. 1985). SP and CGRP may be involved in neurogenic plasma extravasation in the rat and guinea pig dura mater (Moskowitz & Cutrer, 1993) and increased cerebral blood flow in the anaesthetised cat (Goadsby et al. 1988), when they are released from the trigeminal nerve terminals. Only CGRP, however, is reported to be increased in patients during a migraine attack (Goadsby et al. 1990; Goadsby & Edvinsson, 1993; Gallai et al. 1995). Nerve fibres immunoreactive for CGRP and SP are found on the middle meningeal artery, and in the trigeminal ganglion and dorsal root ganglion cells projecting to the middle meningeal artery (O'Connor & van der Kooy, 1988; Keller & Marfurt, 1991). A differential projection of CGRP and SP-like immunoreactive neurons may have interesting implications in view of data showing differential $5HT_{1B}$ and $5HT_{1D}$ distribution in trigeminal ganglion cells immunoreactive for CGRP and

SP (Bonaventure et al. 1998) and the recent report of CGRP infusion producing delayed migraine in susceptible patients (Lassen et al. 1998).

In conclusion, we have observed bilateral activation of second order neurons after unilateral stimulation of the pain-producing middle meningeal artery in both cat and monkey. In both species the distribution of the activation is broadly similar, involving the superficial laminae of the trigeminal nucleus caudalis at the level of the cervicomedullary junction and extending reproducibly into the superficial laminae of the dorsal horns of C_1 and C_2. The distribution for this arterial structure is exactly similar to that which we have seen for the large venous sinus, the superior sagittal sinus, in cat and monkey. These data form part of the anatomical basis for the throbbing nature of primary neurovascular headaches, such as migraine, suggesting that, broadly speaking, there is a final common anatomical pathway for intracranial vascular structures rather than a primary defect in the vessels. The caudal extension of these cells provides an anatomical explanation for the varied cutaneous distribution of the cranial pain symptomatology of these disorders and reinforces the absolute need to bear the central processing and control of the cranial vessels in mind in any formulation of the pathophysiology of these syndromes.

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REFERENCES

APKARIAN AV, HODGE CJ (1989) Primate spinothalamic pathways: I. A quantitative study of the cells of origin of the spinothalamic pathway. *Journal of Comparative Neurology* **288**, 447–473.

ARBAB MA-R, WIKLUND L, SVENDGAARD NA (1986) Origin and distribution of cerebral vascular innervation from superior cervical, trigeminal and spinal ganglia investigated with retrograde and anterograde WGA-HRP tracing in the rat. *Neuroscience* **19**, 695–708.

ARBAB MA-R, DELGADO T, WIKLUND L, SVENDGAARD NA (1988) Brain stem terminations of the trigeminal and upper spinal ganglia innervation of the cerebrovascular system: WGA-HRP transganglionic study. *Journal of Cerebral Blood Flow and Metabolism* **8**, 54–63.

BERMAN AL (1968) *The Brain Stem of the Cat. A Cytoarchitectonic Atlas with Stereotaxic Co-ordinates*. London, WI: University of Wisconsin Press.

BONAVENTURE P, VOORN P, LUYTEN WHML, LEYSEN JE (1998) $5HT_{1B}$ and $5HT_{1D}$ receptor mRNA differential colocalization with peptide mRNA in the guinea pig trigeminal ganglion. *NeuroReport* **9**, 641–645.

BORGES LF, MOSKOWITZ MA (1983) Do intracranial and extracranial trigeminal afferents represent divergent axon collaterals? *Neuroscience Letters* **35**, 265–270.

BULLITT E (1991) Somatotopy of spinal nociceptive processing. *Journal of Comparative Neurology* **312**, 279–290.

BULLITT E, LEE CL, LIGHT AR, WILLCOCKSON H (1992) The effect of stimulus duration on noxious-stimulus induced c-fos expression in the rodent spinal cord. *Brain Research* **580**, 172–179.

BURTON H, LOEWY AD (1976) Descending projections from the marginal cell layer and other regions of the monkey spinal cord. *Brain Research* **116**, 485–491.

CLARKE WB, BOWSHER D (1962) Terminal distribution of primary afferent trigeminal fibers in the rat. *Experimental Neurology* **6**, 372–383.

DARIAN-SMITH I, PHILLIPS G, RYAN RD (1963*a*) Functional organization in the trigeminal main sensory and rostral spinal nuclei of the cat. *Journal of Physiology* **168**, 129–146.

DARIAN-SMITH I, PROCTOR R, RYAN RD (1963*b*) A single-neurone investigation of somatotopic organisation within the cat's trigeminal brain-stem nuclei. *Journal of Physiology* **168**, 147–157.

DAVIS KD, DOSTROVSKY JO (1986) Activation of trigeminal brainstem nociceptive neurons by dural artery stimulation. *Pain* **25**, 395–401.

DUBBELDAM JL, KARTEN HJ (1978) The trigeminal system in the pigeon (Columba livia). I. Projections of the gasserian ganglion. *Journal of Comparative Neurology* **180**, 661–678.

EDVINSSON L, McCULLOCH J, UDDMAN R (1981) Substance P: immunohistochemical localization and effect upon cat pial arteries in vitro and in situ. *Journal of Physiology* **318**, 251–258.

EDVINSSON L, MACKENZIE ET, McCULLOCH J (1993) *Cerebral Blood Flow and Metabolism*. New York: Raven Press.

FANG HCH (1961) Cerebral arterial innervations in man. *Archives of Neurology* **4**, 6561–656.

FEINDEL W, PENFIELD W, McNAUGHTON F (1960) The tentorial nerves and localisation of intracranial pain in man. *Neurology* **10**, 555–563.

FUKUDA H, KOGA T (1991) The Botzinger complex as the pattern generator for retching and vomiting in the dog. *Neuroscience Research* **12**, 471–485.

GALLAI V, SARCHIELLI P, FLORIDI A, FRANCESCHINI M, CODINI M, TREQUATTRINI A et al. (1995) Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. *Cephalalgia* **15**, 384–390.

GIBSON SJ, POLAK JM, BLOOM SR, WALL PD (1981) The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). *Pain* **201**, 65–79.

GOADSBY PJ (1997) Current concepts of the pathophysiology of migraine. In *Neurologic Clinics of North America*, vol. 15 (ed. Mathew NT), pp. 27–41. Philadelphia: W. B. Saunders.

GOADSBY PJ, EDVINSSON L (1993) The trigeminovascular system and migraine: studies characterising cerebrovascular and neuroptide changes seen in man and cat. *Annals of Neurology* **33**, 48–56.

GOADSBY PJ, EDVINSSON L, EKMAN R (1988) Release of vasoactive peptides in the extracerebral circulation of man and the cat during activation of the trigeminovascular system. *Annals of Neurology* **23**, 193–196.

GOADSBY PJ, EDVINSSON L, EKMAN R (1990) Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Annals of Neurology* **28**, 183–187.

GOADSBY PJ, HOSKIN KL (1997) The distribution of trigeminovascular afferents in the non-human primate brain *Macaca*

nemestrina: a c-fos immunocytochemical study. *Journal of Anatomy* **190**, 367–375.

GOBEL S, FALLS WM, HOCKFIELD S (1977) The division of dorsal and ventral horns of the mammalian caudal medulla into eight layers using anatomical criteria. In *Pain in the Trigeminal Region* (ed. Anderson DJ, Matthews B), pp. 443–453. Amsterdam: Elsevier Biomedical.

GRANT G, ARVIDSSON J (1975) Transganglionic degeneration in trigeminal primary sensory neurons. *Brain Research* **95**, 265–79.

HAMMOND DL, PRESLEY R, GOGAS KR, BAUSBAUM AI (1992) Morphine suppresses Fos protein-like immunoreactivity in the spinal cord and nucleus tractus solitarii evoked by a noxious visceral stimulus in the rat. *Journal of Comparative Neurology* **315**, 244–253.

HOSKIN KL, KAUBE H, GOADSBY PJ (1996*a*) Central activation of the trigeminovascular pathway in the cat is inhibited by dihydroergotamine: a c-*Fos* and electrophysiology study. *Brain* **119**, 249–256.

HOSKIN KL, KAUBE H, GOADSBY PJ (1996*b*) Sumatriptan can inhibit trigeminal afferents by an exclusively neural mechanism. *Brain* **119**, 1419–1428.

HOSKIN KL, GOADSBY PJ (1999) Exposure and isolation of the superior sagittal sinus elicits Fos in the trigeminal nucleus caudalis and dorsal horn of the cervical spinal cord: how long should you wait? *Brain Research*, in press.

HU JW, DOSTROVSKY JO, SESSLE BJ (1981) Functional properties of neurons in the cat trigeminal subnucleus caudalis (medullary dorsal horn). I. Responses to oro-facial noxious and nonnoxious stimuli and projections to thalamus and subnucleus oralis. *Journal of Neurophysiology* **45**, 173–191.

HUNT SP, PINI A, EVAN G (1987) Induction of c-fos like protein in spinal cord neurons following sensory stimulation. *Nature* **328**, 1686–1704.

INGVARDSEN BK, LAURSEN H, OLSEN UB, HANSEN AJ (1997) Possible mechanism of c-fos expression in trigeminal nucleus caudalis following spreading depression. *Pain* **72**, 407–415.

JACQUIN MF, SEMBA K, RHOADES RW, EGGER MD (1982) Trigeminal primary afferents project bilaterally to dorsal horn and ipsilaterally to cerebellum, reticular formation, and cuneate, solitary, supratrigeminal and vagal nuclei. *Brain Research* **246**, 285–291.

KAUBE H, KEAY K, HOSKIN KL, BANDLER R, GOADSBY PJ (1993) Expression of c-fos-like immunoreactivity in the trigeminal nucleus caudalis and high cervical cord following stimulation of the sagittal sinus in the cat. *Brain Research* **629**, 95–102.

KELLER JT, SAUNDERS MC, BEDUK A, JOLLIS JG (1985) Innervation of the posterior fossa dura of the cat. *Brain Research Bulletin* **14**, 97–102.

KELLER JT, MARFURT CF, DIMLICH RV, TIERNEY BE (1989). Sympathetic innervation of the supratentorial dura mater of the rat. *Journal of Comparative Neurology* **290**, 310–321.

KELLER JT, MARFURT CF (1991) Peptidergic and serotoninergic innervation of the rat dura mater. *Journal of Comparative Neurology* **309**, 515–534.

KERBER CW, NEWTON TH (1973) The macro and microvasculature of the dura mater. *Neuroradiology* **6**, 175–179.

KERR FW, KRUGER L, SCHWASSMANN HO, STERN R (1968) Somatotopic organization of mechanoreceptor units in the trigeminal nuclear complex of the macaque. *Journal of Comparative Neurology* **134**, 127–144.

KRUGER L, SAPORTA S, FELDMAN SG (1977) Axonal transport studies of the sensory trigeminal complex. In *Pain in the Trigeminal Region*. (ed. Anderson DJ, Matthews B), pp. 191–200. Amsterdam: Elsevier.

LAMBERT GA, ZAGAMI A, BOGDUK N, LANCE JW (1991) Cervical spinal cord neurons receiving sensory input from the cranial vasculature. *Cephalalgia* **11**, 75–85.

LANCE JW, GOADSBY PJ. (1998) *Mechanism and Management of Headache*, 6th edn. London: Butterworth-Heinemann.

LASSEN LH, JACOBSEN VB, PETERSEN P, SPERLING B, IVERSEN HK, OLESEN J (1998) Human calcitonin gene-related peptide (hCGRP)-induced headache in migraineurs. *European Journal of Pharmacology* **6** (Suppl. 3), S63.

MARFURT CF (1981) The central projections of trigeminal primary afferent neurons in the cat as determined by the transganglionic transport of horseradish peroxidase. *Journal of Comparative Neurology* **203**, 785–98.

MARTINS IP, BAETA E, PAIVA T, CAMPO T, GOMES L (1993) Headaches during intracranial endovascular procedures: a possible model of vascular headache. *Headache* **33**, 227–233.

MAYBERG M, LANGER RS, ZERVAS NT, MOSKOWITZ MA (1981) Perivascular meningeal projections from cat trigeminal ganglia: possible pathway for vascular headaches in man. *Science* **213**, 228–230.

MAYBERG MR, ZERVAS NT, MOSKOWITZ MA (1984) Trigeminal projections to supratentorial pial and dural blood vessels in cats demonstrated by horseradish peroxidase histochemistry. *Journal of Comparative Neurology* **223**, 46–56.

MENETREY D, GANNON A, LEVINE JD, BASBAUM AI (1989) Expression of c-fos protein in interneurons and projection neurons of the rat spinal cord in response to noxious somatic, articular and visceral stimulation. *Journal of Comparative Neurology* **285**, 177–195.

MOSKOWITZ MA, CUTRER FM (1993) Sumatriptan: a receptor-targeted treatment for migraine. *Annual Review of Medicine* **44**, 145–154.

MOSKOWITZ MA, NOZAKI K, KRAIG RP (1993) Neocortical spreading depression provokes the expression of C-fos protein-like immunoreactivity within the trigeminal nucleus caudalis via trigeminovascular mechanisms. *Journal of Neuroscience* **13**, 1167–1177.

NICHOLS, FT, MAWAD M, MOHR JP, HILAL S, STEIN B, MICHELSON J (1990) Focal headache during balloon inflation in the internal carotid and middle cerebral arteries. *Stroke* **21**, 555–559.

NICHOLS FT, MAWAD M, MOHR JP, HILAL S, ADAMS RJ (1993) Focal headache during balloon inflation in the vertebral and basilar arteries. *Headache* **33**, 87–89.

NOMURA S, YASUI Y, TAKADA M, MIZUNO N (1984) Trigeminal primary afferent neurons projecting directly to the solitary nucleus in the cat: a transganglionic and retrograde horseradish peroxidase study. *Neuroscience Letters* **50**, 257–262.

NORD SG (1967) Somatotopic organization in the spinal trigeminal nucleus, the dorsal column nuclei and related structures in the rat. *Journal of Comparative Neurology* **130**, 343–356.

NOZAKI K, BOCCALINI P, MOSKOWITZ MA (1992) Expression of c-fos-like immunoreactivity in brainstem after meningeal irritation by blood in the subarachnoid space. *Neuroscience* **49**, 669–680.

O'CONNOR TP, VAN DER KOOY D (1986) Pattern of intracranial and extracranial projections of trigeminal ganglion cells. *Journal of Neuroscience* **6**, 2200–2207.

O'CONNOR TP, VAN DER KOOY D (1988) Enrichment of a vasoactive neuropeptide (calcitonin gene related peptide) in trigeminal sensory projection to the intracranial arteries. *Journal of Neuroscience* **8**, 2468–2476.

OLSZEWSKI J, BAXTER D. (1954) *Cytoarchitecture of the Human Brainstem*. Philadelphia: J. B. Lippincott.

PENFIELD W, McNAUGHTON FL (1940) Dural headache and the innervation of the dura mater. *Archives of Neurology and Psychiatry* **44**, 43–75.

PFALLER K, ARVIDSSON J (1988) Central distribution of trigeminal and upper cervical primary afferents in the rat studied

by anterograde transport of horseradish peroxidase conjugated to wheat germ agglutinin. *Journal of Comparative Neurology* **268**, 91–108.

RAY BS, WOLFF HG (1940) Experimental studies on headache. Pain sensitive structures of the head and their significance in headache. *Archives of Surgery* **41**, 813–856.

REXED B (1954) A cytoarchitectonic atlas of the spinal cord in the cat. *Journal of Comparative Neurology* **96**, 297–380.

ROLAND J, BERNARD C, BRACARD S, CZORNY A, FLOQUET J, RACE JM et al. (1987) Microvascularization of the intracranial dura mater. *Surgical and Radiological Anatomy* **9**, 43–9.

RUSKELL GL, SIMONS T (1987) Trigeminal nerve pathways to the cerebral arteries in monkeys. *Journal of Anatomy* **155**, 23–37.

SIEGEL S (1956) *Non-parametric Statistics for the Behavioural Sciences*. Kogakusha, Tokyo: McGraw-Hill.

SILBERSTEIN SD, LIPTON RB, GOADSBY PJ. (1998) *Headache in Clinical Practice*. Oxford: ISIS Medical Media.

STEIGER HJ, TEW JM, KELLER JT (1982) The sensory representation of the dura mater in the trigeminal ganglion of the cat. *Neuroscience Letters* **31**, 231–236.

STEIGER HJ, MEAKIN CJ (1984) The meningeal representation in the trigeminal ganglion—an experimental study in the cat. *Headache* **24**, 305–309.

STORER RJ, BUTLER P, HOSKIN KL, GOADSBY PJ (1997) A simple method, using 2-hydroxypropyl-β-cyclodextrin, of administering α-chloralose at room temperature. *Journal of Neuroscience Methods* **77**, 49–53.

STRASSMAN A, MASON P, MOSKOWITZ M, MACIEWICZ R (1986) Response of brainstem trigeminal neurons to electrical stimulation of the dura. *Brain Research* **379**, 242–250.

STRASSMAN AM, MINETA Y, VOS BP (1994) Distribution of fos-like immunoreactivity in the medullary and upper cervical dorsal horn produced by stimulation of dural blood vessels in the rat. *Journal of Neuroscience* **14**, 3725–3735.

TORVIK A (1956) Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures. An experimental study in the rat. *Journal of Comparative Neurology* **106**, 51–141.

UDDMAN R, EDVINSSON L, EKMAN R, KINGMAN T, MCCULLOCH J (1985) Innervation of the feline cerebral vasculature by nerve fibres containing calcitonin gene-related peptide: trigeminal origin and co-existence with substance P. *Neuroscience Letters* **62**, 131–136.

WALL PD, TAUB A (1962) Four aspects of the trigeminal nucleus and a paradox. *Journal of Neurophysiology* **25**, 110–126.

WANAKA A, MATSUYAMA T, YONEDA S, KIMURA K, KAMADA T, GIRGIS S et al. (1986) Origins and distribution of calcitonin gene-related peptide-containing nerves in the wall of the cerebral arteries of the guinea pig with special reference to the coexistence with substance P. *Brain Research* **369**, 185–192.

WESTRUM LE, CANFIELD RC, BLACK RG (1976) Transganglionic degeneration in the spinal trigeminal nucleus following removal of tooth pulps in adult cats. *Brain Research* **101**, 137–140.

WOLFF HG. (1963). *Headache and Other Head Pain*. New York: Oxford University Press.

YAMAMOTO K, MATSUYAMA T, SHIOSAKA S, INAGAKI E, SENBA Y, SHIMIZU I et al. (1983) Overall distribution of substance P containing nerves in the wall of the cerebral arteries of the guinea pig and its origins. *Journal of Comparative Neurology* **215**, 421–426.

ZAGAMI AS, HOSKIN KL, GOADSBY PJ (1997) Middle meningeal artery stimulation evokes c-FOS expression in the trigeminal nucleus caudalis. *Cephalalgia* **17**, 346.