

Fos expression in the midbrain periaqueductal grey after trigeminovascular stimulation

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(Accepted 1 July 2000)

ABSTRACT

There is an accumulating body of evidence suggesting that the periaqueductal grey (PAG) is involved in the pathophysiology of migraine. Positron emission tomography (PET) studies in humans have shown that the caudal ventrolateral midbrain, encompassing the ventrolateral PAG, has activations during migraine attacks. The PAG may well be involved not only through the descending modulation of nociceptive afferent information, but also by its ascending projections to the pain processing centres of the thalamus. In this study the intranuclear oncogene protein Fos was used to mark cell activation in the PAG following stimulation of the trigeminally-innervated superior sagittal sinus (SSS) in both cats and in nonhuman primates (*Macaca nemestrina*). Fos expression in the PAG increased following stimulation to a median of 242 cells (interquartile range 236–272) in the cat and 155 cells (range 104–203) in the monkey, compared with control levels of 35 cells (21–50) and 26 cells (18–33), respectively. Activation was predominantly in the ventrolateral area of the caudal PAG suggesting that the PAG is involved following trigeminally-evoked craniovascular pain.

Key words: Sagittal sinus; craniofacial pain; migraine.

INTRODUCTION

Migraine is an episodic disorder involving headache and other sensory disturbances, such as sensitivity to movement, light and sound (Headache Classification Committee of The International Headache Society, 1988). Based on in vivo animal studies brainstem regions (Goadsby et al. 1991) are good candidates for the basic lesion in migraine with the capacity to influence a diverse range of CNS functions (Lance & Goadsby, 1998). Human functional imaging studies during acute migraine attacks have further pointed to the brainstem with activation in PET studies in areas of the pons and midbrain (Weiller et al. 1995).

Migraine is associated with a range of behavioural manifestations, such as mood changes, food cravings, yawning and polyuria, during the premonitory phase (Drummond & Lance, 1984; Rasmussen & Olesen, 1992), visual disturbances, sensitivity to light, scent and sound, as well as nausea and vomiting mark later

stages of an attack (Lance & Goadsby, 1998). This symptomatology suggests the involvement of centrally placed structures in migraine pathophysiology. The midbrain and particularly the periaqueductal grey (PAG) are uniquely placed within the control, modulating and integrating centre of the central nervous system with this region being directly or indirectly connected with a multitude of sensory loci (Depaulis & Bandler, 1991) that could control the development of the migrainous syndrome during an attack.

The first evidence in humans to indicate a central source for the pain of migraine arose from the work of Raskin (1987), who reported a group of nonmigraine patients developing migraine-like headache after implantation of a stimulating electrode into the PAG. These observations were confirmed in a subsequent report of a larger number of patients (Veloso et al. 1998). Isolated clinical findings, such as a plaque of multiple sclerosis in the PAG producing migraine-like

headache (Hass et al. 1993) have added weight to the general view that brainstem structures have a pivotal role in migraine. Weiller et al. (1995) then demonstrated contralateral rostral brainstem activation, measured using PET as increased blood flow marked by the tracer $H_2^{15}O$, in patients during a migraine attack. In all patients the pain was right sided and neuronal activity was increased on the contralateral side in a region encompassing ventrolateral PAG and dorsal raphe nucleus. Activation in this region was found both during and following successful treatment of the head pain with sumatriptan. This ongoing activation after the cessation of headache raises the question as to the role of the PAG in the attack and may start to provide a biological basis for the certain phenomena of migraine headache, including recurrence after acute attack treatment (Ferrari, 1998).

Previous studies have shown that stimulation of the intracranial, extracerebral, trigeminally innervated superior sagittal sinus increases Fos expression in the caudal medulla and upper cervical spinal cord both in the cat (Hoskin et al. 1996*a, b*) and the monkey (Goadsby & Hoskin, 1997). Given that mechanical or electrical stimulation of the superior sagittal sinus (SSS) in humans is pain-producing (Ray & Wolff, 1940) we used our experimental design of SSS stimulation to examine the midbrain and pons for neuronal Fos expression.

MATERIALS AND METHODS

Anaesthesia and surgery

Cats ($n = 5$) weighing 2.5–3.5 kg and monkeys ($n = 6$) weighing 8–14 kg were used in this study. Animals were fasted prior to experimentation. Monkeys were transported to the laboratory under ketamine (10 mg/kg intramuscular injection) anaesthesia. An initial dose of α -chloralose (Calbiochem or Sigma) 60 mg/kg was administered by intraperitoneal injection. During surgical procedures halothane (0.5–2%) was administered. The femoral artery and vein were cannulated for continuous monitoring of the blood pressure and heart rate and for administration of anaesthetic, pharmaceuticals and fluid, respectively. Cardiovascular parameters and pupillary reaction to noxious pinching of the forepaw were used to determine the need for supplementary anaesthetic. The animals were intubated endotracheally, ventilated with 40% oxygen while body temperature and expired CO_2 were monitored continuously. Animals were mounted into a stereotaxic frame and a midline craniotomy (1.5–2 cm) was performed to expose the SSS. On

Table 1. Mean data for some physiological values taken from all cats and monkeys included in these studies

	Weight (kg)	pH	pCO ₂ (mmHg)	pO ₂ (mmHg)
Cat ($n = 5$)	3.4 ± 1.0	7.40 ± 0.03	29 ± 4	274 ± 49
Monkey ($n = 6$)	8.6 ± 1.2	7.47 ± 0.04	32 ± 3	226 ± 24

completion of surgery halothane was discontinued and blood gases were analysed (Table 1), after which animals were monitored for 24 h prior to stimulation. This rest period provides an excellent signal-to-noise ratio for Fos studies (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 with intravenous α -chloralose dissolved either in water, which requires heating, or in β -cyclodextrin (Storer et al. 1997), and given at 2 hourly intervals (10–20 mg/kg). Blood pressure and heart rate were stable and within physiological range for all animals throughout the whole experiment.

To activate the trigeminal primary afferents the SSS was suspended from bipolar hook electrodes and stimulated, for a period of 2 h in the cat and 1 h in the monkey (0.3 Hz, 150 V, 250 μ s duration). The stimulation applied was sufficient to activate trigeminally derived nociceptive neurons and elicit Fos protein in the trigeminal nucleus caudalis and upper cervical spinal cord (Kaube et al. 1993; Goadsby & Hoskin, 1997). Controls were performed by suspending the vessel over the hook electrodes with no current applied.

Perfusion

One hour after stimulation 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 μ m) of the mid-brain were cut on a freezing microtome or cryostat and saved for processing.

Immunohistochemical detection of Fos

The Fos protein was detected using a locally produced research antibody *fos-x* (Gerrard Evan, Imperial

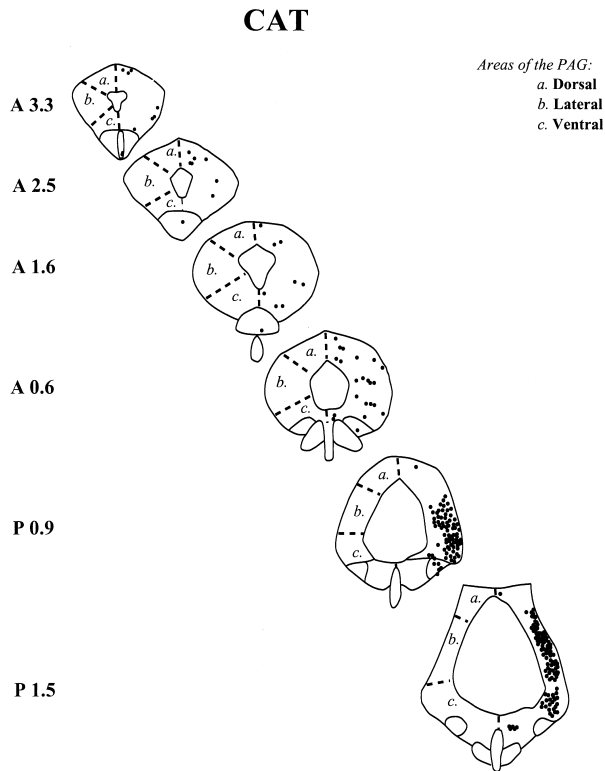


Fig. 1. Representative sections from the midbrain periaqueductal grey, levels P1.5, P0.9, A0.6, A1.6, A2.5 and A3.3 anterior (A) and posterior (P) to the interaural line of the cat after electrical stimulation of the superior sagittal sinus. Data for Fos-positive cells (●) are plotted onto one side since the SSS is a unilateral structure and is innervated equally by trigeminal ganglia from both sides. There is a robust increase in Fos expression in the ventrolateral division of the caudal PAG after superior sagittal sinus stimulation.

Cancer Research, London); free-floating sections were incubated at 4 °C for 3–7 d in a 1:1000 dilution. Fos-like immunoreactivity was visualised using standard avidin-biotin peroxidase immunohistochemical techniques, and staining was enhanced with nickel (0.5–2%). The sections were mounted onto gelatinised slides and cover-slipped. Sections were subsequently examined under a light microscope ($\times 20$ objective). The DAB reaction product is seen as a black precipitate due to the presence of the 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); the cell nucleus must be distinguishable from the background throughout a range of $\times 20$, 10 and 5 magnification. Cells were plotted onto schematic sections of the midbrain modified from the atlas of Berman (1968) for the cat, and were drawn by hand for *Macaca nemestrina*, using Adobe Illustrator (v. 4.0).

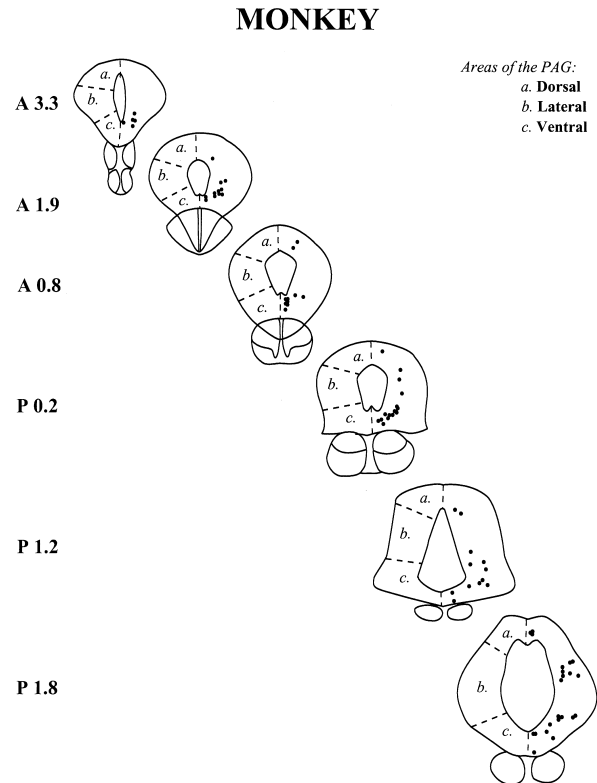


Fig. 2. Representative sections from the midbrain periaqueductal grey, levels P1.8, P1.2, P0.2, A0.8, A1.9 and A3.3 anterior (A) and posterior (P) to the interaural line of *Macaca nemestrina*, after electrical stimulation of the superior sagittal sinus. Data for Fos-positive cells (●) are plotted onto one side since the SSS is a unilateral structure and is innervated equally by the trigeminal ganglia on both sides. Fos expression increases in the ventrolateral caudal PAG following superior sagittal sinus stimulation in a distribution similar to that seen in the cat (Fig. 1).

Plotting and statistics

The cells were plotted onto the schematic transverse sections of the midbrain (Figs 1, 2). A map of cell activation in this region was compiled and the distribution of Fos positive cells was determined semiquantitatively by summing the number of cells from 5 sections and plotting this over 6 levels of the brainstem (Kaube et al. 1993). The data are reported as a median with interquartile (25%, 75%) ranges for 30 sections, 5 sections per level for each of 6 levels, because of the inherently noninterval nature of the Fos methodology (Siegel, 1956).

RESULTS

Animals reported in this study maintained good physiological parameters, blood pressure and respiratory function, during the period of the study (Table 1). Fos expression in the brainstem due to stimulation of the superior sagittal sinus was only observed in significant quantities in the periaqueductal grey. Fos expression was not observed in the locus

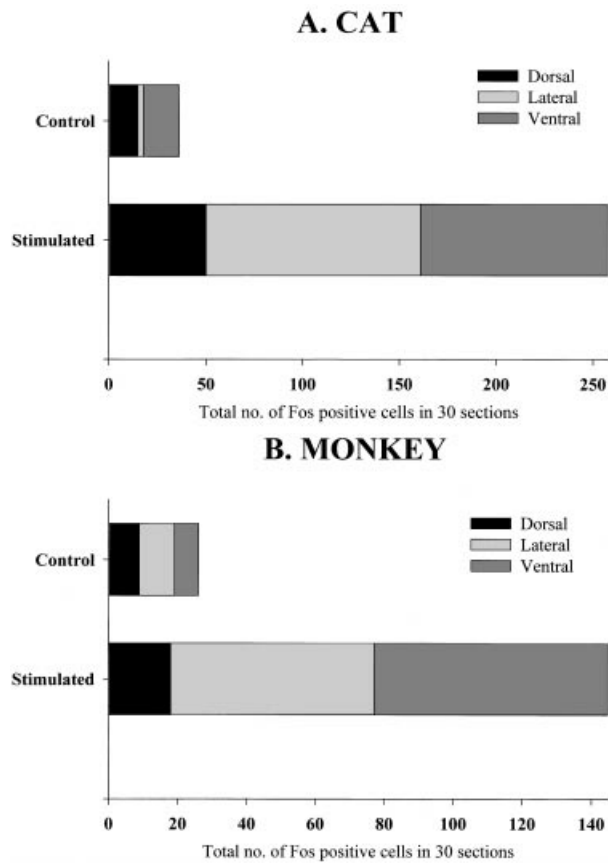


Fig. 3. Bar graphs summarising the population effect of stimulation of the superior sagittal sinus of Fos expression in the midbrain periaqueductal grey consisting of the dorsal, lateral and ventral area in the cat (A) and monkey (B).

coeruleus, nucleus raphe magnus, parabrachial area or to any significant degree in the dorsal raphe nucleus, to mention areas of potential interest for headache.

For expression was marked in all regions of the PAG in both cat and monkey compared with the control unstimulated animals. Within the PAG the ventrolateral area showed the greatest increase in Fos expression. Fos expression in the PAG in both the cat and the monkey was mostly present at the most caudal compared with the rostral level.

Fos expression in cat

In the cat, the dorsal area had a median of 15 cells (range 9–22) in control animals which increased to 50 cells (range 43–51) in stimulated animals, the lateral area 3 cells (range 2–3) in control animals increased to 111 cells (range 110–124) following stimulation and in the ventral area 18 cells (range 10–25) increased to 97 (range 80–106) following 2 h of sinus stimulation.

In the sinus stimulated cats 113 cells (range 110–119) were found at the level posterior to

Table 2. Total number of Fos positive cells in the dorsal, lateral and ventral regions of the periaqueductal grey following electrical stimulation of the superior sagittal sinus in cat (A) and monkey (B)*

	Control	Stimulated
A. Cat	(n = 2)	(n = 3)
Dorsal	15 (9–22)	50 (43–51)
Lateral	3 (2–3)	111 (110–124)
Ventral	18 (10–25)	97 (80–106)
B. Monkey	(n = 2)	(n = 4)
Dorsal	9 (6–11)	18 (11–27)
Lateral	10 (7–14)	59 (41–69)
Ventral	7 (6–7)	68 (48–98)

* Cells were summated from 30 sections of the periaqueductal grey, 5 sections taken from each of the 6 midbrain levels (Figs 1, 2). Data are presented as a median with the quartile ranges.

Table 3. Total number of Fos positive cells in the rostro-caudal dimensions of the periaqueductal grey following electrical stimulation of the superior sagittal sinus in cat (A) and monkey (B)*

Level compared with interaural line	Control	Stimulated
A. Cat	(n = 2)	(n = 3)
P1.5	6 (4–7)	113 (110–119)
P0.9	3 (2–5)	69 (44–78)
A0.6	6 (4–7)	42 (32–43)
A1.6	6 (3–8)	22 (16–22)
A2.5	10 (5–14)	13 (11–15)
A3.3	6 (4–9)	21 (15–24)
B. Monkey	(n = 2)	(n = 4)
P1.8	9 (7–10)	26 (21–31)
P1.2	4 (3–6)	17 (14–28)]
A0.2	3 (2–4)	32 (26–36)
A0.8	3 (2–5)	27 (20–32)
A1.9	5 (3–6)	25 (17–34)
A3.3	2 (2–3)	7 (5–18)

* Data are presented as a median with the quartile ranges as in Table 2.

stereotaxic zero-P1.5 (in 5 sections) compared with a total of 242 cells (range 236–272) in 30 sections over the length of the PAG. At the more rostral levels the number of cells present in 5 sections started to decline at P0.9 69 cells (range 44–78), at A0.6 42 cells (range 32–43), at A1.6 22 (16–22), at A2.5 13 (11–15), and finally, at A3.3 21 cells (range 15–24; Fig. 3A, Table 3).

Fos expression in monkey

Increases were observed in the monkey from 9 cells (range 6–11) to 18 cells (range 11–27) in the dorsal area, from 10 cells (range 7–14) to 59 cells (range

41–69) in the lateral area and from 7 cells (range 6–7) to 68 cells (range 48–98) in the ventral area following 1 h stimulation of the superior sagittal sinus (Table 2). The caudal accentuation was not as obvious in the monkey study which had a reduced period of stimulation compared with the cat (Fig. 3B).

DISCUSSION

This study demonstrates robust Fos expression in the midbrain periaqueductal grey matter following stimulation of the superior sagittal sinus in both cat and monkey. Specifically, within the PAG Fos expression was greatest in the ventrolateral area of the most caudal PAG. This pattern of activation is consistent with PET observations in migraine and regions in which direct electrode placement in humans has provoked migraine-like headaches and suggests a pivotal role for the PAG in migraine. Moreover, the PAG has been functionally subdivided into a series of longitudinal neuronal columns extending along its rostrocaudal axis (Depaulis & Bandler, 1991) and the 2 most extensively studied columns include the areas in which Fos expression occurred in this study, the ventrolateral and the lateral columns. The ventrolateral PAG is reported to be involved in processing deep noxious pain and stimulation of this region produces quiescence, bradycardia and hypotension, which has some similarities to symptoms seen in migraine. This series of responses to deep pain is thought to facilitate recovery following injury, whereas the more dorsally located lateral PAG processes cutaneous, superficial pain and stimulation of this region produces defensive behaviour, tachycardia and hypertension, the so called ‘flight-fight’ response (Bandler & Keay, 1996).

Ascending nociceptive pathway

Some of the Fos-positive cells observed are probably activated directly by second order neurons projecting from the spinal cord and caudal medulla. In the rat retro and anterograde tracing studies show that the upper cervical spinal cord cells project to the lateral and ventrolateral regions of the PAG (Menetrey et al. 1982; Yeziarski, 1991). Neurons in lamina I of the dorsal horn of the cervical spinal cord relay nociceptive, thermoreceptive and visceroreceptive information to the PAG via a contralateral projection travelling in the spinomesencephalic tract (Blomqvist & Craig, 1991). Another projection is reported from the nucleus of the solitary tract and its caudal extension lamina X of the cervical spinal cord (Bandler

& Shipley, 1994). Both regions have been found to contain significant Fos expression due to nociceptive stimulation of the trigeminal system (Kaube et al. 1993; Strassman et al. 1993). Cells projecting from spinal cord to the lateral PAG were mostly found in the superficial laminae and in the lateral cervical nucleus while those projecting to the ventrolateral PAG were found in the ventral horn, perhaps reflecting the behaviour-integrating role of the PAG. Cells in laminae IV and V of the spinal cord sent afferents to both regions of the PAG but few cells were found to project to both regions (Keay & Bandler, 1992).

Similar studies looking at Fos expression in the periaqueductal grey following noxious stimulation determined that deep and cutaneous pain causes Fos expression in different regions of the PAG. Painful cutaneous stimulation results in Fos expression in the lateral PAG while that due to deep, visceral pain produces Fos in the ventrolateral PAG (Keay & Bandler, 1993), consistent with the previously mentioned tracing studies.

Descending pain control pathway

It is also possible that these Fos-positive cells in the PAG are involved in modulating pain at the level of the spinal cord as a reflex response to incoming nociception. Cell bodies within the PAG are known to give rise to descending inhibitory pathways that act on the primary afferents within the dorsal horn of the spinal cord. Microinjection of excitatory amino acids (EAAs), such as glutamate, into the PAG inhibit responses in dorsal horn neurons (Jones & Gebhart, 1988; Sandkuhler et al. 1988, 1991). In the rat the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin, injected into the PAG showed terminal labelling bilaterally in the principal sensory trigeminal nucleus and the oral, interpolar and caudal subnuclei of the spinal trigeminal nucleus. This projection was predominantly ipsilateral (Li et al. 1993). In rat hot plate tests, electrical stimulation of the PAG is more effective at blocking ipsilateral nociception than contralateral nociception (Levine et al. 1991) which is in agreement with much earlier findings by Basbaum et al. (1977). This, unfortunately, was not something we could study in this experimental design. On a larger scale, behavioural analgesia can be induced in conscious laboratory animals by electrically stimulating the PAG (Reynolds, 1969; Mayer et al. 1971; Mayer & Liebeskind, 1974; Marek et al. 1991).

In man, electrical stimulation of the ventrolateral PAG was found to produce the most effective

analgesia for the treatment of chronic pain (Baskin et al. 1986). In relation to migraine, it was reported by Raskin (1987) that placement and the subsequent use of the stimulating electrode in the ventrolateral PAG, to treat chronic pain caused, in a few isolated instances, the development of migraine-like headaches in some patients, although these patients were previously unaffected by the condition suggesting that disrupting the activity within the brain can produce migraine. A larger study of Veloso et al. (1998) has confirmed these observations. This suggests that perturbation of the PAG may either by activation, or more likely in our view by dysfunction and thus disinhibition, produce headache.

Overall, even though the proto-oncogene Fos has been widely used as a marker of nociceptive activation this is not the only type of cell perturbation that can cause Fos expression within the central nervous system. In this study the nature of the nuclei producing Fos have not been classified to any degree. Therefore, it is difficult to state precisely the function of the cells eliciting Fos due to sinus stimulation. They may either be relaying nociceptive information to higher centres, acting to modulate the incoming primary afferents at the level of the trigeminal nucleus caudalis and upper cervical spinal cord or producing a secondary response to the given stimuli.

ACKNOWLEDGEMENTS

The authors thank Paul Hammond for excellent technical assistance and Yolande Knight and R. James Storer for assistance with the conduct of the studies. The work has been supported by the Wellcome Trust and the Migraine Trust. PJG is a Wellcome Senior Research Fellow. These results were presented in part to the Xth International Headache Congress, Barcelona, Spain, 24–26 June, 1999 (Hoskin et al. 1999).

REFERENCES

BANDLER R, KEAY KA (1996) Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. *Progress in Brain Research* **107**, 285–300.

BANDLER R, SHIPLEY MT (1994) Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? *Trends in Neuroscience* **17**, 379–389.

BASBAUM AI, MARLEY NJ, J OK, CLANTON CH (1977) Reversal of morphine and stimulus-produced analgesia by subtotal spinal cord lesions. *Pain* **3**, 43–56.

BASKIN DS, MEHLER WR, HOSOBUCHI Y, RICHARDSON DE, ADAMS JE, FLITTER MA (1986) Autopsy analysis of the safety, efficacy and cartography of electrical stimulation of the central gray in humans. *Brain Research* **371**, 231–236.

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

BLOMQUIST A, CRAIG AD (1991) Organization of spinal and trigeminal input to the PAG. In *The Midbrain Periaqueductal*

Gray Matter: Functional, Anatomical and Neurochemical Organisation. (ed. Depaulis A, Bandler R), pp. 345–363. New York and London: Plenum Press.

DEPAULIS A, BANDLER R (eds.) (1991) *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organisation*. New York: Plenum Press.

DRUMMOND PD, LANCE JW (1984) Neurovascular disturbances in headache patients. *Clinical and Experimental Neurology* **20**, 93–99.

FERRARI MD (1998) Migraine. *Lancet* **351**, 1043–1051.

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.

GOADSBY PJ, ZAGAMI AS, LAMBERT GA (1991) Neural processing of craniovascular pain: a synthesis of the central structures involved in migraine. *Headache* **31**, 365–371.

HAAS DC, KENT PF, FRIEDMAN DI (1993) Headache caused by a single lesion of multiple sclerosis in the periaqueductal gray area. *Headache* **33**, 452–455.

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.

HEADACHE CLASSIFICATION COMMITTEE OF THE INTERNATIONAL HEADACHE SOCIETY (1988) Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalalgia* **8** (Suppl. 7), 1–96.

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

HOSKIN KL, BULMER DCE, JONKMAN A, GOADSBY PJ (1999) The role of the periaqueductal gray (PAG) in the pathogenesis of migraine. *Cephalalgia* **19**, 314.

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* **824**, 133–135.

JONES SL, GEBHART GF (1988) Inhibition of spinal nociceptive transmission from the midbrain, pons and medulla in the rat: activation of descending inhibition by morphine, glutamate and electrical stimulation. *Brain Research* **460**, 281–296.

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.

KEAY KA, BANDLER R (1992) Anatomical evidence for segregated input from the upper cervical spinal cord to functionally distinct regions of the midbrain periaqueductal gray of the cat. *Neuroscience Letters* **139**, 143–148.

KEAY KA, BANDLER R (1993) Deep and superficial noxious stimulation increases Fos-like immunoreactivity in different regions of the midbrain periaqueductal gray of the rat. *Neuroscience Letters* **154**, 23–26.

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

LEVINE R, MORGAN MM, CANNON JT, LIEBESKIND JC (1991) Stimulation of the periaqueductal gray matter of the rat produces preferential ipsilateral antinociception. *Brain Research* **567**, 140–144.

LI YQ, TAKADA M, SHINONAGA Y, MIZUNO N (1993) Direct projections from the midbrain periaqueductal gray and

- dorsal raphe nucleus to the trigeminal sensory complex in the rat. *Neuroscience* **54**, 431–443.
- MAREK P, YIRMIYA R, LIEBESKIND JC (1991) Stimulation-produced analgesia in the mouse: evidence for laterality of opioid mediation. *Brain Research* **541**, 154–156.
- MAYER DJ, LIEBESKIND JC (1974) Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioral analysis. *Brain Research* **68**, 73–93.
- MAYER DJ, WOLFLE TL, AKIK H, CARDER B, LIEBESKIND J (1971) Analgesia from electrical stimulation in the brain stem of the rat. *Science* **174**, 1351–1354.
- MENETREY D, CHAOUCH A, BINDER J, BESSON JM (1982) The origin of the spinomesencephalic tract of the rat: an anatomical study using the retrograde transport of horseradish peroxidase. *Journal of Comparative Neurology* **206**, 193–207.
- RASKIN NH, HOSOBUCHI Y, LAMB S (1987) Headache may arise from perturbation of brain. *Headache* **27**, 416–420.
- RASMUSSEN BK, OLESEN J (1992) Migraine with aura and migraine without aura: an epidemiological study. *Cephalalgia* **12**, 221–228.
- 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.
- REYNOLDS DV (1969) Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* **164**, 444–445.
- SANDKUHLE J, HELMCHEN C, FU QG, ZIMMERMANN M (1988) Inhibition of spinal nociceptive neurons by excitation of cell bodies or fibers of passage at various brainstem sites in the cat. *Neuroscience Letters* **93**, 67–72.
- SANDKUHLE J, WILLMANN E, FU QG (1991) Characteristics of midbrain control of spinal nociceptive neurons and nonsomatosensory parameters in the pentobarbital-anesthetized rat. *Journal of Neurophysiology* **65**, 33–48.
- SIEGEL S (1956) *Non-parametric Statistics for the Behavioural Sciences*. Kogakusha, Tokyo: McGraw-Hill.
- STORER RJ, BUTLER P, HOSKIN KL, GOADSBY PJ (1997) A simple method, using 2-hydroxypropyl- β -cyclodextrin, of administering a-chloralose at room temperature. *Journal of Neuroscience Methods* **77**, 49–53.
- STRASSMAN AM, VOS BP, MINETA Y, NADERI S, BORSOOK D, BURSTEIN R (1993) Fos-like immunoreactivity in the superficial medullary dorsal horn induced by noxious and innocuous thermal stimulation of the facial skin in the rat. *Journal of Neurophysiology* **70**, 1811–1812.
- VELOSO F, KUMAR K, TOTH C (1998) Headache secondary to deep brain implantation. *Headache* **38**, 507–515.
- WEILLER C, MAY A, LIMMROTH V, JUPTNER M, KAUBE H, SCHAYCK RV et al. (1995) Brain stem activation in spontaneous human migraine attacks. *Nature Medicine* **1**, 658–660.
- YEZIERSKI RP (1991) Somatosensory input to the periaqueductal gray: a spinal relay to a descending control center. In *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organisation* (ed. Depaulis A, Bandler R), pp. 365–386. New York: Plenum Press.