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The periaqueductal gray in the cat projects to lamina VIII and the medial part of lamina VII throughout the length of the spinal cord

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Abstract The periaqueductal gray (PAG) plays an important role in analgesia as well as in motor activities, such as vocalization, cardiovascular changes, and movements of the neck, back, and hind limbs. Although the anatomical pathways for vocalization and cardiovascular control are rather well understood, this is not the case for the pathways controlling the neck, back, and hind limb movements. This led us to study the direct projections from the PAG to the spinal cord in the cat. In a retrograde tracing study horseradish peroxidase (HRP) was injected into different spinal levels, which resulted in large HRP-labeled neurons in the lateral and ventrolateral PAG and the adjacent mesencephalic tegmentum. Even after an injection in the S2 spinal segment a few of these large neurons were found in the PAG. Wheat germ agglutinin-conjugated HRP injections in the ventrolateral and lateral PAG resulted in anterogradely labeled fibers descending through the ventromedial, ventral, and lateral funiculi. These fibers terminated in lamina VIII and the medial part of lamina VII of the caudal cervical, thoracic, lumbar, and sacral spinal cord. Interneurons in these laminae have been demonstrated to project to axial and proximal muscle motoneurons. The strongest PAG-spinal projections were to the upper cervical cord, where the fibers terminated in the lateral parts of the intermediate zone (laminae V, VII, and the dorsal part of lamina VIII). These laminae contain the premotor interneurons of the neck muscles. This distribution pattern suggests that the PAG-spinal pathway is involved in the control of neck and back movements. Comparing the location of the PAG-spinal neurons with the results of stimulation experiments leads to the supposition that the PAG-spinal neurons play a role in the control of the axial musculature during threat display.

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Introduction

The mesencephalic periaqueductal gray (PAG) can be considered as a part of the limbic system (Holstege 1990). The PAG is best known for its relation to nociception control (Mayer et al. 1971; Liebeskind et al. 1973; Fardin et al. 1984; Oliveras and Besson 1988; Levine et al. 1991), but physiological experiments in rat and cat have shown that stimulation in the PAG also produces motor activities, such as vocalization (Kanai and Wang 1962; Jürgens and Pratt 1979; Larson 1985; Bandler et al. 1991; Jürgens and Chang-Lin 1993), cardiovascular changes (Lindgren 1955; Abrahams et al. 1960; Lovick 1985a; Bandler et al. 1991; Carrive and Bandler 1991) and movements of neck, back, and limbs (Liebeskind et al. 1973; Fardin et al. 1984; Bandler and Carrive 1988; Bandler et al. 1991).

The question arises, through which pathways does the PAG control these motor output systems? The most simple explanation would be that it projects directly to the motoneurons innervating the muscles involved. Another possibility is that the PAG projects to premotor interneurons in the brainstem or in the spinal cord. It has been demonstrated that the PAG does not influence motoneurons directly, but indirectly via interneurons in caudal brainstem and cervical cord. With respect to nociception control the PAG projects to the rostral ventromedial medulla, specifically the midline nucleus raphe magnus and adjacent reticular formation, which maintains direct connections with laminae I and V of the spinal cord (Abols and Basbaum 1981; Basbaum and Fields 1984; Mason et al. 1985; Holstege 1988a). In respect to vocalization the PAG uses the nucleus retroambiguus as a relay to motoneurons of for example the larynx, pharynx and abdominal muscles (Holstege 1989; Zhang et al. 1992). Considering the influence of the PAG on cardiovascular control it appears that neu-

rons in the rostral ventrolateral medulla play the role of relay between the PAG and the sympathetic motoneurons in the intermediolateral cell column of the spinal cord (Lovick 1985b; Carrive et al. 1989; Lovick 1991).

Regarding the neck movements elicited by stimulation in the PAG, the exact pathways are not yet understood. Retrograde tracing studies in the cat and monkey have shown that the PAG projects to the cervical and upper thoracic spinal cord (Castiglioni et al. 1978; Huerta and Harting 1982; Mantyh 1983; Holstege 1988b). Anterograde autoradiographical tracing studies in the opossum and in the cat have shown that these PAG-spinal neurons terminate on interneurons in laminae V, VII, and the dorsal part of lamina VIII in the upper cervical cord and in laminae VII and VIII in the more caudal cervical and upper thoracic cord (Martin et al. 1979; Holstege 1988a). It is suggested that these interneurons are the premotor interneurons of the neck muscle motoneurons which are responsible for neck movements after PAG stimulation (Holstege 1988a). Direct projections from the PAG to neck muscle motoneurons have never been demonstrated.

Stimulation in the PAG can elicit movements of the back and hind limbs also. The motoneurons involved in these movements are located in the cervical, thoracic, lumbar, and sacral segments of the spinal cord. The question arises whether the PAG projects to these motoneurons and/or to their premotor interneurons. Retrograde studies in opossum and monkey revealed a few labeled PAG neurons projecting to the caudal thoracic and the lumbar spinal cord (Castiglioni et al. 1978; Martin et al. 1979), but their termination pattern is still unknown. In the cat only a very light direct PAG projection to the upper lumbar levels has been reported (Holstege 1988b), but projections to more caudal spinal segments have never been demonstrated. The present retrograde and anterograde tracing study in the cat tries to precisely determine the location of the PAG-spinal neurons and their distribution pattern.

Materials and methods

A total of 12 adult male cats were used, and the surgery procedures, pre- and postoperative care, handling and housing of the animals followed protocols approved by the Faculty of Medicine of the University of Groningen. For surgery, animals were initially anesthetized with intramuscular ketamine (Nimatek, 0.1 ml/kg) and xylazine (Sedamun, 0.1 ml/kg), after which they were kept anesthetized by ventilation with a mixture of O_2, N_2O, and halothane. During surgery electrocardiographic activity (ECG) and body temperature were monitored. Following a survival time of 3 days the animals were initially anesthetized with Ketamin (0.1 ml/kg) and xylazine (0.1 ml/kg) i.m., followed by 6 ml 6% pentobarbital sodium i.p. The cats were perfused transcardially with 2 l of 0.9% saline at 37° C, directly followed by 2 l of 0.1 M phosphate buffer, containing 4% sucrose, 1% paraformaldehyde, and 2% glutaraldehyde.

Retrograde tracing study

In nine anesthetized cats after laminectomy approximately 80 µl 10% horseradish peroxidase (HRP) in saline was injected into various levels of the spinal cord using a Hamilton microsyringe. In some cases HRP was injected unilaterally (left side), in others bilaterally. Since HRP is transported from both terminals and damaged axons, multiple needle penetrations were made in the spinal gray and white matter. In all cases, except for the upper cervical ones, prior to the injection a right-sided hemisection was made some segments rostral to the injection site. This was done in order to verify that retrogradely labeled neurons in the mesencephalon distributed their axons only through the funiculi of the left half of the spinal cord.

After perfusion the brains and spinal cords were removed, postfixed for 2 h and stored overnight in 20% sucrose in phosphate buffer at 4° C. Subsequently the brainstem was cut in 40-µm frozen sections, of which every fourth section was incubated according to the tetramethylbenzidine method, dehydrated, and coverslipped. The spinal segments with the injection sites were cut in 40-µm sections and every fourth section was processed with diaminobenzidine (DAB), to be able to determine the exact area of injection. The precise extent of the hemisections was also determined. Sections were studied with a Zeiss dark-field stereomicroscope and a Zeiss Axioskop light microscope. In each case labeled neurons were plotted in drawings of different levels of the mesencephalon with the aid of a computer.

Anterograde tracing study

In order to determine where the PAG-spinal fibers terminate, in three cats injections of 20 nl 5% wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) in saline was injected in the left PAG. These injections were placed stereotaxically in the ventrolateral and lateral PAG, which according to the retrograde study contained the PAG-spinal neurons.

The WGA-HRP was injected through glass micropipettes using a pneumatic picopump (World Precision Instruments PV830). The PAG was approached dorsally in case 2239 and dorsolaterally in cases 2248 and 2250. The latter approach was chosen to prevent neurons in the superior colliculus being involved in the injection site. All surgical and histological procedures were similar to the retrograde study and every fourth section of the caudal brainstem and of 10–20 segments throughout the length of the spinal cord were studied. The mesencephalon was cut in 40-µm sections and every fourth section was processed with DAB to determine the exact area of injection. Spinal cord sections were studied with a Zeiss Axioplan microscope using dark-field polarized illumination. The labeled fibers and neurons were plotted using a drawing tube, projecting directly on a digitizer which was connected to a computer.

Results

Retrograde tracing study

Location of injection sites and hemisections

Table 1 gives a schematic representation of the injections and hemisections. The injections involved at least the entire left half of the spinal cord, except for cases 2199 and 2173, in which part of the ventromedial and dorsomedial funiculi were not involved in the injection site. Hemisections involved the right side of the spinal cord and sometimes extended into the left dorsal funiculus. Only in case 2152 did the hemisection extend into the left ventromedial funiculus.

Table 1 Schematic drawings of the location of the injection sites and hemisections at different spinal cord levels of the cat

case	injection	hemisection
2199	C2	---
2173	C3	---
2214	T2	C5
2188	T4	T1
2221	T12	T10
2192	L6	L3
2141	S1	L4
2152	S2	L5
2139	S3	L5

Labeled neurons in the PAG

In all nine cases densely labeled neurons were found ipsilaterally in the ventrolateral and lateral PAG and, except for the sacral cord-injected cases, in the laterally adjacent mesencephalic tegmentum (Fig. 1). In the cases with an injection in the cervical and upper thoracic spinal cord a few labeled neurons were found at the border of the dorsal PAG as well. Relatively few labeled neurons were found on the right (contralateral) side of the PAG, indicating that the PAG-spinal projection is predominantly ipsilateral. After cervical and thoracic HRP injections, the labeled neurons in the ventrolateral PAG were located in a rostrocaudally oriented column, extending caudally from the level of the caudal pole of the decussation of the brachium conjunctivum to rostrally the level of the caudal pole of the oculomotor nucleus. The highest number of labeled neurons per section was found just rostral to the level of the trochlear nucleus. In the cases of the lumbar and sacral injections the labeled neurons in the PAG were found exclusively around the level of the trochlear nucleus.

Per 40-μm section the number of labeled neurons in the left ventrolateral and lateral PAG and adjacent tegmentum varied between 20 neurons, after an HRP injection in the upper cervical cord, and 1 single neuron, after an injection in the sacral cord. As mentioned in Materials and methods, one out of four 40-μm sections was incubated. In these sections the number of labeled neurons in the PAG and the laterally adjacent tegmentum was counted (Table 2). Ipsilaterally, the total number of labeled neurons varied from over 500 neurons, after an injection in C2, to 1 neuron, after an injection in S3. In the cervical and upper thoracic cases about one third of these labeled neurons were located in the laterally adjacent tegmentum. In the lower thoracic cases the fraction of labeled neurons in the tegmentum was much smaller. In the lumbosacral cases almost no labeled neurons were present in the adjacent tegmental field. Contralaterally, the total number of labeled neurons was relatively small. Only in the C2-injected case were more than 100 labeled neurons observed, of which almost 90% were located within the borders of the PAG. In none of the cases with an injection caudal to C3 were labeled neurons observed in the contralateral adjacent tegmentum. In the lumbosacral cases no labeled neurons were found in the contralateral PAG or in the adjacent tegmentum.

The diameter of the ventrolateral PAG-spinal neurons was relatively large and differed from 15 to 40 μm,

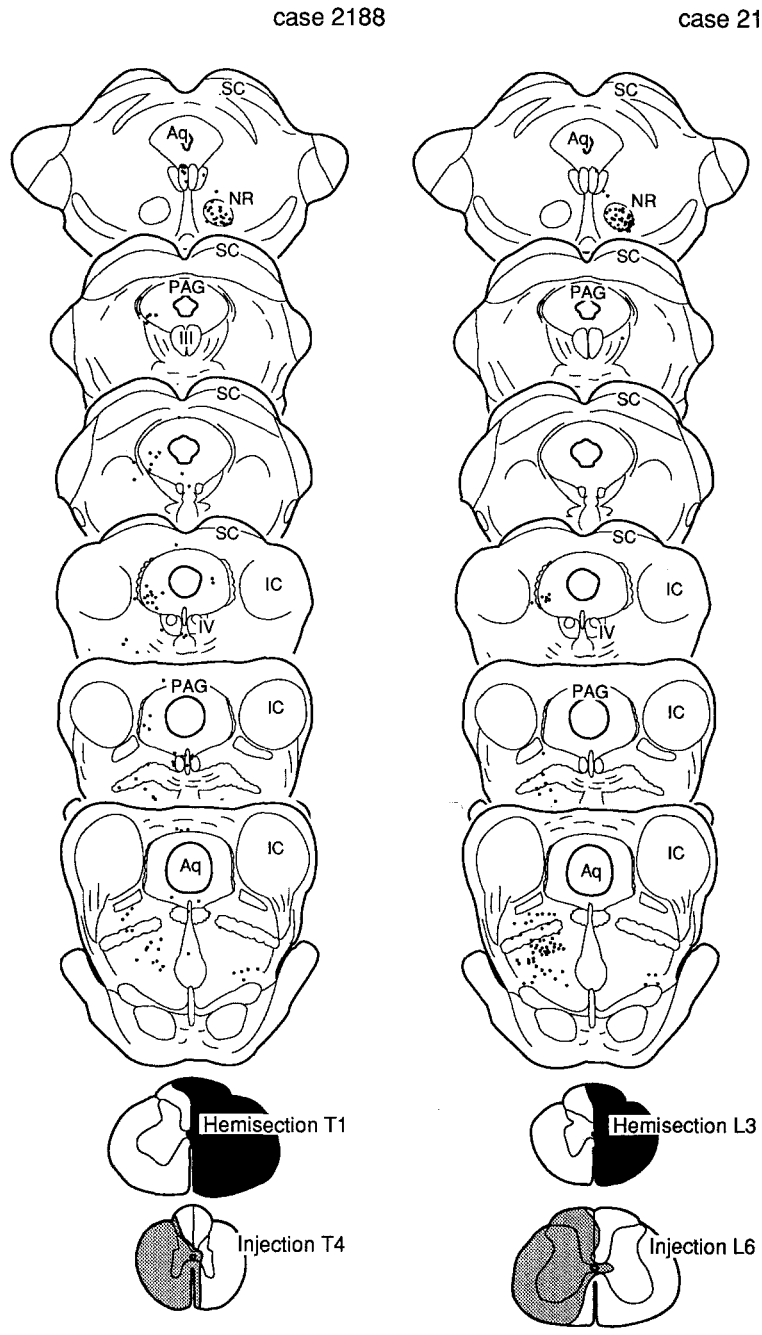


Fig. 1 Schematic drawings of various levels of the cat mesencephalon with labeled periaqueductal gray (*PAG*) neurons after an HRP injection in the left spinal cord and a contralateral hemisection. Each drawing represents one 40-µm-thick section. (*Aq* aqueduct of Silvii, *IC* inferior colliculus, *NR* nucleus ruber, *SC* superior colliculus, *III* oculomotor nucleus, *IV* trochlear nucleus)

Table 2 Numbers of HRP-labeled neurons observed in the ipsilateral and contralateral periaqueductal gray (*PAG*) and adjacent tegmentum

Case	Injection site	Total number of labeled neurons					
		Ipsilateral			Contralateral		
		PAG	Tegmentum	Total	PAG	Tegmentum	Total
2199	C2	320	136	456	93	15	108
2173	C3	181	73	254	24	5	29
2214	T2	126	51	177	13	0	13
2188	T4	62	11	73	7	0	7
2221	T12	37	7	44	1	0	1
2192	L6	22	1	23	0	0	0
2141	S1	5	0	5	0	0	0
2152	S2	2	0	2	0	0	0
2139	S3	1	0	1	0	0	0

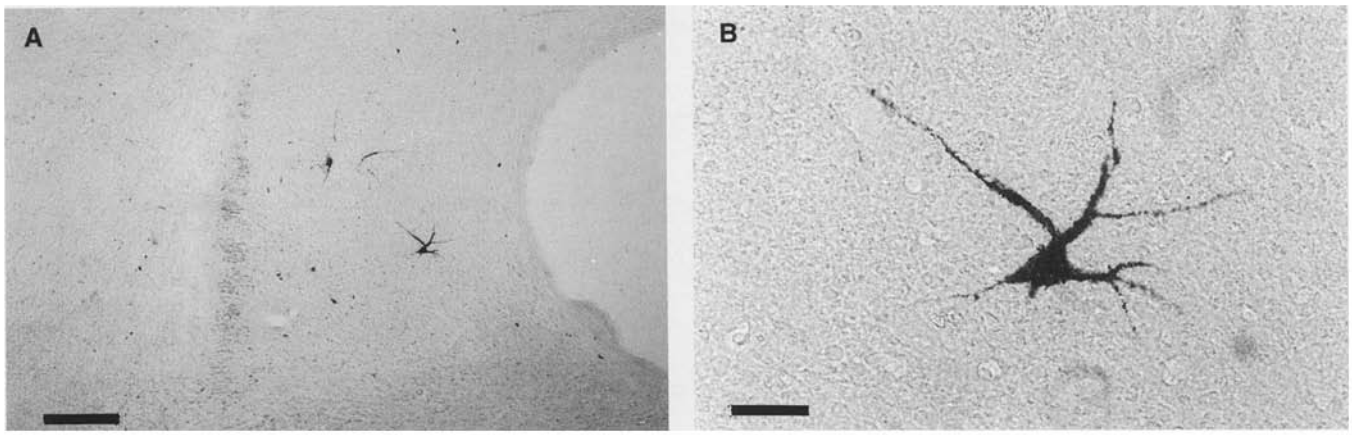


Fig. 2A, B Bright-field photomicrographs of one labeled PAG-spinal neuron after an HRP injection in L6 (case 2192). *Scale bars* **A** 300 μm, **B** 50 μm

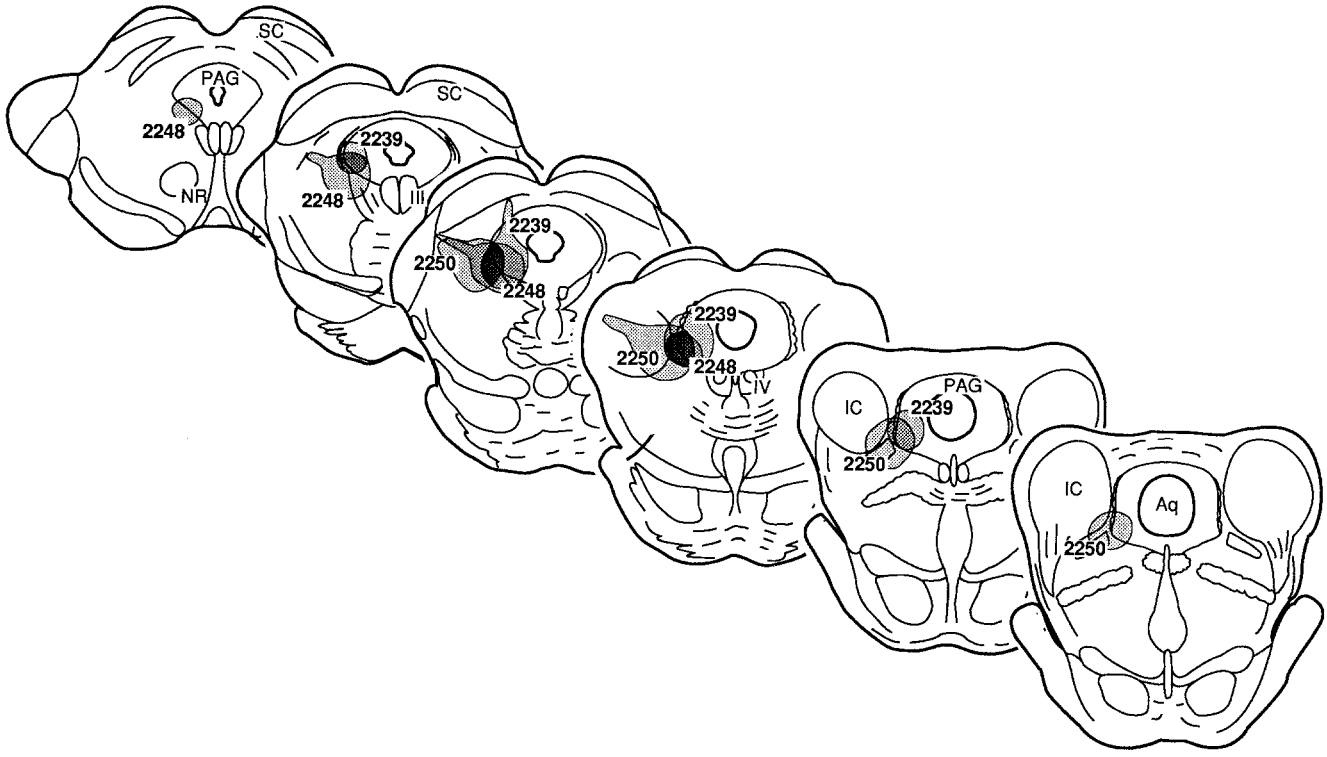


Fig. 3 Schematic drawings of the WGA-HRP injection sites in the ventrolateral PAG and the adjacent tegmentum in cases 2239, 2248, and 2250. (*Aq* aqueduct of Silvii, *IC* inferior colliculus, *PAG* periaqueductal gray, *NR* nucleus ruber, *SC* superior colliculus, *III* oculomotor nucleus, *IV* trochlear nucleus)

with a mean of approximately 33 μm (Fig. 2). In the cases with an injection in the cervical and upper thoracic spinal cord some faintly labeled neurons were observed at the border of the dorsal PAG and in the dorsally adjoining superior colliculus. These labeled dorsal neurons were relatively small, with a diameter of approximately 15 μm, and were located more caudally than the labeled neurons in the ventrolateral and lateral PAG.

Anterograde tracing study

Injection sites

In three cases WGA-HRP injections were placed in the ventrolateral and lateral PAG and adjacent tegmentum (Fig. 3). In case 2239 the injection site involved the ventrolateral and lateral PAG at the level between the trochlear nucleus and the oculomotor nucleus. In case 2248 the injection site was located more rostrally and involved the ventrolateral part of the PAG and the ventrolaterally adjacent tegmentum. In the last case (2250) the injection site was located slightly more caudally than in case 2239. It included the lateral part of the ventrolateral PAG, the laterally adjoining tegmentum, and part of the inferior colliculus.

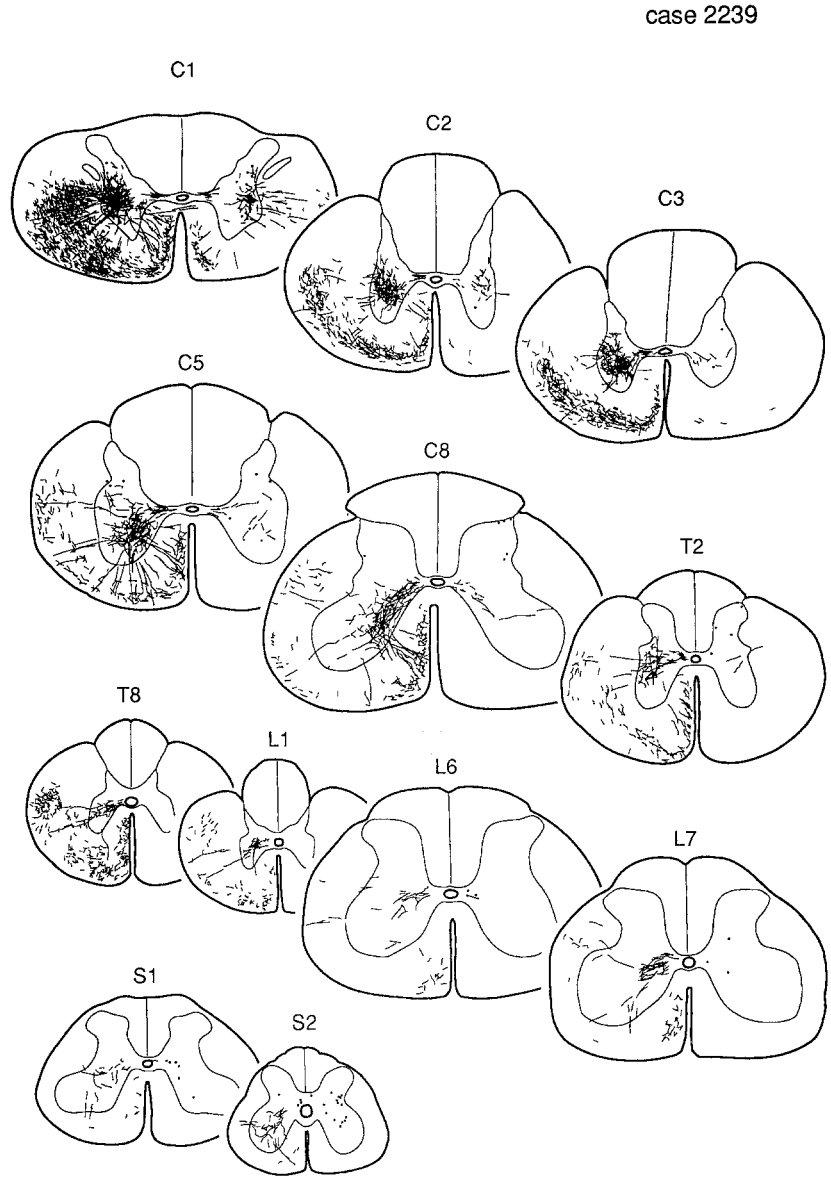


Fig. 4 Schematic drawing of the labeled fibers at various levels of the spinal cord after a WGA-HRP injection in the ventrolateral periaqueductal gray (PAG) of the cat (case 2239). It must be emphasized that each drawing of a spinal cord segment represents six 40-μm-thick sections

Descending pathway

From the injection site many labeled fibers passed ventrally to descend through the lateral tegmental field of caudal mesencephalon and rostral pons. They gradually shifted ventromedially, and at upper medullary levels they were found just medial to the superior olivary complex and facial nucleus and more caudally just lateral to the inferior olive. Many fibers terminated in the ventromedial tegmental field and nucleus raphe magnus. Some fibers continued caudally and came to lie in the ventral funiculus of the caudal medulla. Further caudally half of these labeled fibers continued into the ventral and lateral funiculi of the upper cervical cord, the other half gradually shifted medially to descend in the ventromedial funiculus (Fig. 4). At lower cervical levels descending labeled fibers were present in the lateral, ventrolateral, and ventromedial funiculi. At thoracic and lumbar levels the number of descending fibers diminished gradually. Most of these fibers were found in the ventromedial funiculus and only a very few in the lateral funiculus. At sacral levels no more than two labeled descending fibers per section were observed.

In contrast to other spinal pathways, such as the coeruleo-, raphe-, reticulo- and vestibulospinal pathways (Holstege 1988a, b), the PAG-spinal fibers did not descend in the most peripheral portion of the white matter of the spinal cord.

Distribution pattern in the spinal gray matter

At the upper cervical level many labeled fibers terminated ipsilaterally in the lateral portions of laminae V, VII, and VIII of Rexed (1954; Fig. 5). Contralaterally, some fibers terminated in these same laminae. Caudal to C1 the termination of labeled fibers shifted to more central and medial parts of the ventral horn (medial part of

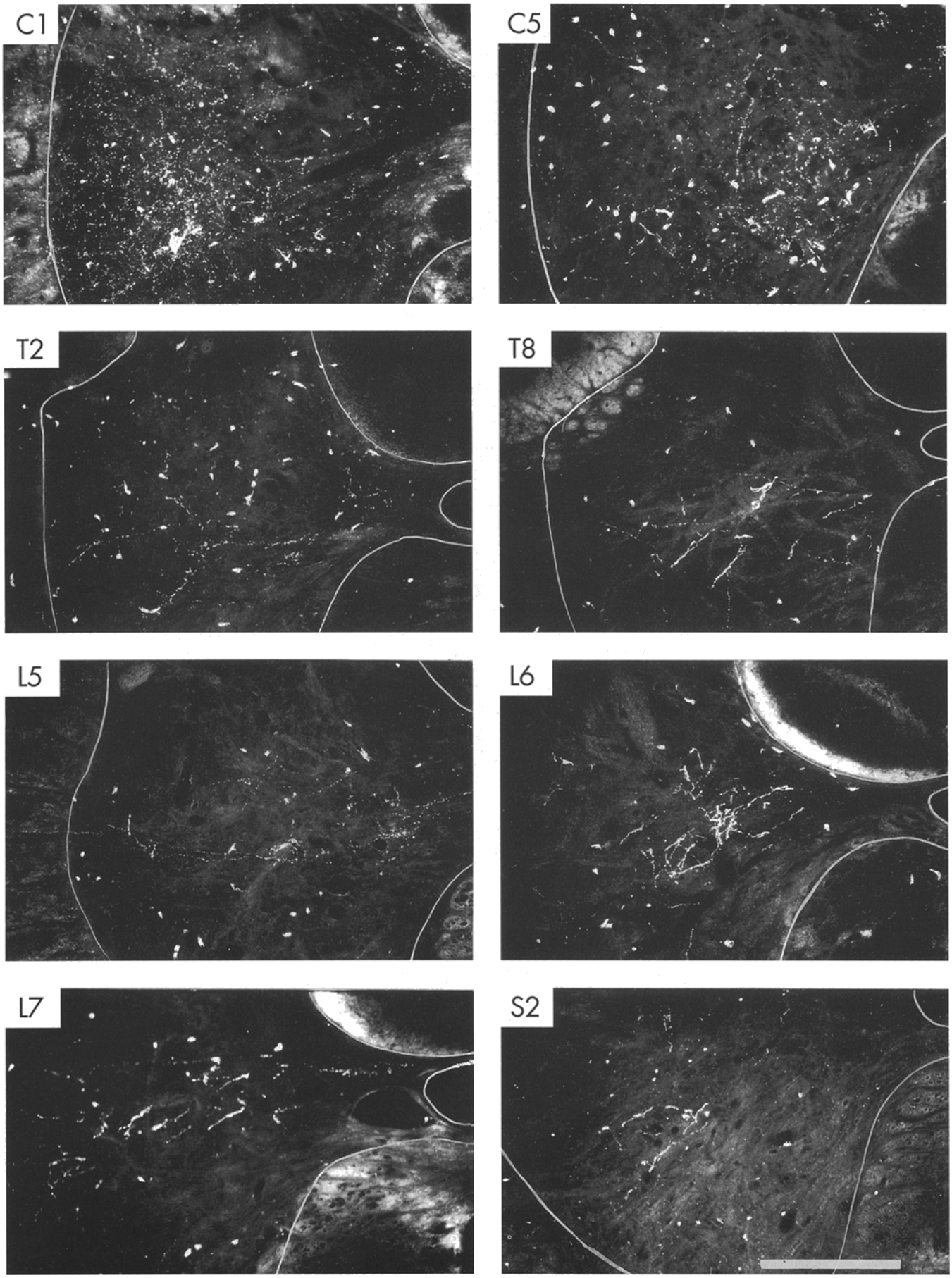


Fig. 5 Dark-field polarized photomicrographs of various levels of the spinal cord of the cat after a WGA-HRP injection in the ventrolateral PAG (case 2239). *Scale bar* 500 µm

lamina VII and lamina VIII). Ipsilaterally, at levels caudal to T2 descending labeled fibers were distributed mainly to medial lamina VII with some fibers terminating in the dorsal part of lamina VIII. Contralaterally no labeled fibers were found beyond the level of T2. No labeled fibers were found to terminate in lamina IX. The labeled fibers also did not terminate in the intermediolateral cell column, except for some thin fibers at upper thoracic levels. Such fibers have been reported by Holstege (1988a, b) also, using autoradiographic tracing techniques.

Discussion

The present study demonstrates that neurons in the PAG project throughout the length of the spinal cord. In earlier anterograde tracing studies in the cat PAG projections to cervical and upper thoracic segments were found, and only a sparse distribution to lower thoracic and upper lumbar segments was reported (Holstege 1988a). PAG projections to the caudal lumbar and sacral spinal cord have never been described before. The numbers of retrogradely labeled neurons, as presented in Table 2, should be interpreted with caution. PAG neurons projecting to, for example, the lumbosacral cord may have been labeled after an HRP injection in the thoracic or cervical cord, since plain HRP is taken up not only by terminating fibers but also by damaged fibers of passage (Kristensson and Olsson 1974). This implies that the number of PAG neurons labeled after an HRP injection in a certain segment can be larger than the number of PAG neurons whose fibers actually terminate there. Irrespective of this, the results clearly show that there are far more PAG neurons projecting to the cervical cord than to the thoracic or lumbar cord and that only a very few PAG neurons project as far as the sacral cord.

From several brainstem-spinal pathways, for instance the interstitiospinal pathway (Fukushima et al. 1978), the vestibulospinal pathway (Abzug et al. 1974), the rubrospinal pathway (Shinoda et al. 1977), and the pontine reticulospinal pathway (Matsuyama et al. 1993), it has been shown that one brainstem neuron sends collateral fibers to more than one segment of the spinal cord. This raises the question of how collateralized the PAG-spinal system is. In other words, do PAG-spinal fibers terminating in the caudal spinal cord, for instance at the lumbar level, have collaterals to more rostral segments, for instance the thoracic and cervical levels, or do all PAG-spinal fibers project to one or a few adjacent segments only? Another possibility is that part of the PAG-spinal fibers are highly collateralized and part of them have a specific projection. Whichever, it is clear that there exist PAG neurons projecting exclusively to the cervical cord. Whether there exist PAG neurons projecting exclusively to the thoracic, lumbar, or sacral cord, respectively, cannot be determined. Double-labeling tracing experiments might solve this problem.

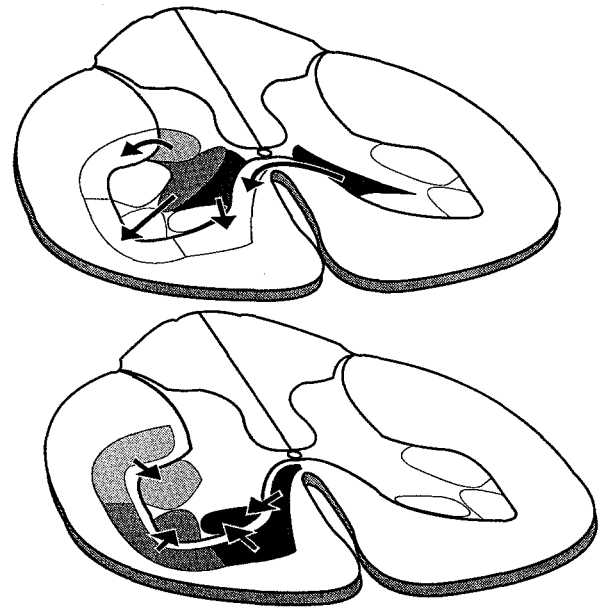


Fig. 6 Schematic illustration of the projections from interneurons in the intermediate zone (lamina V–VIII) via the propriospinal pathways to the motoneurons (from Holstege 1991)

Our study shows that the PAG-spinal fibers do not terminate directly on motoneurons, but on interneurons in the ventral horn. Earlier findings (Rustioni et al. 1971; Sterling and Kuypers 1968; Molenaar et al. 1974; Molenaar 1978) have demonstrated that the dorsolateral part of the intermediate zone (lateral part of lamina V to VII) of the brachial and lumbosacral cord contains interneurons projecting via propriospinal pathways to the motoneurons of distal limb muscles, while interneurons in the medial intermediate zone project bilaterally to axial muscle motoneurons. Interneurons located in between these two areas project to proximal limb muscle motoneurons (Fig. 6). The present results show that the PAG-spinal fibers projecting to the caudal cervical, thoracic and lumbosacral cord terminate in the medial portion of the intermediate zone (lamina VIII and medial part of lamina VII), which would imply that the PAG is involved in the control of axial and proximal musculature.

Projections similar to the PAG-spinal pathway are derived from other brainstem areas, such as the reticular formation adjacent to the interstitial nucleus of Cajal (INC-RF; Nyberg-Hansen 1966; Holstege and Cowie 1989), the reticular formation adjacent to the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF-RF; Holstege 1988b; Holstege and Cowie 1989), the lateral vestibular nucleus (LVN; Nyberg-Hansen and Mascitti 1964; Petras 1967; Holstege and Kuypers 1982), and the medial part of the caudal pontine and upper medullary tegmentum (PMTm; Nyberg-Hansen 1965; Petras 1967; Holstege and Kuypers 1982; Holstege 1988b). All these four brainstem nuclei are in-

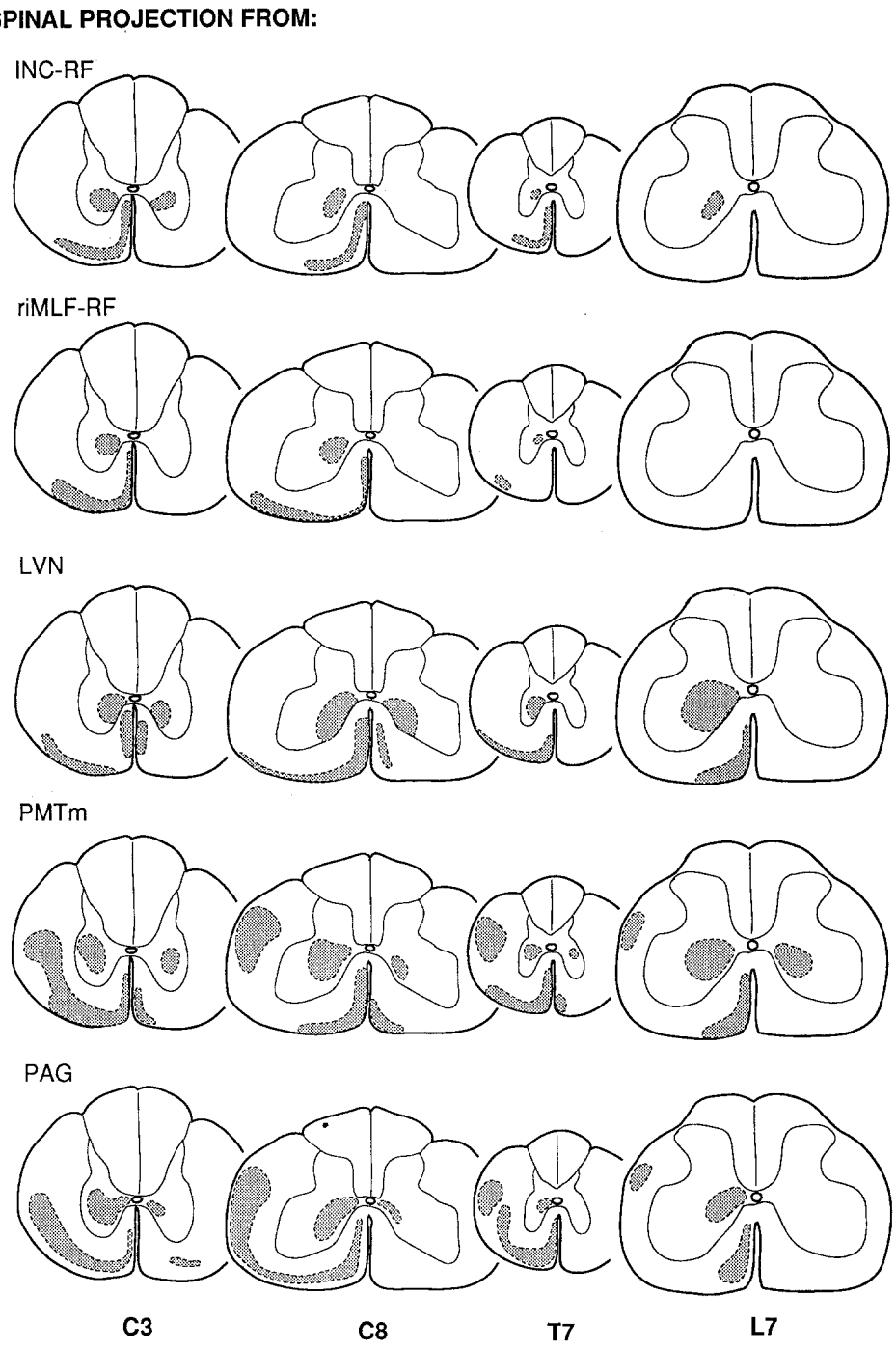


Fig. 7 Schematic drawing of the spinal pathways from reticular formation adjacent to the interstitial nucleus of Cajal (*INC-RF*), the reticular formation adjoining the rostral interstitial nucleus of the medial longitudinal fasciculus (*riMLF-RF*), the lateral vestibular nucleus (*LVN*), the medial pontine tegmentum and the rostral medulla (*PMTm*), and the periaqueductal gray (*PAG*). At each level of the spinal cord both the areas of descending fibers in the white matter and the area of terminating fibers in the gray matter are indicated. Note that this scheme does not give an indication of the number of fibers belonging to the different pathways. (Spinal pathways from INC-RF, riMLF-RF, LVN, and PMTm have been described by Holstege, 1988b)

volved in the control of axial and proximal muscles by way of terminating in the medial part of the intermediate zone (Fig. 7). Considering the function of these different descending systems, physiological and lesion studies make clear that they all have a specialized function within the vestibular-oculomotor framework. The INC-RF is thought to be responsible for the slow, small movements of neck and back muscles controlling the position of the head necessary to integrate vertical gaze shifts and head movements (Hyde and Toczek 1962; Fukushima et al. 1985; Fukushima 1987). The riMLF-RF might control the fast axial movements during rapid vertical eye movements. The LVN is responsible for movements of neck, back, and hind limbs necessary for maintaining the body equilibrium, the position of the head, and the direction of gaze (Wilson and Peterson 1981). Finally, the PMTm takes part in the control of the postural musculature connected with fast horizontal eye movements (Hassler 1972; Büttner-Ennever and Büttner 1988).

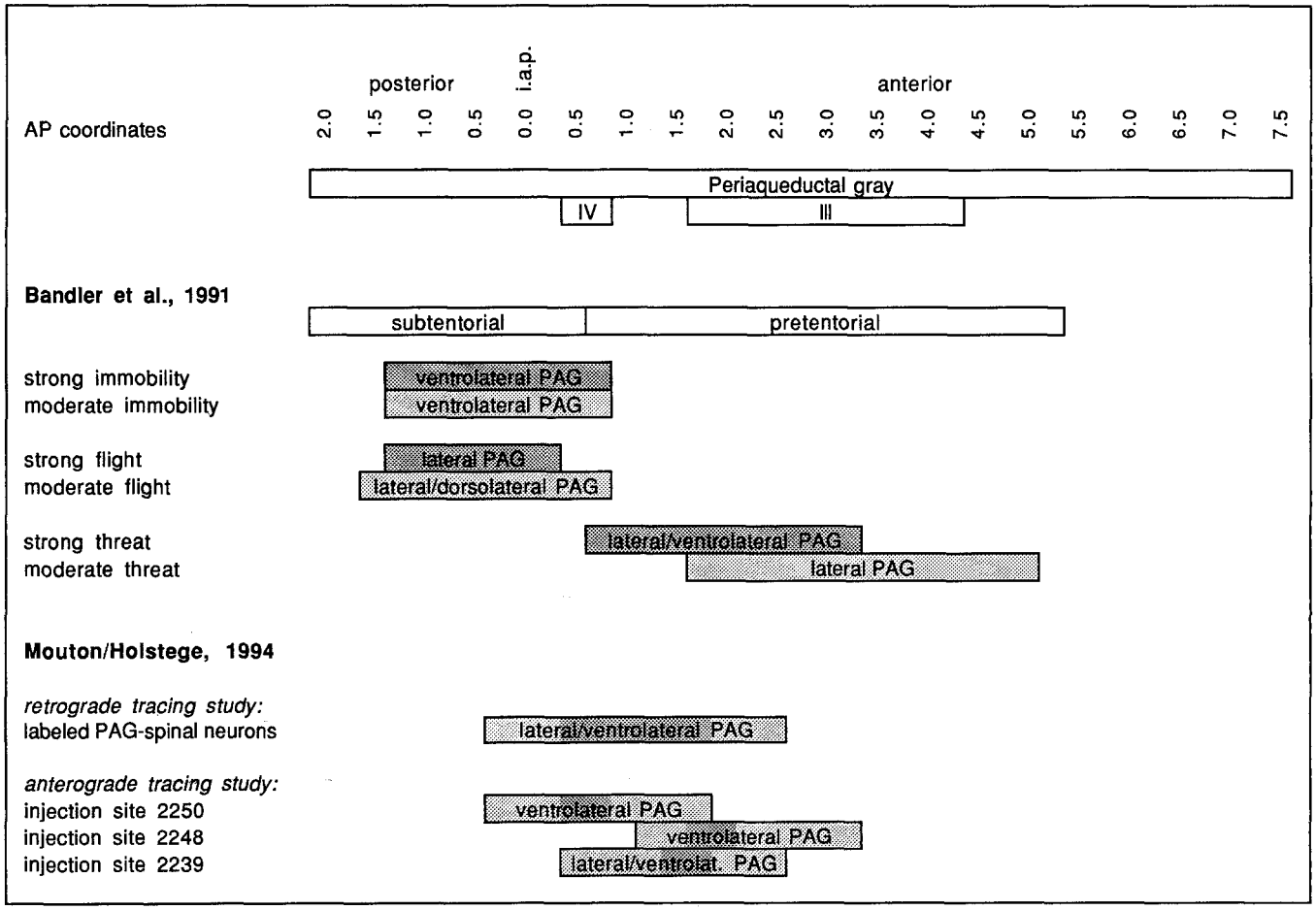
Although the PAG-spinal pathway seems to be involved in axial musculature control, it is not clear in what framework this system functions. The ventrolateral and lateral PAG, in which the PAG-spinal neurons

are located, have never been shown to receive afferents from vestibular or oculomotor structures like each of the other four brainstem areas (Büttner-Ennever and Büttner 1988). The ventrolateral and lateral PAG, however, receives many afferents from limbic structures such as the lateral hypothalamic area (Berk and Finkelstein 1982; Holstege 1987), the central nucleus of the amygdala (Hopkins and Holstege 1978; Price and Amaral 1981; Rizvi et al. 1991), and the bed nucleus of the stria terminalis (Holstege et al. 1985). These limbic structures send fibers to neither the riMLF-RF, the INC-RF, the LVN nor to the PMTm, which suggests that the PAG-spinal pathway does not function in a vestibulo-oculomotor framework.

A more obvious possibility would be that the PAG-spinal pathway controls the axial muscles within the framework of the emotional motor system, as defined by Holstege (1992). The emotional motor system contains the descending pathways from limbic structures to caudal brainstem and spinal cord and can be divided functionally in a medial and a lateral part. The medial part, containing for instance the diffuse coeruleo- and raphe-spinal pathways, has a global effect on the level of activity of the somatosensory neurons and motoneurons in general. The lateral part of the emotional motor system involves some specific parts of the brain, such as the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the lateral hypothalamus, and those cells of the PAG which are thought to be involved in specific functions such as vocalization and blood pressure control during emotional behavior. If one considers the PAG-spinal pathway as part of the lateral component of the emotional motor system, it might control axial movements in specific emotional activities.

Experiments in the freely moving cat (Bandler and Carrive 1988; Zhang et al. 1990; Bandler et al. 1991) have shown that both electrical stimulation and stimulation with excitatory amino acids in the PAG can elicit three basic patterns of behavior, threat display, flight, or immobility. In this respect threat display consists of moderate pupil dilation and piloerection, vocalization (howling usually mixed with hissing, or hissing alone), retraction of the ears and/or arching of the back. Flight is characterized by moderate pupil dilation and piloerection, vocalization (mewing), rapid running, and

Fig. 8 Schematic overview of behavioral patterns after stimulation at different rostrocaudal levels of the periaqueductal gray (*PAG*) in the freely moving cat, related to the location of the PAG-spinal neurons. *Darkest areas* represent the levels with the strongest behavioral reactions (stimulation experiments), with the highest number of labeled neurons per section (retrograde study), and with the centers of the WGA-HRP injections (anterograde study). (*III* oculomotor nucleus, *IV* trochlear nucleus, *i.a.p.* interaural plane according to Berman, 1968)



multiple jumps. Immobility is the situation in which the cat shows a period of profound inactivity. Each of these responses can be elicited in specific parts of the PAG (Fig. 8). Zhang et al. (1990) showed that strong or moderate immobility is found after stimulating the ventrolateral part of the caudal one-third of the PAG (subtentorial PAG), while a strong flight response is observed after stimulating more dorsally, i.e., in the lateral subtentorial PAG around the P 0.5 level of Berman (1968). The area in which stimulation produced moderate flight responses occupied a slightly larger portion of the subtentorial PAG. Threat display was found more rostrally in the PAG (pretentorial or middle third of the PAG). Strong threat display can be elicited by injecting excitatory amino acid in the lateral and ventrolateral parts of the caudal pretentorial PAG. Moderate threat display was evoked from more rostral parts of the lateral pretentorial PAG (Bandler and Carrive 1988).

As described in the results section, the PAG-spinal neurons are located in the lateral and ventrolateral PAG. They form a rostrocaudally oriented column extending caudally from the level of the decussation of the brachium conjunctivum to rostrally around the level of the caudal pole of the oculomotor nucleus. The bulk of labeled neurons was found just rostral to the level of the trochlear nucleus, i.e., in the caudal pretentorial PAG. Comparing the location of the PAG-spinal neurons with the results of the stimulation experiments of Bandler and coworkers (Fig. 8) leads to the supposition that the PAG-spinal neurons play a role in threat display. Possibly, arching of the back as a component of threat display is the result of the activity of PAG-spinal neurons controlling axial musculature.

In conclusion, the present results show that the PAG projects to the lateral parts of the intermediate zone of the upper cervical cord and to the medial parts of the intermediate zone of the caudal cervical, thoracic, lumbar, and sacral spinal cord. In these areas the premotor interneurons of the axial muscles are located. It is hypothesized that the PAG-spinal pathway forms the anatomical framework for arching of the back as part of threat display.

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