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Distinct Patterns of Activated Neurons Throughout the Rat Midbrain Periaqueductal Gray Induced by Chemical Stimulation Within Its Subdivisions

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ABSTRACT

This study provides a map of those neurons in the midbrain periaqueductal gray which are activated by chemical stimulation within different subdivisions of the periaqueductal gray. In pentobarbital anesthetized rats, the expression of the c-FOS protein was detected by immunocytochemistry and was used as a marker of neuronal activity. Microinjections of the γ-aminobutyric acid ($GABA_A$) receptor antagonist bicuculline (200 pmol in 50 nl) were used to increase selectively the firing rate of neurons originating from the injection site. The pattern of c-FOS immunoreactivity was highly specific for different injection sites. Dorsal injections were characterized by an extensive distribution of c-FOS immunoreactivity along the entire rostrocaudal extent of the periaqueductal gray, while ventral injections produced a much more restricted labeling. Following injection into the dorsal subdivision of the rostral periaqueductal gray, c-FOS immunoreactivity was present bilaterally in the dorsal and dorsolateral subdivisions of the rostral periaqueductal gray and was found in all subdivisions of the caudal periaqueductal gray. Dorsolateral injections at the level of the oculomotor nuclei produced strictly ipsilateral labeling in the dorsal and dorsolateral periaqueductal gray at the level of injection and throughout the ipsilateral half of the periaqueductal gray at more caudal levels. Stimulation in the ventrolateral periaqueductal gray induced FOS in the ventrolateral periaqueductal gray and the adjoining reticular formation. At rostral levels c-FOS immunoreactivity was also seen in the lateral periaqueductal gray but was absent caudal to the injection site. The identified patterns of activity in the periaqueductal gray provide a new basis for the interpretation of the diverse functional consequences of stimulation at periaqueductal gray sites.

Indexing terms: PAG, intrinsic connections, functional anatomy, bicuculline, c-fos

Neurons originating from the midbrain periaqueductal gray (PAG) are known to play an important role in a broad spectrum of functions, including antinociception (Reynolds, 1969; Besson and Chaouch, 1987), vocalization (Jürgens and Pratt, 1979), aggressiveness (Bandler, 1988; Shaikh and Siegel, 1990), sexual behavior (McCarthy et al., 1991), defence reaction (Carrive, 1993; Lovick, 1993), and cardiovascular control (Carrive and Bandler, 1991). Widespread efferent connections to well-defined brainstem sites (e.g., Mantyh, 1983a,b) are the anatomical substrate for the diversity of output functions. It has been shown that the PAG can be divided into subdivisions on the basis of cytoarchitectural, myeloarchitectural, biochemical, pharmacological, or functional criteria (Lewis and Gebhart, 1977; Moss and Basbaum, 1983; Moss et al., 1983; Prieto et al., 1983; Fardin et al., 1984b; Moskowitz and Goodman, 1984; Beitz, 1985; Beitz and Shepard, 1985; Gioia et al., 1985; Reichling et al., 1988; Gerrits et al., 1993). It is well established that these subdivisions project, at least in part, to different target sites outside the PAG (Mantyh, 1983a,b). In contrast, little is known about the intrinsic functional organization within and between the subdivisions of the PAG (Tredici et al., 1983). Because of this lack of knowledge, rather than because of available evidence, it is generally assumed that the effects which can be evoked by focal chemical (or electrical) stimulation at PAG sites are due to the activation of efferents leaving the PAG from the site of stimulation. The possibility that intrinsic neurons might

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also recruit neurons in various subdivisions of the PAG remote from the site of stimulation is often neglected.

To address directly the question as to which neurons are recruited in the PAG by focal chemical stimulation, we have used the expression of the proto-oncogene c-*fos* as a cellular marker of activated neurons (Dragunow and Faull, 1989). For focal chemical stimulation we used microinjection of small amounts of the $GABA_A$ receptor antagonist bicuculline, since this is known to increase the firing rate of most PAG neurons (Behbehani et al., 1990) and which has been demonstrated to activate maximally output functions of the PAG (Sandkühler et al., 1989, 1991). The data demonstrate for the first time that a very distinct pattern of activated neurons throughout the entire rostrocaudal extent of the PAG results from focal stimulation at different PAG sites.

MATERIALS AND METHODS

Preparation of animals

Housing and anesthesia. Experiments were performed on 17 adult male Sprague-Dawley rats (200–300 g body weight) under pentobarbital anesthesia. The animals were housed in groups of two in one Macrolone type III cage in an air-conditioned room (temperature 22 ± 2°C, water saturation 55 ± 5%) with a 12 hour light-dark cycle (light on at 7:00 a.m.). Food pellets and water were given ad libitum. The animals were allowed to adapt to this environment for at least 7 days before the experiments were begun. On the day of the experiments, the animals were placed in a large Perspex chamber with the air containing halothane to induce anesthesia within 10–15 seconds. Pentobarbital was then injected intraperitoneally at a dose of 60 mg kg^{-1}. The animals were transported to the laboratory under deep pentobarbital anesthesia (see Sandkühler, 1991).

The core body temperature was measured continuously with a rectal probe and was kept constant at 37.5 ± 0.5°C by means of a feedback controlled heating blanket underneath the ventral surface of the animals. A continuous infusion of pentobarbital (15 mg in 1 ml tyrode) into an external jugular vein was used to maintain a constant level of anesthesia (withdrawal reflexes absent, corneal reflex present, 5–10 mg kg^{-1} $hour^{-1}$).

Stereotaxic implantation. The animals were placed with their heads in a stereotaxic frame with the surface of the skull in a horizontal position. The skin overlaying the parietal bones was incised sagittally after shaving and following application of a gel which contained 2% lidocaine. A small craniotomy (3 mm in diameter) was made with an electric drill in the left parietal bone 5 to 8 mm caudal to the bregma and 1–4 mm left of the midline. The underlying dura mater was incised to allow insertion of a fine, multibarrel glass probe at a mediolateral angle of 10°. The tip of the pipette was lowered to the desired injection site in the PAG by means of an electronically controlled microstepping motor (Narishige, Japan, 1,000 μm in 15 seconds). The atlas of Paxinos and Watson (1982) was used.

Microinjections. The multibarrel glass probe was constructed as described in detail previously (Sandkühler and Gebhart, 1991). One barrel was filled with either bicuculline methiodide (Sigma, Deisenhofen, Germany, 4 mM in 0.9% saline, pH adjusted to 2.5 with HCl) or with the vehicle alone. Another pipette was filled with saturated fast green dye. For stimulation, 200 pmol bicuculline (in 50 nl) was injected by applying positive pressure via a 1 ml syringe which was connected to PE 10 tubing, both filled with air. The volume injected was determined by watching the meniscus in the pipette through a microscope as it moved along a calibrated scale. In sham-treated animals, 50 nl vehicle was injected. The pipette was left in place for 90 min, and then 100–200 nl fast green dye was injected to mark the injection site.

Immunocytochemistry. Ninety minutes after the injections into the PAG, the animals were killed by an overdose of pentobarbital and were immediately thereafter transcardially perfused with 100 ml phosphate-buffered saline at room temperature followed by 200 ml ice-cold paraformaldehyde (4%) in phosphate buffer. The brain was removed and postfixed overnight in the same fixative and then stored for 48 hours in 30% sucrose for cryoprotection. The brain was cut in a cryostat in 50 μm thick coronal sections through the midbrain. Free-floating sections were incubated with normal goat serum (2% in phosphate buffered saline and 0.2% Triton-X-100) for 1 hour, followed by the primary antiserum at 1:6,000 for 24 hour. The polyclonal rabbit antibody was raised against bacterially expressed fusion protein and was generously provided by Dr. R. Bravo, The Squibb Institute for Medical Research, Princeton, NJ. This antibody was characterized recently (Kovary and Bravo, 1991). After washing, the sections were incubated in biotylinated goat anti-rabbit antiserum followed by an avidin-peroxidase complex (Vectastain, Vector Laboratories) for 1 hour. The sections were then developed in 0.02% diaminobenzidine with 0.02% hydrogen peroxide and intensified by addition of 0.02% cobalt chloride and nickel ammonium sulfate.

RESULTS

Sham-treated animals

Five sham-treated rats received an injection of vehicle into the dorsal PAG at the level of the posterior commissure (n = 2) or the dorsolateral (n = 1) or ventrolateral or medial subdivision (n = 2) of the PAG at the level of the oculomotor nuclei, respectively.

In all sham-treated animals, the number of cells with c-FOS IR was low or zero in most subdivisions of the PAG, including the injection site. A few labeled cells were scattered bilaterally throughout the PAG, mainly in its ventral half (see Fig. 1A for an example). However, c-FOS IR was consistently found bilaterally in cells of the nucleus of Darkschewitz (Fig. 2A), the dorsal raphe nucleus (Fig. 2B,C), and along the track of the pipette in the cerebral cortex and the superior colliculus (Figs. 1A, 2D). No difference in the distribution of c-Fos positive cells was apparent between animals of different sham treated groups.

c-FOS IR induced by bicuculline microinjections

Increasing the firing rate of neurons which originate at well-defined areas of the midbrain PAG by microinjections of bicuculline produced a characteristic pattern of c-FOS expression throughout the entire rostrocaudal extent of the PAG. Dorsal and dorsolateral injections were characterized by extensive c-FOS labeling. Ventrolateral injections induced much more restricted labeling. For the following description, it has been found convenient to divide the PAG into the subdivision described by Beitz (1985) in the rat based on cluster analysis of cytoarchitectural features. Similar subdivisions have been described earlier in the cat (Hamilton, 1973).

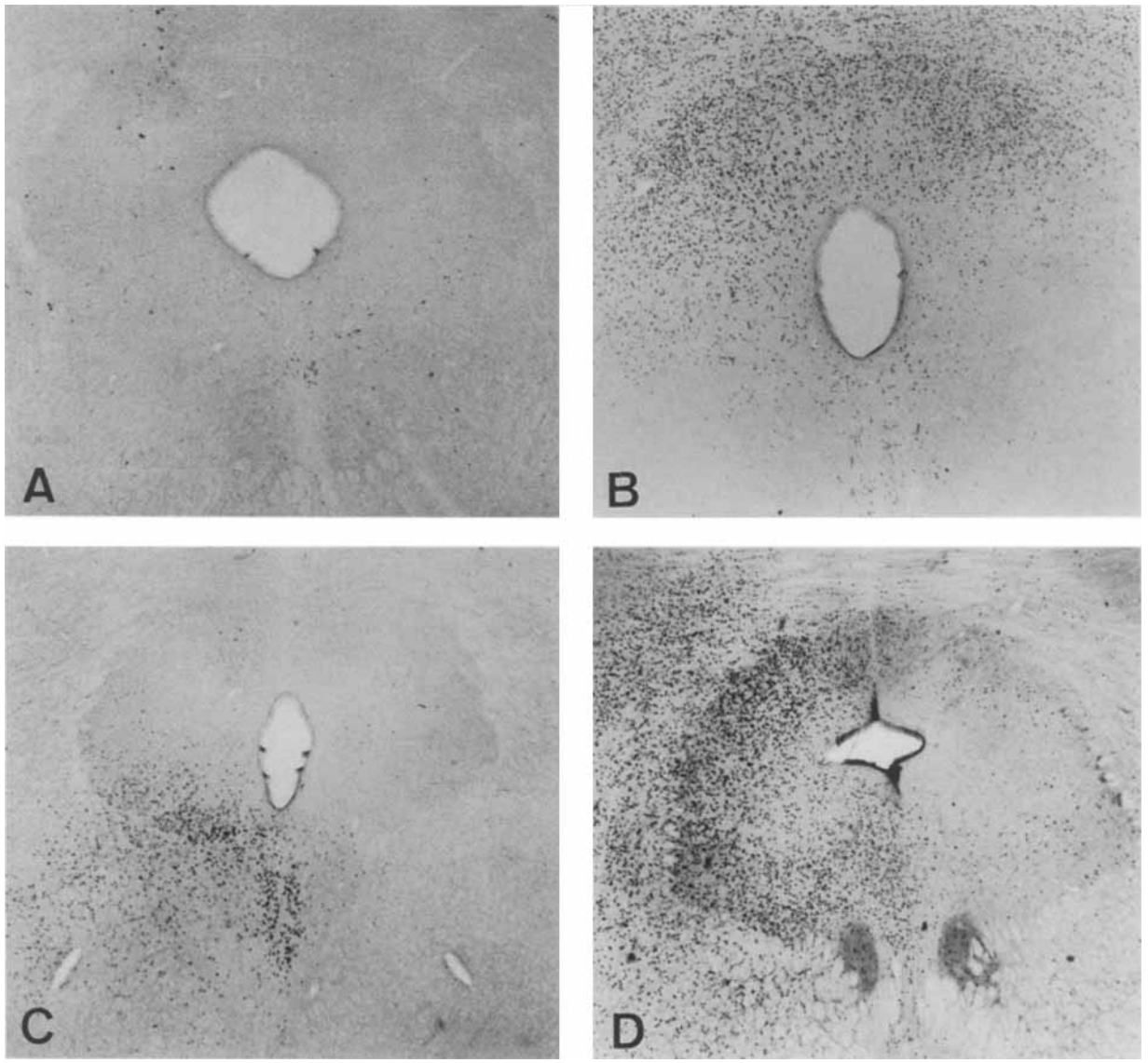

Fig. 1. c-FOS immunoreactivity in 50 μm thick sections through the midbrain periaqueductal gray (PAG) of four different rats. Representative examples illustrate the distribution of c-FOS positive cells following microinjection of 50 nl physiological saline into the ventrolateral PAG (**A**), or 200 pmol bicuculline (in 50 nl) into the dorsal PAG (**B**), the ventrolateral PAG (**C**), or the dorsolateral PAG (**D**). The calibration bar at the bottom equals 1,000 μm (B,C) or 850 μm (A,D). Approximate anterior-posterior levels with reference to bregma are given in mm: A, −6.3; B, −6.3; C, −5.8; D, −7.3.

Stimulation in the dorsal subdivision. In four animals, bicuculline was microinjected into the dorsal part of the rostral PAG at the level of the posterior commissure. Enhanced c-FOS IR was found throughout the entire rostrocaudal extent of the PAG. At rostral levels, c-FOS IR was dense bilaterally in the dorsal PAG (Fig. 1B and Fig. 3B), with very little or no labeling in the medial, the lateral or ventral PAG. In two animals labeling was also enhanced in the nucleus of Darkschewitz (Fig. 3A as compared to Fig. 2A). At the level of the oculomotor (Fig. 3D) and trochlear (Fig. 3E) nuclei, c-FOS IR was also prominent bilaterally in the dorsal and dorsolateral subdivisions and, to a lesser extent, in the medial PAG. Caudal to the decussation of the superior cerebellar peduncle (Fig. 3G,H) c-FOS IR was distributed in all subdivisions. The total number of c-FOS positive cells/subdivision/section varied between the four animals. Figure 3 illustrates the pattern of c-FOS IR in one of the animals with a medium number of c-FOS positive cells. The pattern of c-FOS IR is, however, representative for the four injected animals.

Stimulation in the dorsolateral subdivision. In four animals, bicuculline was microinjected into the dorsolateral PAG at the level of the oculomotor nuclei. Strong c-FOS IR was found only ipsilateral and caudal to the injection sites in all these animals. At rostral levels (Fig. 4A,B), the number of c-FOS positive cells was not significantly higher than in sham-treated animals (compare Fig. 2A,B with Fig. 4A,B), and the labeled cells were scattered throughout the

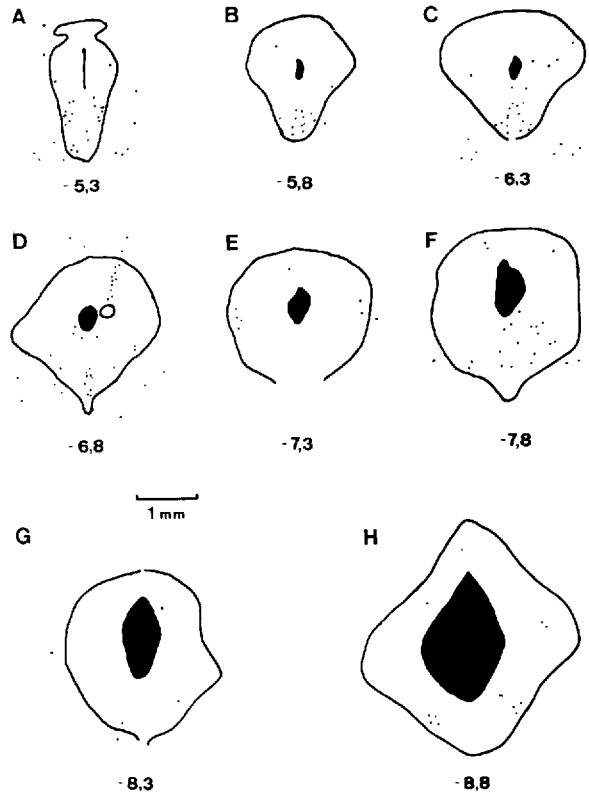

Fig. 2. c-FOS immunoreactivity in the midbrain periaqueductal gray (PAG) of a sham-treated animal, which received an injection of 50 nl vehicle into the medial subdivision of the PAG (open circle in part **D**) at the level of the oculomotor nuclei. Each dot represents four strongly labeled neurons in a 50 μm transverse section through the midbrain. Sections are shown from rostral (**A**) to caudal (**H**). The track of the pipette is indicated by some FOS positive cells (D). The black vertical bar equals 1,000 μm. The borderline of the PAG is indicated by the solid lines, the aqueduct is filled. Approximate anterior posterior levels of the sections with reference to bregma are given in mm.

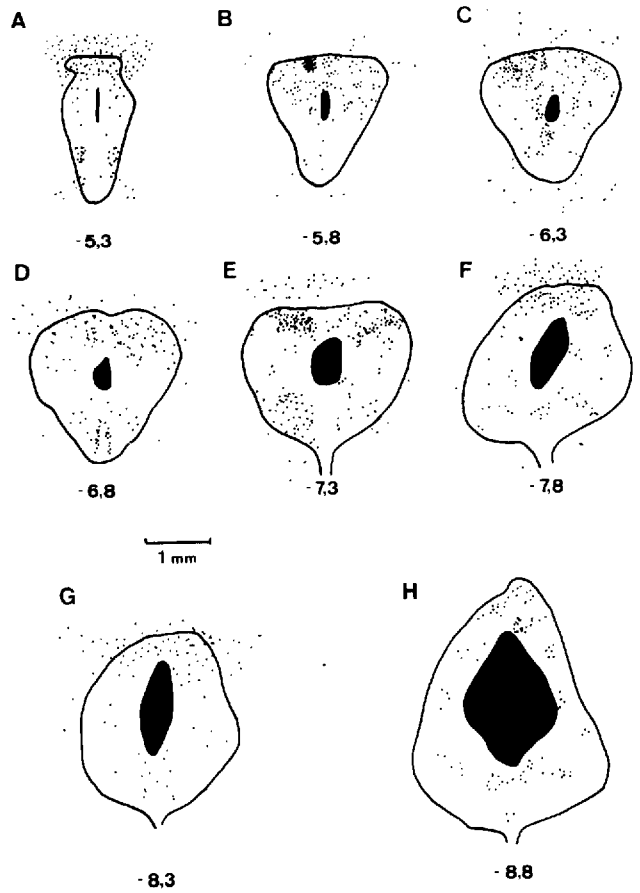

Fig. 3. c-FOS immunoreactivity within the PAG and adjacent midbrain structures following stimulation in the dorsal PAG at the level of the posterior commissure (**B**). The site of microinjection of bicuculline was within the high density area of labeled cells at the left hand site of the dorsal part of the PAG. The black vertical bar equals 1,000 μm. Approximate anterior posterior levels of the sections with reference to bregma are given in mm.

dorsal and ventral subdivisions. In one of the animals, labeling rostral to the injection site was almost absent (not shown). At the level of the injection, c-FOS IR was strong in the dorsolateral and dorsal PAG, while c-FOS-positive cells were sparse in the medial and ventrolateral PAG (Fig. 1D and Fig. 4C). Caudal to the injection site, labeling was very dense throughout the ipsilateral half of the PAG, with significantly less labeling in the medial subdivision (Fig. 4D–F). In three of the animals, only a few labeled cells were present at the contralateral site, while contralateral labeling was somewhat stronger in one animal (not shown). At the caudal most levels of the PAG, most c-FOS-positive cells were located in the ventrolateral PAG and significantly fewer labeled cells were seen in the dorsal PAG (Fig. 4G,H).

Stimulation in the ventrolateral subdivision. In four animals, bicuculline was microinjected into the ventrolateral PAG at the level of the oculomotor nuclei (Fig. 5C) and induced c-FOS predominantly ipsilaterally to the injection site in the lateral and ventrolateral PAG at the level of the injection site and further rostrally. Rostral to the injection site, labeled cells were found bilaterally in the ventrolateral subdivision. On the ipsilateral site, labelling extended into the lateral and dorsolateral PAG, but not including the dorsal PAG (Fig. 5A,B). Caudal to the level of stimulation, labeling was progressively reduced and disappeared almost completely caudal to the level of the trochlear nuclei (Fig. 5F–H).

DISCUSSION

Meaning of c-FOS expression in PAG neurons

The c-FOS protein belongs to a group of transcription factors which are formed by members of the *fos* and *jun* multigene families (see Bravo, 1990 for a review). These proteins are characterized by formation of the so-called AP-1 complex which interacts with specific DNA binding sites in the promotors of target genes (Sonnenberg et al., 1989a). It is generally assumed that the expression of c-FOS indicates transcriptional operations following a defined stimulus. Some of putative target genes of the c-FOS protein are presently under investigation, such as prodynorphin (Naranjo et al., 1991) and preproenkephalin (Sonnenberg et al., 1989b). Interestingly, both members of the endorphin family are expressed in PAG neurons (Fallon and Leslie, 1986). This suggests that c-FOS could also

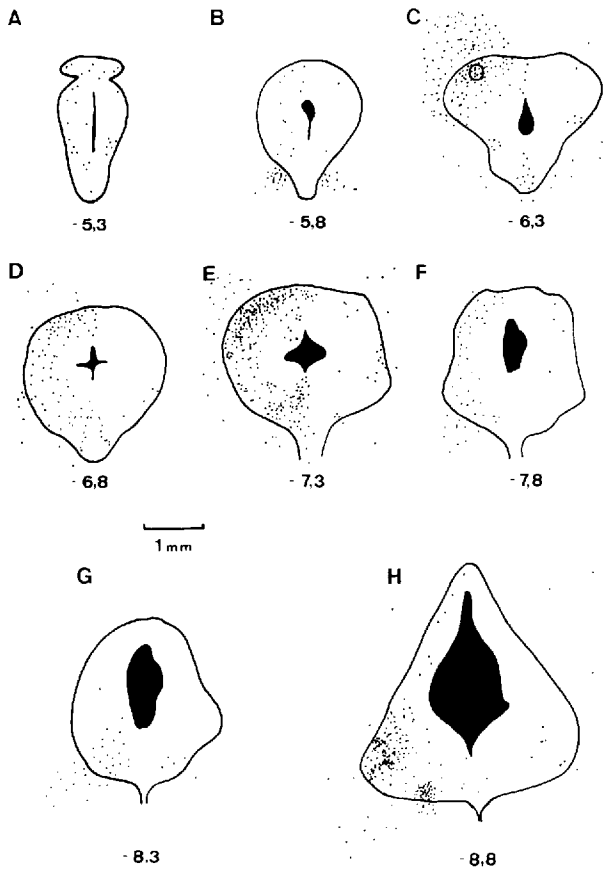

Fig. 4. c-FOS immunoreactivity following stimulation within the dorsolateral PAG at the level of the oculomotor nuclei (open circle in **C**). The black horizontal bar indicates 1,000 μm. Anterior-posterior levels are given in mm with reference to bregma.

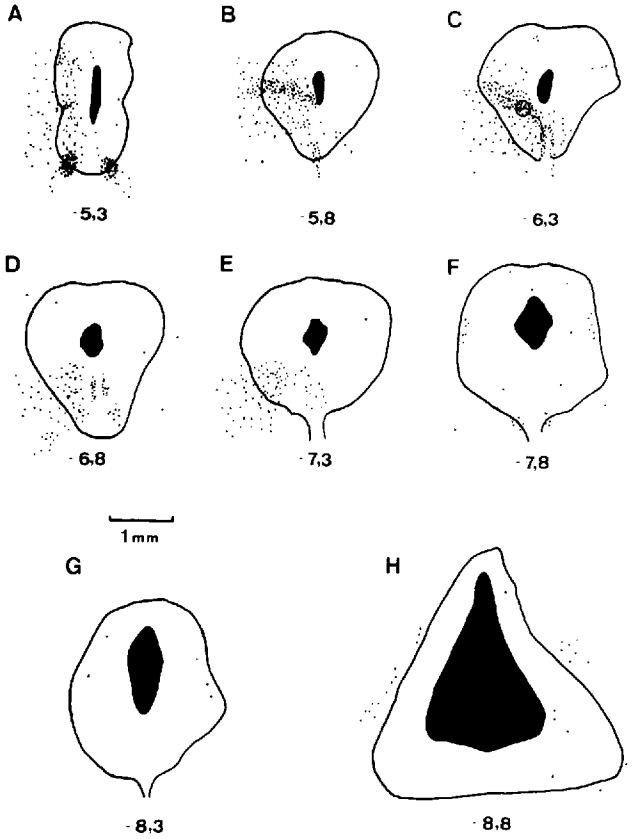

Fig. 5. c-FOS immunoreactivity following stimulation within the ventrolateral PAG at the level of the oculomotor nuclei (open circle in **C**). The horizontal bar indicates 1,000 μm.

mediate the expression of theses genes in neurons of the PAG.

In vitro studies have shown that c-FOS alone is not effective in transcriptionally activating target genes, but needs formation of heterodimeric protein complexes with a member of the *jun* family such as c-JUN, JUN B, or JUN D (Chiu et al., 1988). Several studies have demonstrated that the expression of c-FOS is not a single event but is paralleled by the expression of numerous transcription factors of the *fos, jun,* and other gene families (e.g., Almendral et al., 1988; Herdegen et al., 1991). Thus, expression of c-FOS does not only indicate the activation of central neurons but also suggests that transcriptional processes take place which are responsible for reactive alterations in gene expression.

c-FOS as a cellular marker of activated neurons in the PAG

In the present study, we have used the expression of c-FOS, detected by immunocytochemistry, to map neurons in the PAG which were activated during focal chemical stimulation at selected sites within the PAG. When interpreting the results, some caveats have to be taken into account. First, c-FOS may be induced not only by depolarizing stimuli, but also by receptor-mediated activation of the appropriate second messengers, such as free intracellular calcium and cyclic adenosine monophosphate (c-AMP), bypassing changes in membrane potential (Morgan and Curran, 1986). In the central nervous system, c-FOS is not only expressed in neurons, but also in glial cells in response to heat shock (Dragunow et al., 1989) or following cortical trauma (Dragunow and Robertson, 1988), but not by depolarizing stimuli (Hisanaga et al., 1990). The number and the distribution of c-FOS positive cells in the present study suggests, however, that the vast majority of labeled cells were indeed neurons. Not all central neurons may express c-FOS, no matter what stimuli are used (Dragunow and Faull, 1989). This could cause false negative results in our maps of activated neurons. It does, however, seem unlikely that a significant proportion of PAG neurons was unresponsive in this study, as dense FOS labeling was seen in all subdivisions throughout the entire rostrocaudal extent when pooling the results from all experiments. Keay and Bandler (1993) also found c-FOS positive cells in all subdivisions of the PAG following cutaneous noxious stimulation. False negative results due to stimulation in a refractory period for c-FOS expression (Morgan et al., 1987) seem unlikely since stimulation was performed only once per animal and background labeling was negligible. Hyperpolarizing agents or actions have not been reported to induce c-FOS, i.e., inhibitory pathways were probably not traced.

We have previously reported that in awake, drug free animals environmental stimuli such as novel and poten-

tially stressful handling procedures 6–0.5 h prior to the perfusion may induce c-FOS in neurons throughout the PAG (Sandkühler, 1991), suggesting that not only direct chemical stimulation, but also natural and mildly stressful environmental stimuli, may induce c-FOS in neurons of the PAG. This may account for the fact that in sham-treated animals some labeled cells were always seen. However, no consistent pattern was apparent in sham-treated animals, except that the labeling was always bilateral and the number of labeled cells was always low. In groups of animals which were stimulated at similar sites of the PAG, the patterns of c-FOS IR were highly reproducible, characteristic, and very distinct from the scattered distribution of labeled cells in sham-treated animals. Thus, we conclude that the selective excitation of cells originating from the stimulation sites induced the specific patterns of activated neurons throughout the PAG.

Bicuculline microinjection at PAG sites

For the purpose of the present study, stimulation at PAG sites should fulfill the following criteria:

— selective activation of cell bodies and dendrites, but not fibers of passage,
— strong and long lasting increase in the firing rate of as many neurons as possible at the stimulation site,
— direct effects restricted to a small area around the stimulation site,
— similar efficacy at all stimulation sites,
— proved efficacy on output functions of the PAG, and
— minimal mechanical irritation of surrounding tissue by insertion of the stimulation device.

To meet these criteria, we microinjected small amounts of the $GABA_A$ receptor antagonist bicuculline through fine multibarrel glass pipettes (Sandkühler et al., 1991). Bicuculline has been shown to strongly enhance the firing rate of a large proportion of PAG neurons, both in vivo and in vitro (Behbehani et al., 1990), probably by removing tonic GABAergic inhibition. The effects produced by bicuculline in the PAG are most likely due to a specific blockade of $GABA_A$ receptors, as the administration of other $GABA_A$ receptor antagonists such as picrotoxin were shown to produce qualitatively identical effects (e.g., Depaulis and Vergnes, 1986; Moreau and Fields, 1986). Further, the effects of bicuculline in the PAG can be antagonized by GABA and vice versa (Behbehani et al., 1990).

In a previous study, we have compared the effects of bicuculline (40, 200, or 400 pmol) with the effect of glutamic acid (10, 30, or 50 nmol) injected into identical PAG sites of the rat on the descending inhibition of nociceptive spinal dorsal horn neurons and on mean pressure in one carotid artery. In those experiments, bicuculline was found always to produce longer-lasting effects (20 minutes vs. 2 minutes) and was apparently more potent in activating these output functions (Sandkühler et al., 1991). GABAergic neurons and terminals are distributed throughout all subdivisions of the PAG (Barbaresi and Manfrini, 1988); consequently, effective stimulation sites were found in all subdivisions of the PAG (Sandkühler et al., 1991). Bicuculline microinjections at PAG sites have also been used in other studies (e.g., Di Scala et al., 1984; Moreau and Fields, 1986; Jacquet et al., 1987).

In most previous studies which have measured the spread of an injected substance within the central nervous system an injection volume of 0.5 μl or more was used (Myers and Hoch, 1978), i.e., volumes at least ten times larger than the 50 nl injected in the present study. These studies and our own results (Sandkühler and Gebhart, 1991) suggest that 0.5 μl may spread up to 1,000 μm from the injection site. Nicholson (1985) has calculated that 10 nl microinjected into brain tissue may spread 130–230 μm from the injection site. Thus, in the present study bicuculline may have had direct effects on neuronal discharge rates at a distance of more than 130 μm, but clearly less than 1,000 μm from the site of injection.

c-FOS labeling in sham-treated animals

Major damage to tissue along the track of the injection device could be avoided by the use of fine glass micropipettes with tip diameters of less than 40 μm. Consequently, little or no c-FOS labeling was seen in four of the sham-treated animals, either at the injection site or elsewhere in the PAG. Apparently under the present experimental conditions, the induction of anesthesia and surgery did not produce a significant expression of c-FOS in neurons of the PAG. This supports our earlier findings (Sandkühler, 1991). In one animal, labeling was somewhat more pronounced, especially bilaterally in the ventrolateral PAG, possibly due to undetected environmental stimuli (vide supra).

c-FOS labeling in stimulated animals

The pattern of c-FOS IR following chemical stimulation within the various subdivisions of the PAG is consistent with the axonal and dendritic orientation of PAG neurons. It further suggests that di- or poly-synaptic pathways were activated to recruit neurons throughout the entire rostrocaudal extent of the PAG. The extensive recruitment of neurons along the longitudinal axis of the PAG is consistent with the hypothesis of a functional columnar organisation of the PAG (Carrive and Bandler, 1991).

Stimulation in the dorsal subdivision. Stimulation in the dorsal subdivision of the rostral PAG induced strong bilateral labeling in the dorsal and dorsolateral PAG, a finding which is consistent with the orientation of dendrites and axonal projections parallel to the fibers in the posterior commissure (Beitz and Shepard, 1985). The additional labeling in all subdivisions at caudal levels of the PAG may be due to excitatory projections via a relay in the dorsolateral PAG which sends axonal projections in dorsomedial, ventral and ventrolateral directions (Beitz and Shepard, 1985). This conclusion is consistent with the results obtained by direct stimulation in the dorsolateral subdivision (vide infra).

Stimulation in the dorsolateral subdivision. Stimulation of neurons in the dorsolateral subdivision evoked dense labeling throughout all subdivisions of the caudal PAG. This labeling was strictly ipsilateral which is consistent with anatomical data showing no evidence for axonal projections crossing the sagittal plane (Beitz and Shepard, 1985). In addition, our results suggest that stimulation in the dorsolateral PAG at the level of the oculomotor nuclei did not recruit major ascending excitatory projections, the anatomical correlate of which awaits identification. In awake animals, stimulation in the dorsal or dorsolateral PAG produced a variety of effects, including antinociception (Prieto et al., 1983; Fardin et al., 1984a), aversive behavior (Atrens et al., 1977; Fardin et al., 1984a), aggressive behavior (Shaikh and Siegel, 1990), and lordosis (Sakuma and Pfaff, 1980). In anesthetized animals, stimulation in

the dorsolateral subdivision produced descending inhibition of nociceptive spinal dorsal horn neurons, tachypnoe, abdominal and fascial muscle contraction, and cardiovascular responses (Sandkühler et al., 1991). This range of effects may now be explained by the widespread activation of neurons throughout the entire rostrocaudal extent of the PAG.

Stimulation in the ventrolateral subdivision. Neurons located in the ventrolateral subdivision of the PAG project strongly to the neighboring reticular formation, but much less to the other subdivisions of the PAG (Beitz, 1985). Interestingly, stimulation in the ventrolateral PAG induced strong labeling in this subdivision and the adjoining reticular formation but failed to induce c-FOS in other parts of the PAG. In awake animals, electrical stimulation in the ventrolateral PAG or the raphe dorsalis was shown to produce antinociception without any other detectable effects (Fardin et al., 1984b). These stimulations sites were therefore considered to be "purely analgetic." The apparent absence of "side effects" may now be explained by the lack of excitatory projections to dorsal and dorsolateral areas of the PAG. This conclusion is further supported by the complete absence of c-FOS labeling in the PAG following stimulation in the raphe dorsalis (data not shown), an area from which stimulation may also produce "pure analgesia" (Fardin et al., 1984b). Of course, the ventrolateral part of the PAG may also be involved in functions, which are not readily detected in freely moving rats, e.g., in the facilitation of the arterial baroreflex (Inui et al., 1994).

Conclusions

1) The present data support the concept of subdivisions of the PAG and provide direct evidence for specific intrinsic excitatory projections between subdivisions. 2) Focal chemical stimulation at different subdivisions of the PAG may recruit a large number of neurons in the PAG, well away from the stimulation site. The pattern of activation is of high topological order. 3) The large rostrocaudal spread of neurons activated from some of the subdivisions is consistent with the hypothesis of a columnar organization in the PAG. 4) The functional consequences of stimulation at PAG sites may now be interpreted not only on the basis of efferents leaving the PAG from the site of stimulation, but also of efferents leaving the PAG from all other activated areas.

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