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THE ORGANIZATION AND FUNCTION OF ENDOGENOUS ANTINOCICEPTIVE SYSTEMS

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Abstract—Much progress has been made the understanding of endogenous pain-controlling systems. Recently, new concepts and ideas which are derived from neurobiology, chaos research and from research on learning and memory have been introduced into pain research and shed further light on the organization and function of endogenous antinociception. These most recent developments will be reviewed here. Three principles of endogenous antinociception have been identified, as follows. (1) Supraspinal descending inhibition: the patterns of neuronal activity in diencephalon, brainstem and spinal cord during antinociceptive stimulation in midbrain periaqueductal gray (PAG) or medullary nucleus raphe magnus have now been mapped on the cellular level, using the c-Fos technique. Results demonstrate that characteristic activity patterns result within and outside the PAG when stimulating at its various subdivisions. The descending systems may not only depress mean discharge rates of nociceptive spinal dorsal horn neurons, but also may modify harmonic oscillations and nonlinear dynamics (dimensionality) of discharges. (2) Propriospinal, heterosegmental inhibition: antinociceptive, heterosegmental interneurons exist which may be activated by noxious stimulation or by supraspinal descending pathways. (3) Segmental spinal inhibition: a robust long-term depression of primary afferent neurotransmission in Aδ fibers has been identified in superficial spinal dorsal horn which may underlie long-lasting antinociception by afferent stimulation, e.g. by physical therapy or acupuncture. Copyright © 1996 Elsevier Science Ltd.

CONTENTS

ABBREVIATIONS

2-DG	2-Deoxyglucose	IR	Immunoreactivity
5-HT	5-Hydroxytryptamine	LTP	Long-term potentiation
CD	Coefficient of dispersion	NMDA	*N*-methyl-D-aspartate
D-APV	D-2-amino-5-phosphonovalerate	NRM	Nucleus raphe magnus
EPSP	Excitatory postsynaptic potential	NRPGα	Nucleus reticularis paragigantocellularis pars α
GABA	γ-Aminobutyric acid	PAG	Periaqueductal gray

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1. INTRODUCTION

Reynolds had reported that an exploratory laparotomy could be performed in unanesthetized rats when nociceptive responses are suppressed by electrical stimulation at midbrain sites, mainly the periaqueductal gray and adjoining periventricular gray (Reynolds, 1969). Since this discovery, endogenous antinociceptive mechanisms have been investigated intensively in numerous mammals, including man (Hosobuchi *et al.*, 1977; Peyron *et al.*, 1995). It is now well established that endogenous antinociception can be activated by environmental stimuli, such as stress (Millan *et al.*, 1980; Jayaram *et al.*, 1995) e.g. on the battle-field (Beecher, 1946) or during long-distance running (Henry, 1982) or sexual activity (Henry, 1982; Watkins *et al.*, 1984). Conditioning stimuli of peripheral tissues, e.g. some forms of acupuncture (Bing *et al.*, 1990; Ishimaru *et al.*, 1995; Du and Chao, 1976), also may activate endogenous antinociception. Some endogenous antinociceptive systems may display a circadian rhythm (Berge, 1982; Oliverio *et al.*, 1982) leading to regular, systematic fluctuation in nociceptive thresholds (Oliverio *et al.*, 1982; Grabfield and Martin, 1913; Wright, 1981) which may be correlated with mood states (Rogers and Vilkin, 1978). Animals may learn to activate their endogenous antinociceptive systems by classical conditioning (Chance *et al.*, 1977; Watkins *et al.*, 1982). The most powerful analgesic substances, the opioids, exert their effects in part by activating endogenous antinociceptive systems (Gogas *et al.*, 1991; Gebhart *et al.*, 1984; see, however, Duggan *et al.*, 1980; Dickenson and Le Bars, 1987). A major component of antinociception present under these various conditions disappears when the spinal cord is separated functionally from the brainstem (Watkins *et al.*, 1984; Kelly and Franklin, 1984; Du and Chao, 1976; Berge, 1982). This suggests that pathways descending from supraspinal sites to the spinal cord are responsible for a portion of endogenous antinociception. In addition, an afferent-induced segmental form of antinociception has been described (Melzack and Wall, 1965). Both segmental and supraspinal descending antinociceptive systems are now used clinically to alleviate pain in humans (Long and Hagfors, 1975; Hosobuchi *et al.*, 1977).

The midbrain periaqueductal gray (PAG) and the medullary nucleus raphe magnus (NRM) have attracted most attention, and descending inhibition from these brainstem sites has been analyzed in the greatest detail. However, numerous additional antinociceptive stimulation sites have now been identified in brainstem, diencephalon and cortex. Various aspects of endogenous antinociceptive systems have been reviewed earlier (Fields and Basbaum, 1978; Gebhart, 1982; Besson and Chaouch, 1987; Zieglgänsberger *et al.*, 1986; Fields *et al.*, 1991) and they will not be reproduced here. The present review, rather, will focus on most recent developments in the understanding of organization and function of endogenous antinociceptive systems. Data, mainly from our own laboratory, will be summarized; these show that antinociceptive stimulation at brainstem sites produces a complex activation pattern throughout the central nervous system, strongly suggesting that antinociception is only one of an array of effects of these stimulations. Evidence is now available that not only segmental and supraspinal descending antinociceptive mechanisms exist, but that also propriospinal, heterosegmental neurons are relevant to antinociception. These antinociceptive systems may not only depress discharge rates of nociceptive neurons in the spinal cord, but may also change nonlinear dynamics of discharges and affect discharge patterns of nociceptive neurons such as oscillations and burst-like discharges. Simultaneous recordings from two or more nociceptive neurons at the same site in the spinal dorsal horn revealed that tonic descending excitation and tonic descending depression are active simultaneously and may affect the synchronization of discharges of spinal dorsal horn neurons. Evidence will be summarized showing that long-term potentiation of synaptic transmission of fine primary afferent nerve fibers exists in superficial spinal dorsal horn, possibly mediating plastic changes of spinal nociception and that intact tonic descending systems may prevent such changes. Finally, recent studies have identified a robust long-term depression of primary afferent neurotransmission in the superficial spinal dorsal horn, possibly mediating afferent-induced segmental antinociception.

2. FUNCTIONAL ANATOMY OF ENDOGENOUS ANTINOCICEPTIVE SYSTEMS

Much progress has been made in elaborating topography and pathways of endogenous antinociceptive systems (see reviews by Fields and Basbaum, 1978; Basbaum and Fields, 1984; Willis, 1988; Besson and Chaouch, 1987). In most of the previous studies, the functional anatomy of antinociceptive systems has been investigated by means of traumatic lesions or reversible local anesthetic blocks of selected brainstem nuclei or fiber tracts. Only recently it has become possible to map stimulation-produced neuronal activity on the cellular level, using the expression of the c-Fos protein as a marker of activated neurons. We have utilized this method to evaluate activity patterns in diencephalon, brainstem and spinal cord during antinociceptive stimulation.

2.1. Distribution of Antinociceptive Stimulation Sites in Midbrain

To identify the location of antinociceptive neurons in the midbrain of rats, we have varied systematically the stimulation sites in the midbrain and compared heat-evoked responses of lumbar spinal dorsal horn neurons before and during stimulation (Sandkühler *et al.*, 1991b). Microinjection of small quantities of either L-glutamate (10–50 pmol in 60 nl) or bicuculline, a $GABA_A$ receptor antagonist (40–400 pmol in 60 nl) through fine glass micropipettes, were used either to excite neurons directly, but not fibers of passage, or to increase neuronal activity by disinhibition. Effective stimulation sites were found

throughout the PAG (Figs 1 and 2), whereas ineffective injection sites were located mainly in the reticular formation lateral to the PAG (Figs 1 and 2).

Within the PAG, effective and ineffective stimulation sites were intermingled (data not shown).

2.2. Patterns of Activated Neurons During Antinociceptive Stimulation

Electrically, pharmacologically or behaviorally induced antinociception is accompanied typically by additional, often unwanted 'side-effects' including cardiovascular responses, aversion or sedation (Fardin *et al.*, 1984). Even when stimulating at proclaimed 'pure analgesic' brainstem sites, it cannot be excluded that additional, undetected effects were produced. To better describe neuronal systems which can be activated during antinociceptive stimulation in the midbrain PAG or medullary NRM, we have used expression of the proto-oncogene c-*fos* as a cellular marker of activated neurons (Sagar *et al.*, 1988; Dragunow and Faull, 1989). Mono- or polysynaptic activation may induce c-Fos in many, albeit not all, neurons of the central nervous system, probably via modification of open time of voltage-dependent calcium channels (Morgan and Curran, 1986). Inhibitory afferents and hyperpolarization of neurons have not been reported to induce c-*fos*. Neurons are not the only group of cells in the brain which may express c-Fos protein. Glial cells may respond with an enhanced expression of c-Fos to heat-shock stimulation (Dragunow *et al.*, 1989) or following cortical trauma (Dragunow and Robertson, 1988), but not following depolarization (Hisanaga *et al.*, 1990). We have not attempted to prove that all c-Fos positive cells were neurons, the number and pattern of stained cells suggest, however, that the vast majority were indeed neurons.

The uptake of 2-deoxyglucose (2-DG) also has been used as a marker of neuronal activity (Duncan *et al.*, 1987) and, in most brain regions and in the spinal cord, both 2-DG uptake and c-Fos expression are enhanced following stimulation (Duncan *et al.*, 1993). However, at some regions, a mismatch between 2-DG uptake and c-Fos expression was reported. This may be due to the fact that Fos-immunoreactivity (IR) may detect only the activity of the cell body, while 2-DG-uptake also is sensitive to axonal and dendritic activity (Sagar *et al.*, 1988; Dragunow and Faull, 1989). An increased 2-DG-uptake without corresponding c-Fos induction also may indicate that some neurons never express c-Fos, no matter what stimulus is applied (Dragunow and Faull, 1989). Possibly, stimulation was given during a 'refractory period' for the expression of c-Fos, which also would yield false negative results (Morgan *et al.*, 1987). Conversely, Fos-IR may detect activated neurons in some regions of the brain, e.g. in the hypothalamic magnocellular nuclei, which failed to show an elevated 2-DG uptake upon stimulation (Sagar *et al.*, 1988). The general anesthetic pentobarbital, which was given in part of our experiments, may not induce c-*fos* by itself, but rather may depress its expression (Morgan *et al.*, 1987; Menétrey *et al.*, 1989).

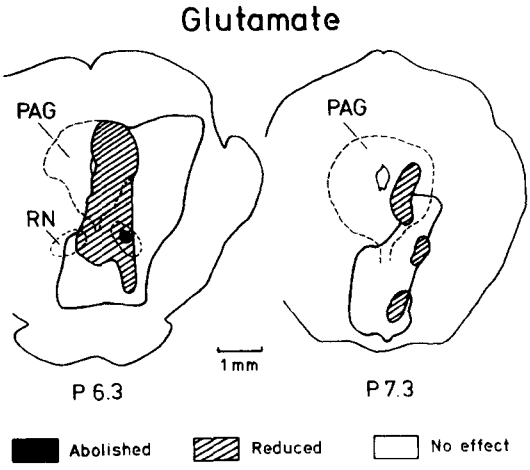

Fig. 1. Descending inhibition of noxious heat-evoked responses of lumbar spinal dorsal horn neurons by glutamate microinjection at midbrain sites. Summary of the effects of 80 microinjections of L-glutamic acid (60 nl, 0.5 μM). Sites at which glutamate microinjections abolished noxious heat-evoked responses in spinal dorsal horn are indicated by the black area; sites at which microinjections reduced heat-evoked responses are represented by hatched areas; open areas indicate absence of effective injection sites. In addition, ineffective injection sites also were found in hatched areas. Inhibition by more than 20% of control was considered effective. Anatomic boundaries of the periaqueductal gray (PAG) and the red nucleus (RN) are drawn as broken lines.

2.2.1. *Background C-Fos Immunoreactivity in Brainstem Neurons*

We have described recently the distribution of c-Fos-positive cells in brainstem (Sandkühler, 1991) including PAG (Sandkühler and Herdegen, 1995) and spinal cord (Bett and Sandkühler, 1995) following chemical stimulation in the PAG or NRM, respectively. Adult rats, which were anesthetized deeply in the animal house prior to transportation to the laboratory and which were perfused 15–30 min later, served as controls and had no or only very faint background Fos-IR, mainly in the superior colliculus. Sham-treated animals, which received a stereotaxic surgery and a microinjection of vehicle into the PAG, showed additional c-Fos expression throughout the ipsilateral cortex, mainly in the primary olfactory cortex in the islands of Cajal. This may be due to a cortical spreading depression (Dragunow and Robertson, 1988; Herdegen *et al.*, 1993). At the injection sites, no or only faint staining was present in sham-treated animals [Fig. 3(A)].

When animals are exposed to unspecific, eventually stressful stimuli such as transporting naive, awake animals to the laboratory or applying intraperitoneal injection, Fos-IR may be enhanced strongly in numerous brainstem areas, including the PAG, the medial hypothalamus and the nuclei of the solitary tract. This pattern overlapped with Fos-IR following stimulation in the dorsal PAG. Thus, no firm conclusions may be drawn from background labeling on a basal constitutive expression of c-Fos without a detailed description of the immediate past history of

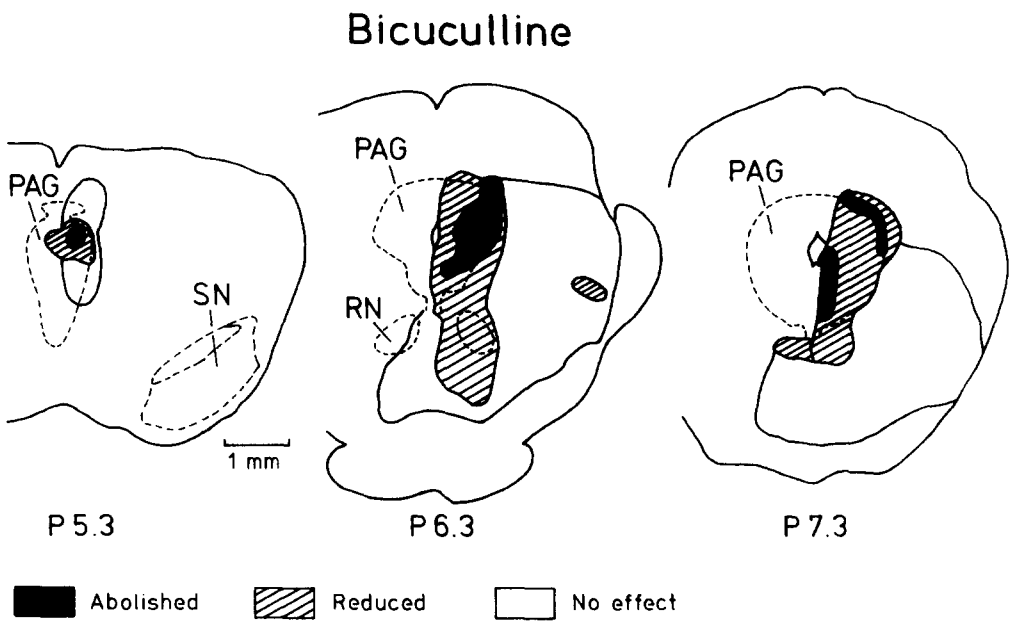

Fig. 2. Descending inhibition of noxious heat-evoked responses of lumbar spinal dorsal horn neurons by bicuculline microinjection at midbrain sites. The summary is based on 120 microinjections of 40–400 pmol bicuculline in 60 nl. Complete inhibition of noxious heat-evoked responses was produced by microinjections into the black areas; significant reduction of heat-evoked responses by more than 20% of control were evoked by microinjections into the hatched areas. No effective sites were found within the open areas. The broken lines indicate boundaries of the substantia nigra (SN), red nucleus (RN) and periaqueductal gray (PAG).

the animals. The procedure of handling, the route of drug application and control of body temperature need to be described.

2.2.2. *Activity Patterns Within the Periaqueductal Gray*

Disinhibition of neurons by blockage of $GABA_A$-receptors with bicuculline is a very powerful method for activation of descending inhibitory pathways from the PAG (Sandkühler *et al.*, 1989; Sandkühler *et al.*, 1991b) and for depressing nociceptive reflexes (Moreau and Fields, 1986). It is likely that the activation of descending inhibition by substances such as morphine (Gebhart *et al.*, 1984; Morgan *et al.*, 1992) or the neuropeptide somatostatin (Helmchen *et al.*, 1995) is due also to disinhibition (Zieglgänsberger *et al.*, 1979). Here, we use the microinjection of small quantities of bicuculline to disinhibit neurons within the various subdivisions of the PAG (Sandkühler and Herdegen, 1995).

2.2.2.1. *Microinjections of bicuculline into the dorsal PAG*

The c-Fos immunoreactivity was found throughout the entire rostrocaudal extend of the PAG (Fig. 4). At rostral levels, c-Fos immunoreactivity was found bilaterally in the dorsal PAG [Fig. 3(B) and Fig. 4(B)] with very little labeling in the medial, the lateral or ventral PAG. At the level of the oculomotor [Fig. 4(D)] and cochlear nuclei [Fig. 4(E)], c-Fos immunoreactivity also was prominent bilaterally in the dorsal and dorsolateral subdivisions and to a lesser extent in the medial PAG. Caudal to the decussation of the superior peduncles [Fig. 4(G) and (H)], c-Fos immunoreactivity was distributed in all subdivisions. The bilateral labeling throughout the dorsal and dorsolateral PAG corresponds well to 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 relays in the dorsolateral PAG which send axonal projections in dorsomedial, ventral and ventrolateral directions (Beitz and Shepard, 1985).

2.2.2.2. *Stimulation in the dorsolateral PAG*

Stimulation in the dorsolateral subdivision at the level of the oculomotor nuclei produced strong c-Fos immunoreactivity only ipsilateral and caudal to the injection sites (Fig. 5). At the level of the injection, c-Fos immunoreactivity was strong in the dorsolateral and dorsal PAG, while c-Fos positive cells were sparse in the medial and ventrolateral PAG [Fig. 5(C)]. 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. 3(D) and Fig. 5(D)–(F)]. At the caudal most levels of the PAG, most c-Fos-positive cells were located in the ventrolateral PAG and significantly fewer cells were labeled within the dorsal PAG [Fig. 5(G) and (H)]. This strict ipsilateral labeling is consistent with anatomical data showing

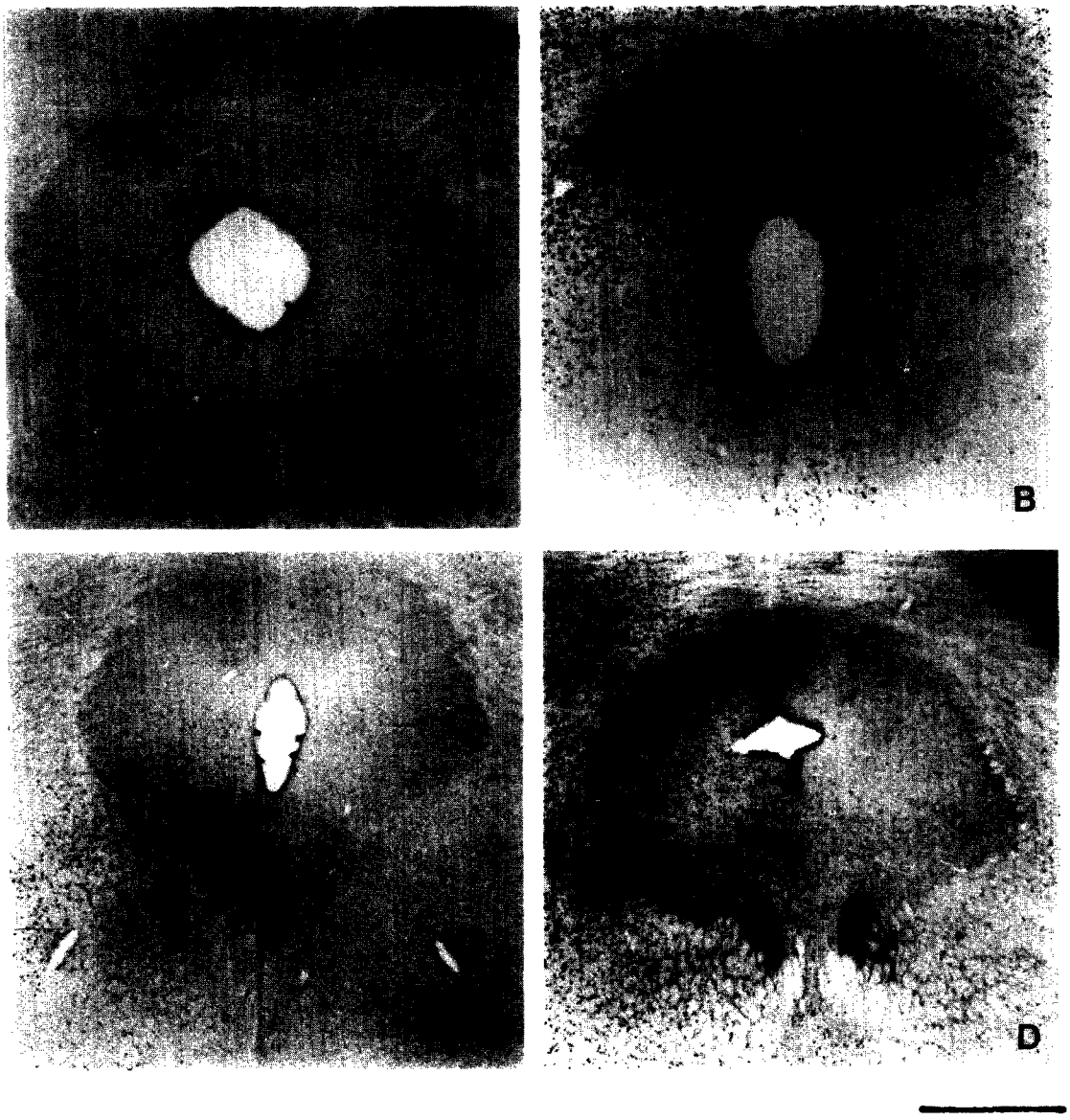

Fig. 3. Representative examples of c-Fos immunoreactivity in sections through the midbrain periaqueductal gray following microinjection of saline (A) or bicuculline [200 pM, 50 nl, (B)–(D)]. Four different rats received either a sham injection into the ventrolateral PAG (A) or bicuculline injections into the dorsal PAG (B), the ventrolateral PAG (C), or the dorsolateral PAG (D). Dorsal injections typically induced a bilateral c-Fos immunoreactivity which was most prominent in the dorsal half of the PAG, while ventrolateral injections produced c-Fos immunoreactivity which was restricted to the ventrolateral quadrant and adjoining reticular formation. Dorsolateral injections produced unilateral c-Fos immunoreactivity in dorsal and ventral PAG and adjoining reticular formation. The calibration bar at the bottom equals 1000 μm [(B) anad (C)] or 850 μm [(A) and (D)]. The approximate anterior–posterior level of the sections with reference to bregma are given in mm: (A) − 6.3; (B) − 6.3; (C) − 5.8; (D) − 7.3.

no evidence for axonal projections crossing the sagittal plane (Beitz and Shepard, 1985). In unanesthetized drug-free animals, stimulation in the dorsal or dorsolateral PAG may produce a variety of effects, including antinociception (Prieto *et al.*, 1983; Fardin *et al.*, 1984), aversive behavior (Atrens *et al.*, 1977; Fardin *et al.*, 1984), agressive behavior (Shaikh and Siegel, 1990) and lordosis (Sakuma and Pfaff, 1980); see Behbehani (1995) for a recent review. In anesthetized animals, stimulation in the dorsolateral subdivision may produce descending inhibition of nociceptive spinal dorsal horn neurons together with tachypnea, abdominal or facial muscle contraction, and cardiovascular responses (Sandkühler *et al.*, 1991b). This wide range of effects may now be explained by the extensive activation of neurons throughout the entire rostrocaudal extent of the PAG.

2.2.2.3. *Stimulation in the ventrolateral PAG*

Stimulation in the ventrolateral subdivision at the level of the oculomotor nuclei induced c-Fos mainly ipsilaterally to the injection site in the lateral and ventrolateral PAG at the level of the injection site and further rostrally (Fig. 6). Rostral to the injection site, labeled cells were found bilaterally in the ventrolateral subdivision [Fig. 6(A) and (B)]. At the ipsilateral site, labeling extended into the lateral and dorsolateral PAG, but not including the dorsal PAG. Caudal to the level of stimulation, labeling was reduced progressively and disappeared almost completely caudal to the level of the cochlear nuclei [Fig. 6(F)–(H)]. This pattern of labeling is explained by the fact that neurons located in the ventrolateral subdivision of the PAG have strong projections to neighboring reticular formation but rather few projections to

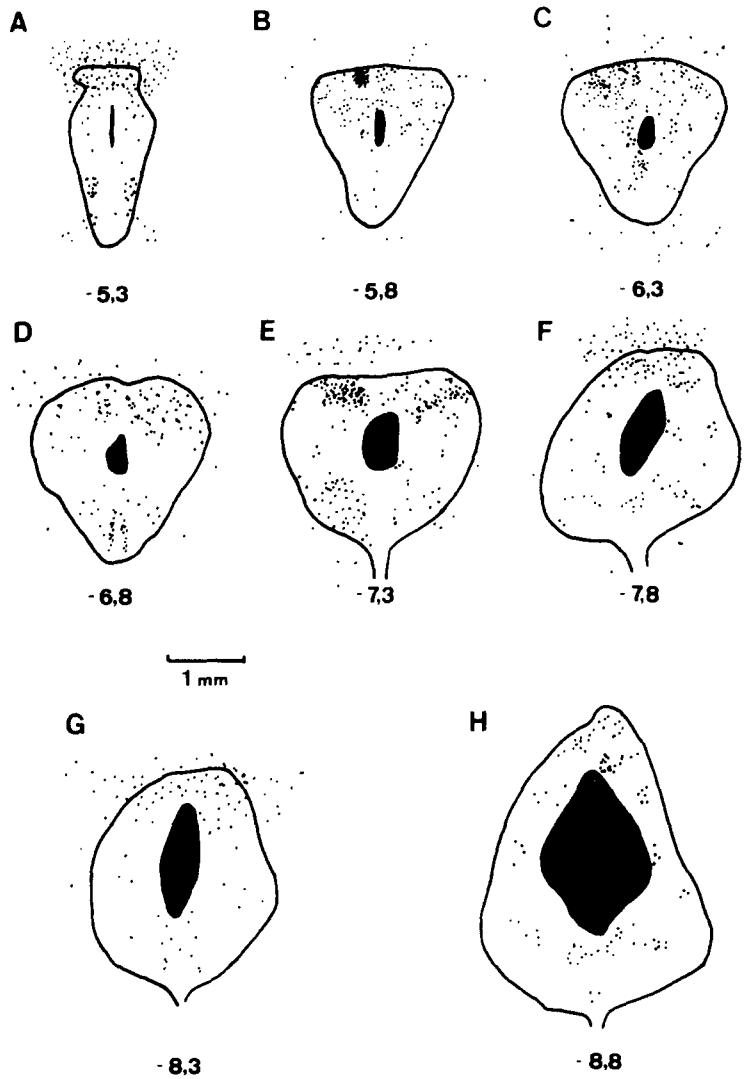

Fig. 4. Schematic illustration of c-Fos immunoreactivity in one rat which received a bicuculline injection into the dorsal PAG at the level of the posterior commissure. Each dot represents one c-Fos positive cell in a 50 μm thick section. Similar results were obtained in three other animals. In (B) the site of injection is marked by the high density of c-Fos positive cells in the dorsal PAG. Anterior–posterior levels of the sections with reference to bregma are given in mm.

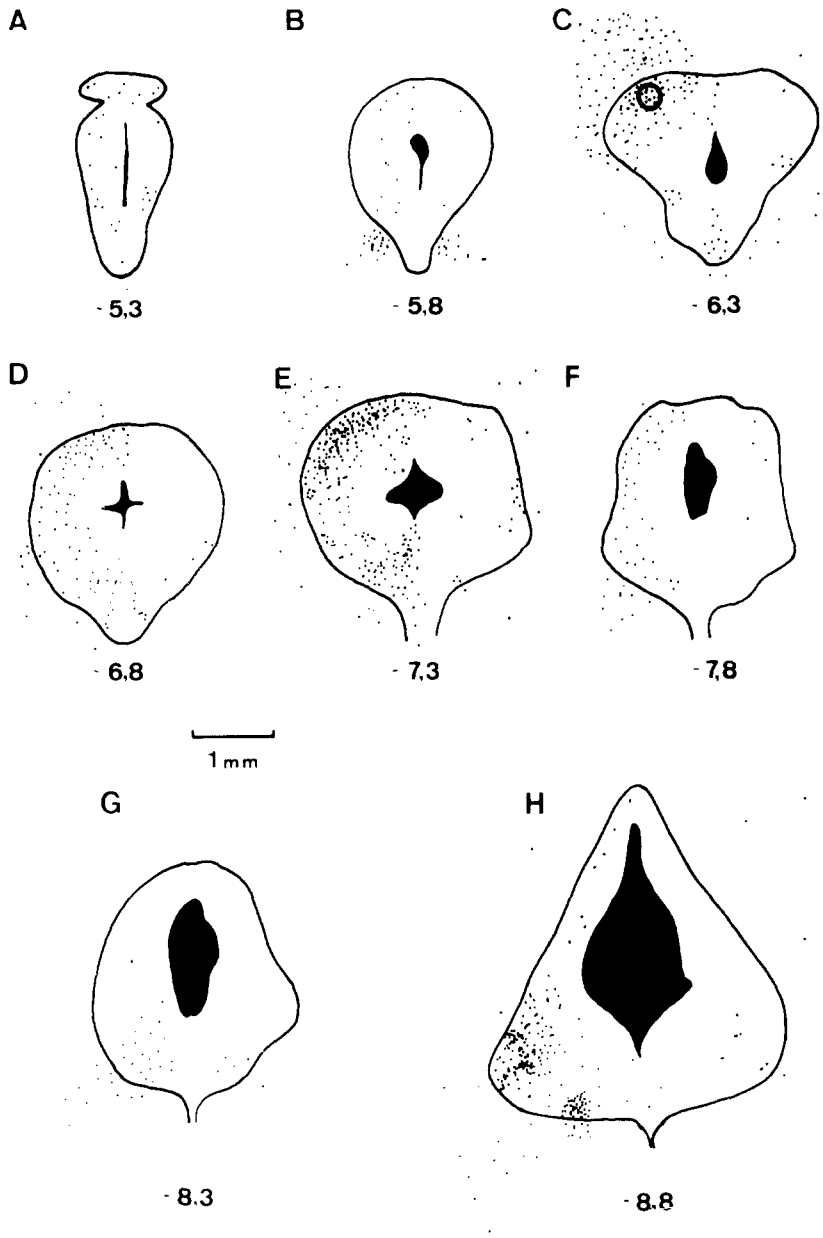

Fig. 5. Distribution of c-Fos positive cells in one representative animal following bicuculline injection into the dorsolateral PAG at the level of the oculomotor nuclei. The injection site is indicated by an open circle in (C). Similar results were obtained in three other animals.

other subdivisions of the PAG (Beitz and Shepard, 1985). Correspondingly, stimulation in the ventrolateral subdivision of the PAG induced strong c-Fos labeling within this subdivision and within the adjoining reticular formation but failed to induce c-Fos in other parts of the PAG. Electrical stimulation in the ventrolateral PAG of awake animals may produce antinociception without any other detectable effects (Gioia *et al.*, 1985). These stimulation sites were considered, therefore, 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. Of course, the ventrolateral PAG also may 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).

2.2.3. *Activation Patterns in Brainstem and Diencephalon*

Stimulation in the dorsal part of the PAG typically produces a wide variety of effects in addition to antinociception, which can be evoked from dorsal and from ventral subdivisions. See Behbehani (1995) for a recent review, and chapters in Depaulis and Bandler (1991). Therefore, we describe the pattern of labeled cells throughout the brainstem and diencephalon in two rats which received a dorsal injection

of bicuculline (Sandkühler, 1991), from rostral to caudal.

2.2.3.1. *Activity pattern in diencephalon*

The supraoptic nuclei contained dense Fos-IR [Fig. 7(B)] after stimulation in the dorsal PAG, compared to sham-treated animals. The pathway for this activation is not known, as neurons in the supraoptic nuclei propably do not receive direct projections from the PAG. Possibly a disynaptic pathway with a relay in the nuclei of the solitary tract (vide infra) may be involved (Raby and Renaud, 1989). The activation of neurons in the supraoptic nuclei may be involved in nonspecific increase in vasopressin release (Shibuki and Yagi, 1986). Labeled cells also were found in the suprachiasmatic nucleus (Fig. 7A).

The medial hypothalamus contained numerous c-Fos positive cells (Fig. 8) overlapping with areas which receive direct projections from the dorsal PAG (Mantyh, 1983a) and overlapping with areas from which defence and flight behavior (Siegel and Pott, 1988), as well as descending inhibition of multireceptive spinal dorsal horn neurons (Carstens, 1986), can be elicited by electrical stimulation. In the lateral hypothalamus, labeling was not elevated above control.

2.2.3.2. *Activity pattern in brainstem*

Labeled cells were also found bilaterally in the zona incerta [Fig. 9(C)] which receives a direct projection from the PAG (Mantyh, 1983a). Labeled cells also were found in dorsal and medial

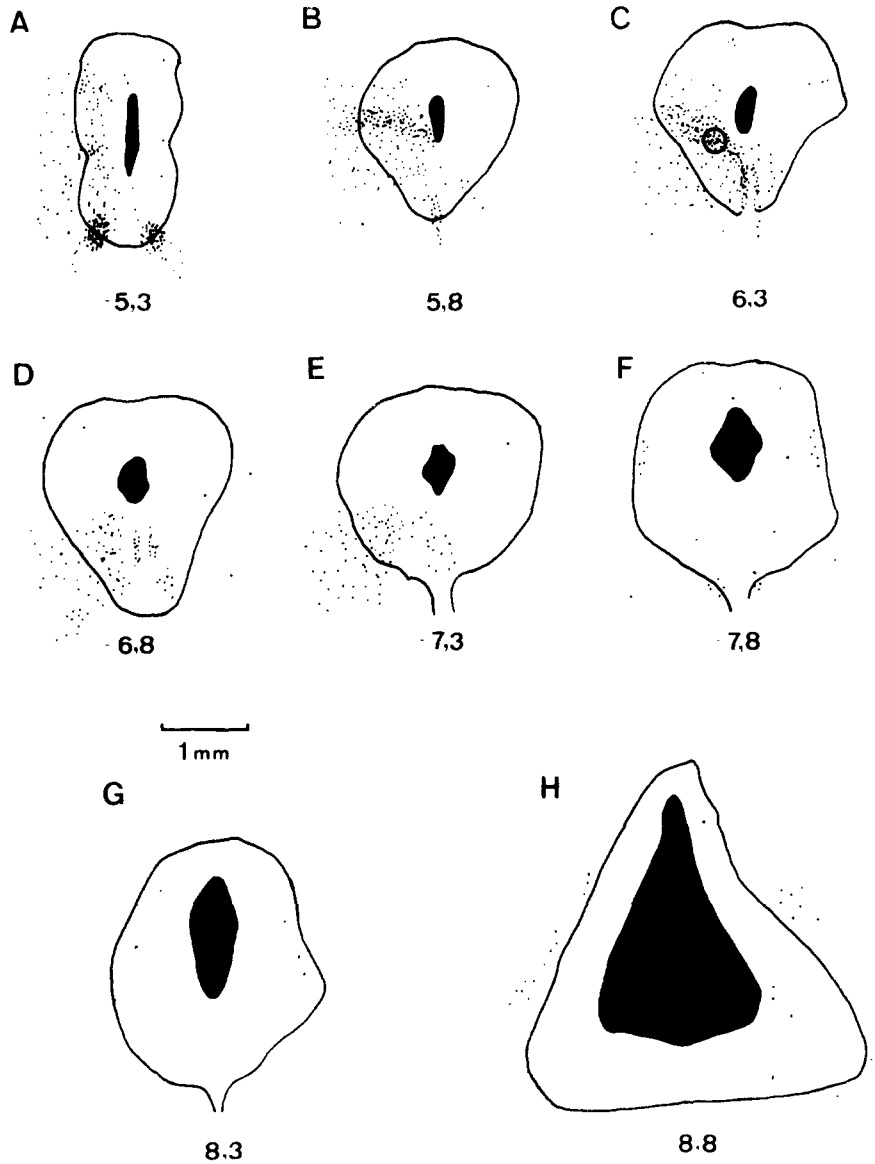

Fig. 6. Pattern of c-Fos immunoreactivity in one rat which received a bicuculline injection into the ventrolateral PAG at the level of the oculomotor nuclei. The injection site is indicated by a circle in C.

parts of the periventricular gray matter [Fig. 9(B)] and dorsolateral to the posterior commissure [Fig. 9(A)].

Dense c-Fos labeling was found in all layers of the superior colliculus which is in line with a strong anatomical projection from the PAG to the superior colliculus (Mantyh, 1983b). Neurons in the superior colliculus may play a role for species specific defence reactions (Dean *et al.*, 1988) and for cardiovascular and respiratory responses (Keay *et al.*, 1988). Some of the neurons in the superior colliculus can be activated by noxious stimuli (Keay and Bandler, 1993).

A direct projection from the PAG to the NRM and to the neighboring nucleus reticularis paragigantocellularis pars alpha (NRPGα) exists which is believed to be an important part of descending antinociceptive systems (see early reviews by Basbaum and Fields, 1979, 1984). Indeed, bilateral local anesthetic blocks of the NRM and the NRPGα led to an increase in threshold for PAG stimulation to inhibit the spinally mediated rat tail-flick reflex (Sandkühler and Gebhart, 1984a). In cats, similar lidocaine blocks reduce the efficacy of PAG-induced descending inhibition of nociceptive spinal dorsal horn neurons (Gebhart *et al.*, 1983). Here, stimulation at PAG sites induced c-Fos in cells of the NRM and the NRPGα (Fig. 10) bilaterally. This provides further evidence that both nuclei are activated by chemical stimulation in the PAG. However, the number of c-Fos positive cells in the ventromedial medulla was relatively low, compared to some densely labeled areas in the brainstem such as the nucleus locus coeruleus (Fig. 11). A similar, moderate increase was observed in 2-DG uptake in the NRM (+8%) following electrical stimulation in the PAG (Beitz and Buggy, 1981). In contrast, 2-DG uptake was strong (+36%) in the NRPGα in the same study. Possibly, relatively few neurons in the NRM are activated during PAG stimulation. Hentall *et al.* (1984) have estimated that less than 30–75 neurons in the ventromedial medulla need to be activated to suppress the rat tail-flick reflex. These few neurons may, however, exert a strong inhibitory effect on spinal processing of nociceptive information. These neurons may be identical to 'OFF-cells' which have a firing pattern during the tail-flick suggestive of an ability to suppress the reflex when excited continuously (Hentall *et al.*, 1991).

The nucleus locus coeruleus displayed densely packed and intensively stained neurons bilaterally (Fig. 11). This region receives a direct projection from the PAG (Mantyh, 1983b); see, however, (Aston-Jones *et al.*, 1986). In sharp contrast to the present strong induction of c-Fos in the nucleus locus coeruleus, 2-DG uptake was not increased significantly during electrical stimulation in the PAG (Beitz and Buggy, 1981). While raphe-spinal neurons are the major source of serotonergic terminals in the spinal cord, neurons in the nucleus locus coeruleus are a primary source of noradrenergic spinal terminals (see Fields *et al.*, 1991, for a review). The α_2-adrenoceptor agonists (Hämäläinen and Pertovaara, 1995) and 5-$HT_{1,2,3}$ receptor agonists (Danzebrink and Gebhart, 1991) may depress spinal nociceptive neurons. The role of these monoamines in spinal nociception has been investigated intensively; see Lima *et al.* (1991) for a recent review. Noradrenergic spinopetal neurons of the nucleus locus coeruleus are believed to mediate descending antinociception elicited by stimulation in the PAG, as α-adrenergic receptors are involved in PAG-induced descending inhibition of the tail-flick reflex (Jensen and Yaksh, 1984; Janss *et al.*, 1987; Aimone and Gebhart, 1987). In contrast, the descending inhibition of single nociceptive spinal dorsal horn neurons was not affected by application of α-adrenergic or serotoninergic receptor blockers near the recording site, either by superfusion of the spinal cord at the recording segment (Sandkühler and Zimmermann, 1988) or by iontophoretic application (Dupont *et al.*, 1982). This may indicate that the relevant spinal monoaminergic synapses may be remote from the recording site in lumbar spinal dorsal horn. Evidence presented in Section 2.4. will show that propriospinal, heterosegmental neurons are capable of inhibiting nociceptive neurons in the lumbar spinal dorsal horn and may be relays for descending inhibition. Possibly, the intrathecal injection of monoaminergic receptor blockers which are used in behavioral studies may reach these heterosegmental relays, while local application in electrophysiological studies do not. This might explain the discrepancy between the well-established blockade of descending inhibition of nociceptive behavior by intrathecal injection of monoaminergic blockers and the failure of the same substances to block descending inhibition of nociceptive lumbar spinal dorsal horn neurons. Noradrenergic neurons in the nucleus locus coeruleus also may be involved in the stress response (Abercrombie and Jacobs, 1987), which may be part of a complex behavioral pattern elicited by stimulation in the PAG.

Labeled cells were found consistently in the caudal ventrolateral medulla including the lateral reticular nucleus [Fig. 12(B)]. This is in line with a direct anatomical projection from the PAG to the lateral reticular nucleus (Mantyh, 1983b). Since electrical stimulation in the lateral reticular nucleus of rat also produces a descending inhibition (Janss *et al.*, 1987) and in cat electrolytic lesions of the lateral reticular nucleus reduce PAG-induced descending inhibition (Morton *et al.*, 1983), spinopetal neurons in the lateral reticular nucleus may be another relay for descending inhibition. Neurons in the lateral reticular nucleus also may mediate cardiovascular responses such as fall in mean arterial blood pressure (Bonham and Jeske, 1989).

The c-Fos immunoreactivity was strong in the solitary tract nuclei [Fig. 12(A)], which suggests that a direct anatomical projection from the PAG to the solitary tract nuclei (Bandler and Tork, 1987) is, at least in part, excitatory. The 2-DG uptake in this region rose moderately (by 14%) following electrical stimulation in the PAG (Beitz and Buggy, 1981). Neurons in the solitary tract nuclei may be involved in the modulation of cardiovascular function and nociception (Du and Zhou, 1990) induced by stimulation at PAG sites.

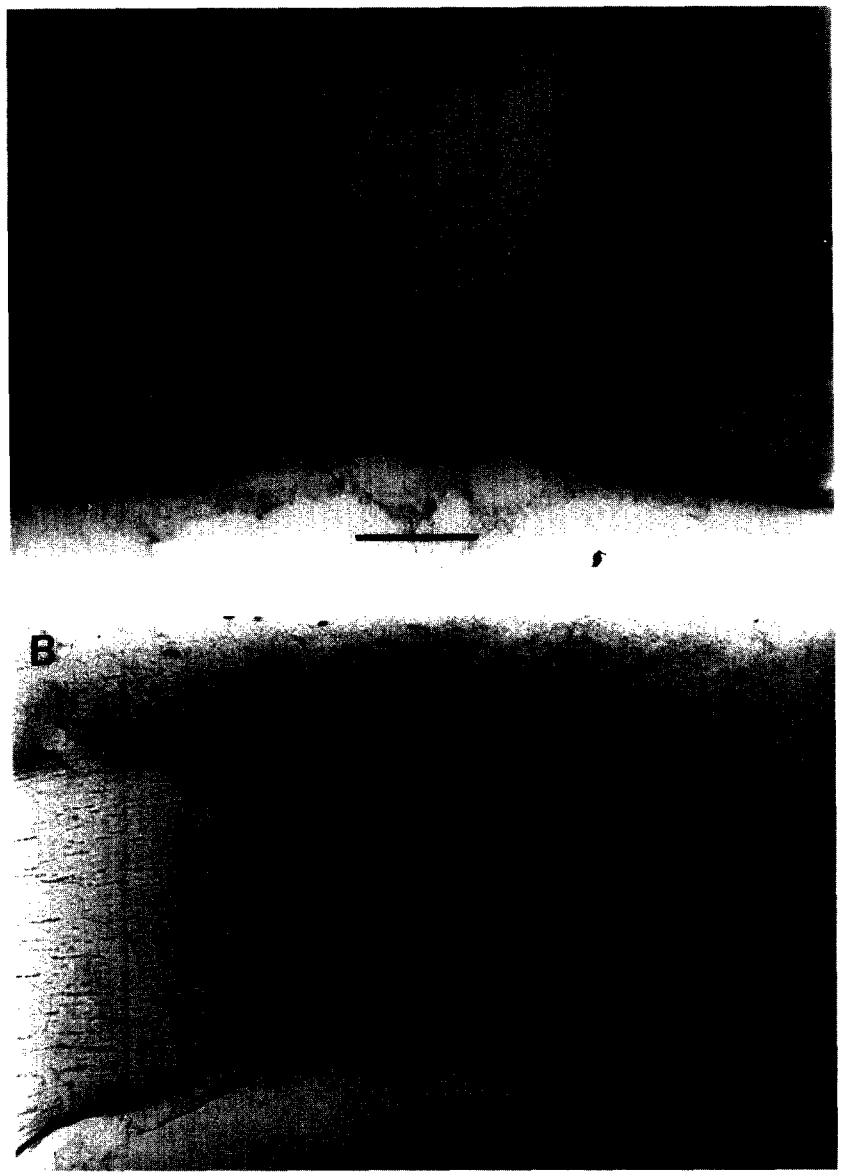

Fig. 7. The c-Fos immunoreactivity in one animal which received a bicuculline injection into the dorsal PAG at the level of the commissure of the superior colliculus, 5.3 mm caudal to bregma. (A) The c-Fos positive cells are shown in suprachiasmatic nucleus bilaterally. (B) Densely stained neurons in the supraoptic nucleus contalateral to the injection site. Similar staining was found on the ipsilateral site. Scale bars indicate 200 μm.

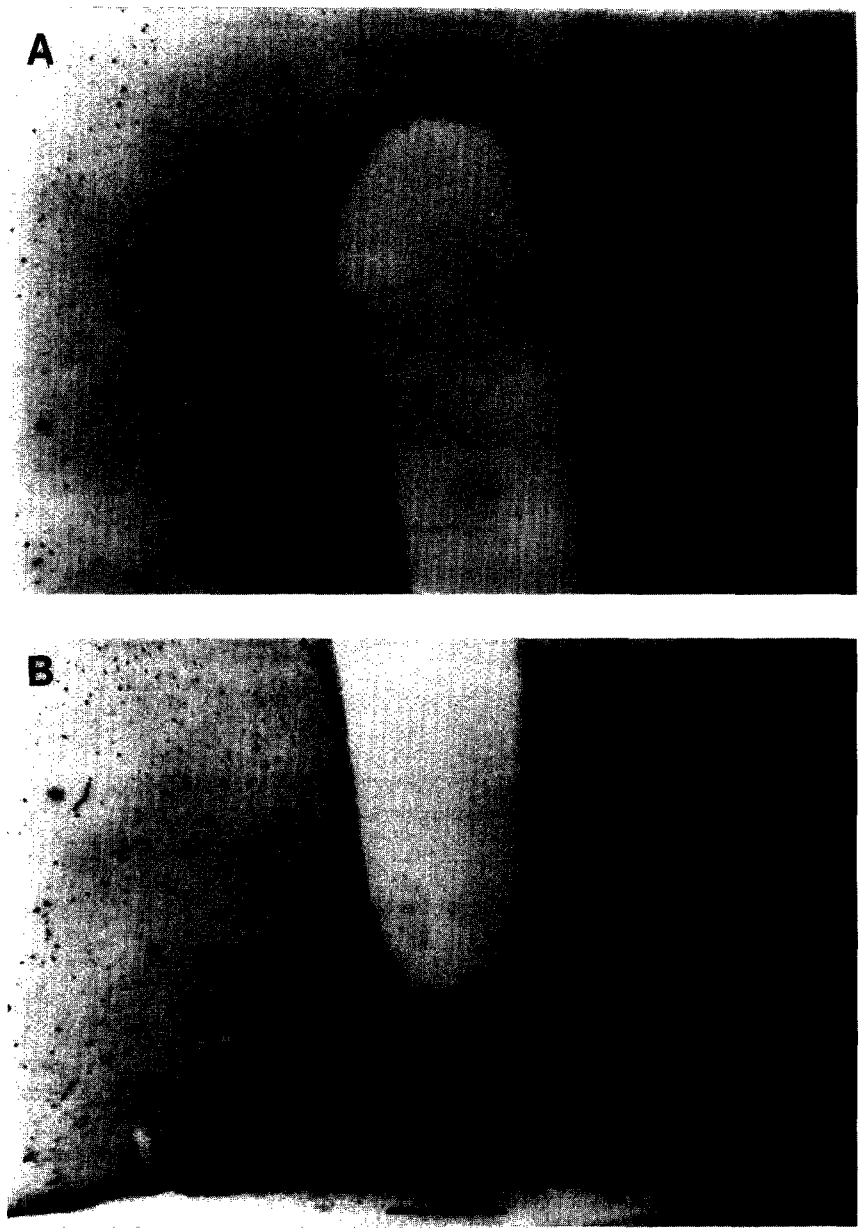

Fig. 8. The c-Fos immunoreactivity in the dorsal part of the medial hypothalamus (A) and in periventricular hypothalamic nucleus and in the median eminence (B) following stimulation in the dorsal PAG. Same animal as in Fig. 7. Scale bar indicates 600 μm.

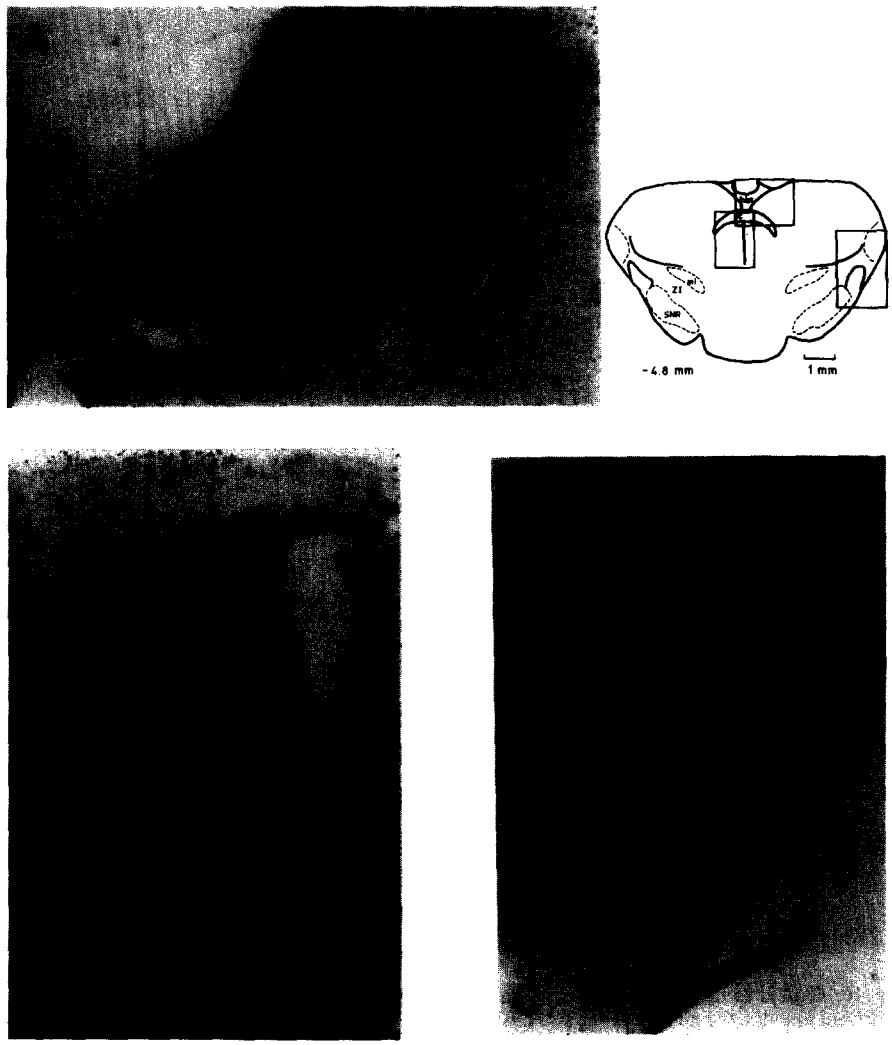

Fig. 9. Distribution of c-Fos positive cells dorsolateral to the posterior commissure (A), in dorsal and medial parts of the periventricular gray matter (B), and in the zona incerta (C). Same animal as in Fig. 7.

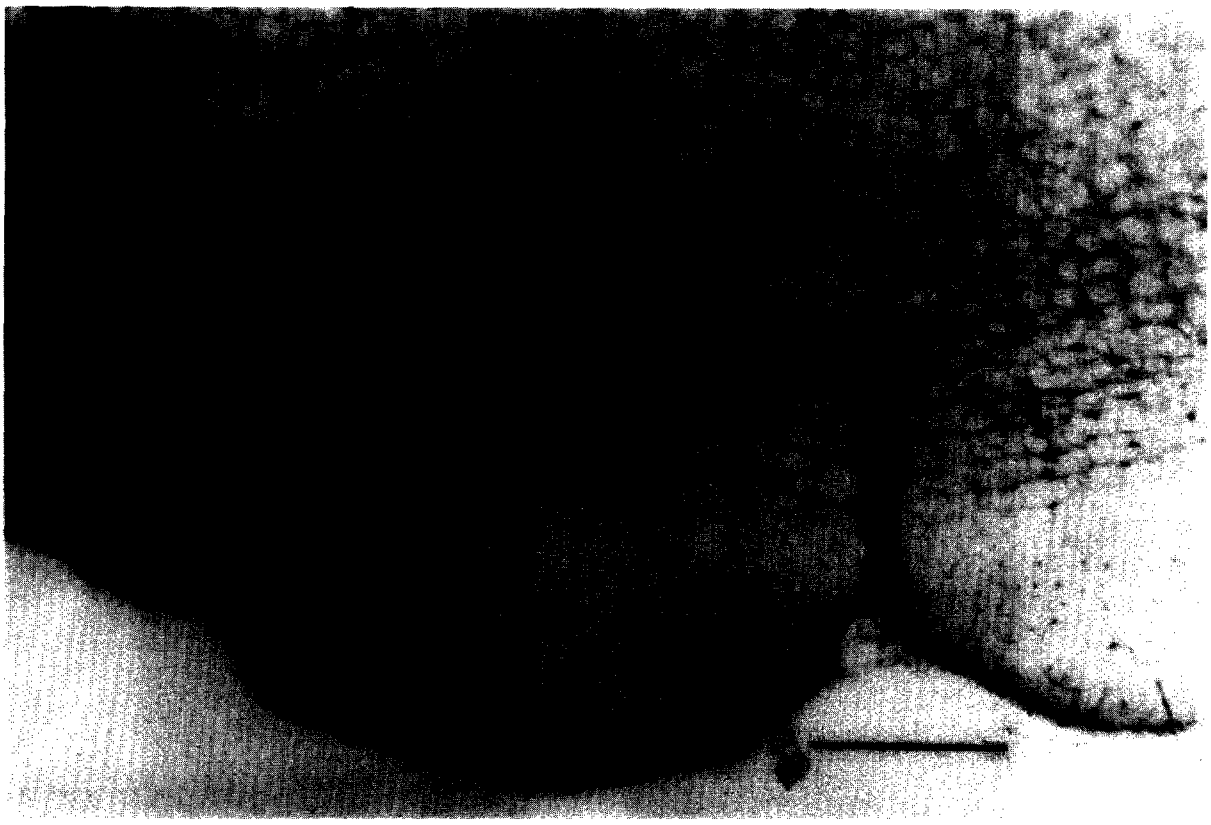

Fig. 10. Caudal to the PAG, c-Fos immunoreactivity was found in cells of the ventral medulla. Labeled cells were found in the nucleus raphe magnus and bilaterally in the nucleus reticularis paragigantocellularis pars alpha (scale bar: 500 μm). Same animal as in Fig. 7.

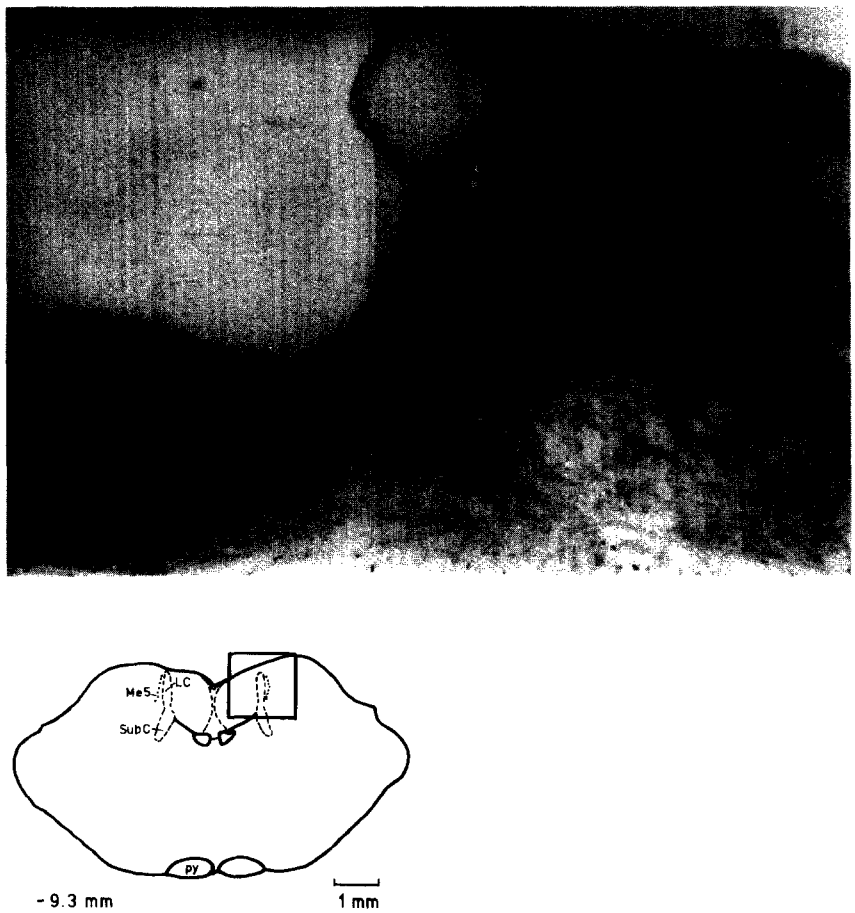

Fig. 11. A dense bilateral labeling was present in the nucleus locus coeruleus. Here, the labeling contalateral to the injection site is shown. Same animal as in Fig. 7.

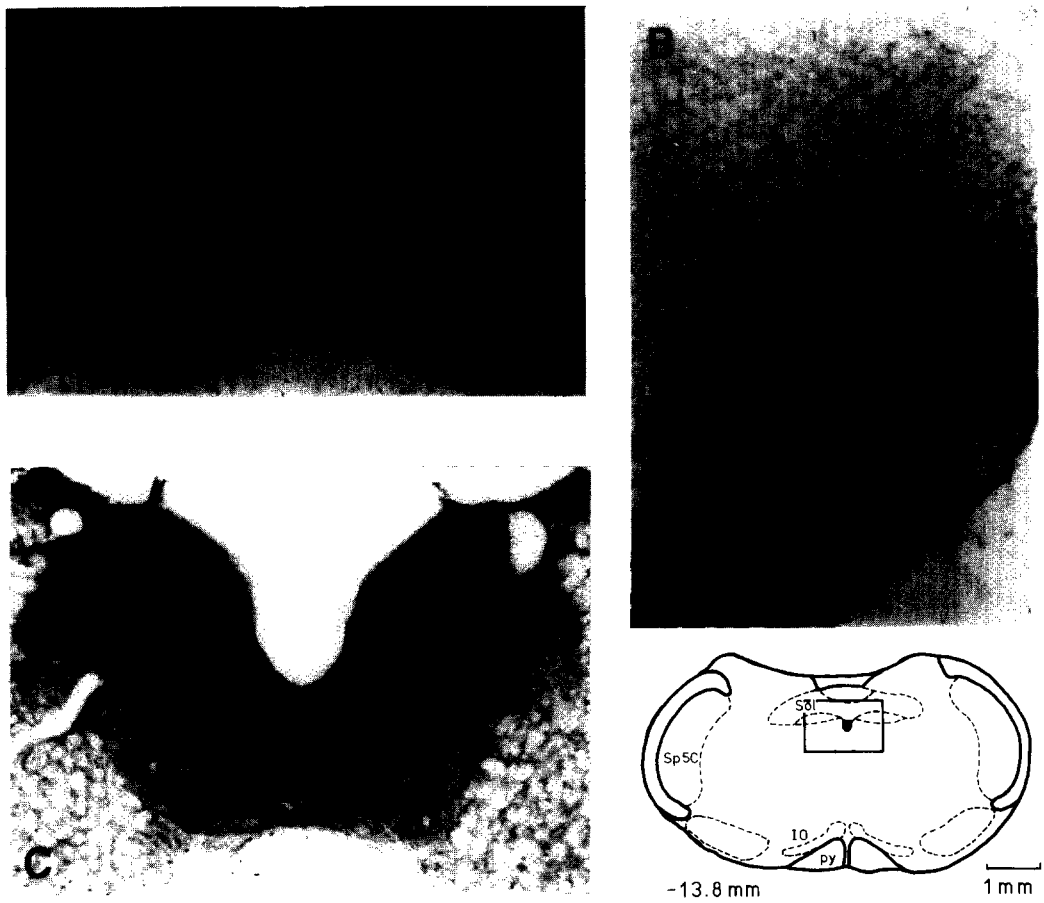

Fig. 12. The c-Fos positive cells were found in the commissural part of the solitary tract nucleus (A) and in the ventrolateral medulla ipsi- and contralateral to the injection site (B). Both sections are approximately 3.8 mm caudal to bregma. The most rostral staining in the solitary tract nucleus was found 3.3 mm caudal to bregma (C). Same animal as in Fig. 7.

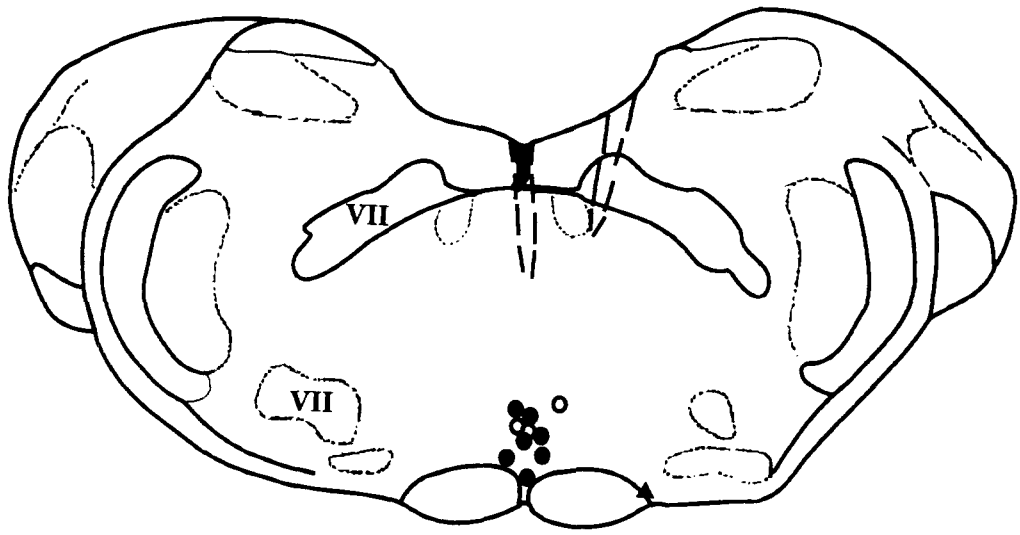

Fig. 13. Schematic illustration of histologically verified injection sites in the ventromedial medulla which are superimposed on a representative frontal section. Anterior posterior levels of the injection sites varied between − 10.3 and − 11.3 mm caudal to bregma. Kainic acid was microinjected at sites indicated by filled circles, open circles represent sites of saline injection. The triangle indicates one injection site of kainic acid outside the boundaries of the nucleus raphe magnus. Dashed lines indicate histologically recoverd tracks of two pipette penetrations which were used for saline injections.

2.3. Pattern of Activated Neurons Following Stimulation in the NRM

It has long been suggested that analgesia by stimulation in the PAG is, at least in part, mediated by raphe-spinal neurons (Basbaum *et al.*, 1976; Fields and Basbaum, 1978). Indeed, the c-Fos labeling of NRM neurons following bicuculline injection into the PAG is consistent with this well-accepted concept. To address which neurons are activated by direct stimulation in the NRM, we have microinjected kainic acid, an amino acid which produces a strong and long-lasting excitation of a large number of central neurons (Ishida and Shinozaki, 1988), into the ventromedial medulla of awake, drug-free adult rats (Fig. 13).

2.3.1. *Activity Pattern in Spinal Cord*

Because neurons in the ventromedial medulla are a major source of medullo-spinal projections, we have analyzed the distribution of c-Fos labeling throughout the spinal cord (Bett and Sandkühler, 1995). All seven rats which received a kainic acid injection (40 pmol in 100 nl) into the ventromedial medulla displayed a significantly enhanced expression of c-Fos bilaterally throughout the rostrocaudal extend of the spinal cord (Fig. 14). The most prominent increase was seen in the inner layer of lamina II (II_i) at the border to lamina III. Significant increases were also found in lamina I, outer lamina II (II_o), lamina III and laminae IV–VI (see Table 1), but not in the ventral horn. The overall pattern of c-Fos labeling, with a predominance in inner lamina II, was seen in all segmental levels investigated. This pattern corresponds well to increased 2-DG uptake in lamina II following activation of descending pathways from the midbrain (Gonzalez-Lima, 1986) and to direct anatomical projection from the NRM to laminae I, II and V (Basbaum *et al.*, 1978; Basbaum and Fields, 1979) and more directly to the projection of physiologically identified neurons in the NRM which are involved in nociceptive modulation (Fields *et al.*, 1995). A strong labeling of laminae IV–VI neurons in the lumbar spinal cord was not paralleled by equally strong labeling at cervical or thoracic levels. Nociceptive specific and multireceptive neurons may be predominantly located in laminae I and II, while low-threshold mechanoreceptive neurons may be clustered in laminae II_i and III. The excitation of neurons in lamina II_i may indicate that descending inhibition from the NRM is exerted via inhibitory interneurons located in these subdivisions (Melzack and Wall, 1965. Fields *et al.*, 1991). Indeed, electrical stimulation of descending fibers in the dorsolateral funiculus, the major pathway of raphe-spinal projections, may increase discharge rates of neurons in these laminae (Dubuisson and Wall, 1980) and, more directly, electrical stimulation in the nucleus raphe magnus also may excite spinal dorsal horn neurons in laminae II_i and III with low threshold input (Light *et al.*, 1986). In Section 2.4, direct evidence will be provided, showing that excitation of propriospinal neurons may depress responses of nociceptive spinal dorsal horn neurons and may mediate descending inhibition of lumbar spinal dorsal horn neurons.

2.3.2. *Activity Pattern in Diencephalon and Brainstem*

The medullary NRM does not only contain neurons with long descending projections to the spinal cord. In addition, numerous sites in brainstem and diencephalon may receive direct excitatory or inhibitory projections from the NRM. Following kainic acid injection into the NRM of awake

drug-free animals, we found c-Fos positive cells at numerous sites. Strong c-Fos immunoreactivity was present in the NRPGα, the nucleus locus coeruleus, in the cortex dorsalis colliculi inferior, the pretectal nucleus, the supramammillary nucleus and the periaqueductal gray (data not shown).

2.4. Spinal Pathways of Descending Inhibition

The widespread excitation of neurons throughout the diencephalon and brainstem by antinociceptive stimulation at discrete brainstem sites such as the ventromedial medulla or the various subdivisions of the PAG suggests that multiple, parallel and possibly independent descending pathways might mediate inhibition of spinal nociception. These various antinociceptive systems may use different descending pathways in spinal cord white matter. Lesions and less traumatic local anesthetic blocks of spinal cord white matter (Sandkühler and Gebhart, 1991) revealed that not only the dorsolateral funiculus ipsilateral to the recording site in the lumbar spinal dorsal horn, but also the contralateral dorsolateral funiculus (Sandkühler *et al.*, 1987b; Sandkühler *et al.*, 1987a) and the ventrolateral funiculus (Jones and Gebhart, 1987) may contain descending inhibitory pathways. Recently, Fields and co-workers (Fields *et al.*, 1995) have shown that physiologically identified neurons in the NRM which are believed to mediate descending antinociception may have axons which descend in spinal cord through the dorsolateral funiculus and cross to the contralateral side where they terminate in the superficial layers of the dorsal horn. Thus, NRM-induced descending modulation of neurons in the superficial spinal dorsal horn would be affected by contralateral blockade of the dorsolateral funiculus. In support, we reported earlier that local anesthetic blocks of the dorsolateral funiculus contralateral to the recording site in the lumbar spinal dorsal horn may reduce descending inhibition by stimulation in the NRM or PAG (Sandkühler *et al.*, 1987a; Sandkühler *et al.*, 1987b).

Even if conduction through fibers in the lateral funiculi at the level of the lower thoracic cord is blocked by multiple bilateral injections of lidocaine or by transaction, descending inhibition of nociceptive neurons in the lumbar spinal dorsal horn is reduced by only about 50% (Fig. 15) (Sandkühler *et al.*, 1987a). This suggests that pathways outside this spinal cord white matter must be involved, possibly via relays in spinal cord gray matter. To address directly the question of whether propriospinal, intersegmental, antinociceptive relay neurons exist, we have searched systematically for propriospinal antinociceptive neurons. Then we have blocked synaptic transmission but not conduction in fibers of passage in selected spinal cord segments rostral to the recording site and have determined the effects of these blocks on the efficacy of descending inhibition (Sandkühler *et al.*, 1993; Sandkühler *et al.*, 1991a).

Table 1. Number of C-FOS Positive Cells

		Sham $n = 5$	Kainic acid $n = 7$
Cervical laminas	I	8 ± 2	35 ± 1
	IIo	2 ± 1	12 ± 3
	IIi	7 ± 3	101 ± 9
	III	1 ± 1	10 ± 3
	IV–VI	0 ± 0	17 ± 5
Thoracic laminas	I	4 ± 1	24 ± 8
	IIo	2 ± 0	8 ± 3
	IIi	6 ± 4	73 ± 6
	III	1 ± 1	10 ± 3
	IV–VI	0 ± 0	16 ± 5
Lumbar laminas	I	1 ± 1	34 ± 10
	IIo	1 ± 1	12 ± 3
	IIi	2 ± 1	124 ± 15
	III	0 ± 0	15 ± 3
	IV–VI	0 ± 0	61 ± 17

Number of c-Fos positive cells (± SEM) as found in in dorsal horn of sham-treated animals (saline microinjections into the NRM) and in stimulated animals (kainic acid microinjections). The total number of immunoreactive cells in five 50 μm thick transverse sections through the spinal cord were counted separately for different spinal laminae. Significantly enhanced numbers of c-Fos positive cells in stimulated animals were verified for all laminae and all segments by the Mann–Whitney *U*-test.

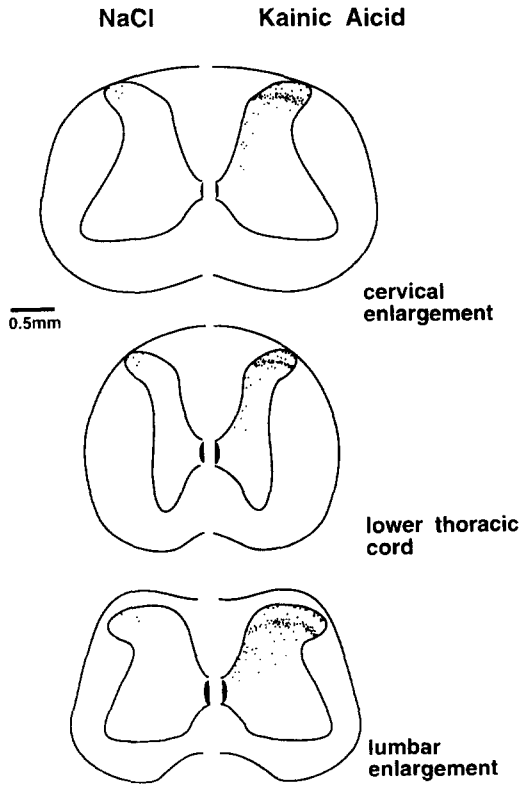

Fig. 14. Pattern of c-Fos immunoreactivity of two different animals which received a sham injection (NaCl, left-hand column) or a kainic acid injection (Kainic acid, right-hand column). Each dot represents one c-Fos positive cell. Results from five 50 μm thick sections are plotted in each diagram. Results are representative for five sham-treated and for seven stimulated animals.

2.4.1. *Propriospinal Antinociception*

Propriospinal neurons outnumber by far all other spinal neurons (Chung and Coggeshall, 1983) and their involvement in motor function is well established, e.g. Schomburg *et al.* (1986). It is surprising, therefore, that only little is known about the role of propriospinal, intersegmental neurons in the modulation of somatosensory information. Anatomical data show that some propriospinal neurons originate (Menétrey *et al.*, 1985) and some terminate in the spinal dorsal horn (Szentagothai and Schadi, 1964). Propriospinal neurons may be activated by afferent stimulation (Skinner *et al.*, 1980) or by supraspinal pathways descending through the dorsolateral funiculus (Alstermark *et al.*, 1991). Conditioning, heterosegmental stimulation of nociceptors, e.g. at the front-paw, may inhibit nociceptive responses in the lumbar spinal dorsal horn, not only with the spinal cord intact, but also in animals with a high cervical spinalization (Cadden *et al.*, 1983; Gerhart *et al.*, 1981; Sandkühler *et al.*, 1993; Pitcher *et al.*, 1995). This suggests that propriospinal neurons mediate this inhibition. To identify the spinal segments from which propriospinal antinociceptive neurons originate, we have recorded noxious heat-evoked responses in the lumbar spinal dorsal horn before and after superfusion of sacral, thoracic or cervical segments with glutamate (Fig. 16) (Sandkühler *et al.*, 1993). These superfusions depressed noxious heat-evoked responses in the dorsal horn of the lumbar spinal cord, especially when performed at lower thoracic or sacral segments, but also when superfusions were made at cervical levels. Incidence of inhibited neurons and degree of inhibition by cervical glutamate superfusion were similar to the inhibition produced by conditioning noxious stimulation at a front-paw (Fig. 16). This does, of course, not suggest that superfusions of spinal cord with a general excitant such as glutamate may mimic in any way the punctate, synaptic excitation by afferent nerve stimulation. These superfusions may, however, be useful to screen for spinal cord segments from which propriospinal antinociceptive efferents originate. Interestingly, superfusions of spinal cord segments with substance P or somatostatin, which may mimic the release and extrasynaptic spread of those neuropeptides after massive excitation of nociceptors (Beck *et al.*, 1995), failed to activate propriospinal antinociception. This suggests that spinal substance P or somatostatin alone cannot reproduce the antinociceptive effects of conditioning noxious skin stimulation. This lack of effect cannot be attributed to a diffusion barrier, as radiolabeled substance P was found to penetrate well into the spinal dorsal horn after superfusion (Beck *et al.*, 1995) and may excite some dorsal horn neurons (Liu and Sandkühler, 1995a) and induce expression of immediate–early genes in spinal dorsal horn cells (Beck and Sandkühler, 1993).

To identify the lamina location of propriospinal antinociceptive neurons, small quantities (60 nmol in 60 nl) of glutamate were microinjected through one barrel of a multibarrel glass pipette into spinal cord

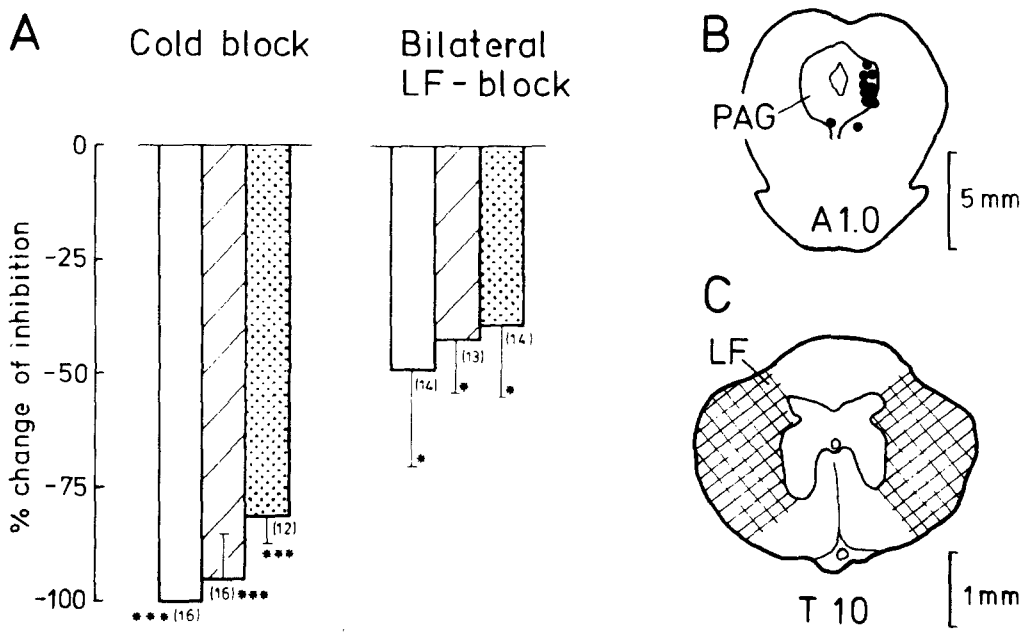

Fig. 15. The efficacy of tonic- or stimulation-produced descending inhibition is not abolished by bilateral lidocaine blocks of the lateral funiculi, but by reversible spinalization at the lower thoracic spinal cord. In (A), vertical bars indicate percent change in efficacy of tonic (open bars) or stimulation-produced descending inhibition from the PAG (hatched bar) or the nucleus raphe magnus (dotted bar) during spinal blocks. Values at − 100% indicate that descending inhibition was blocked completely. In (B), stimulation sites in the PAG are superimposed on a representative section through the midbrain. Hatched areas in (C) illustrate the distribution of fast green dye after microinjection of 2 × 1 µl into the lateral funiculi of each site in one experiment. Supraspinal electrical stimulation consisted of 0.1 msec pulses at 50–500 µA given at 100 Hz for 35 sec.

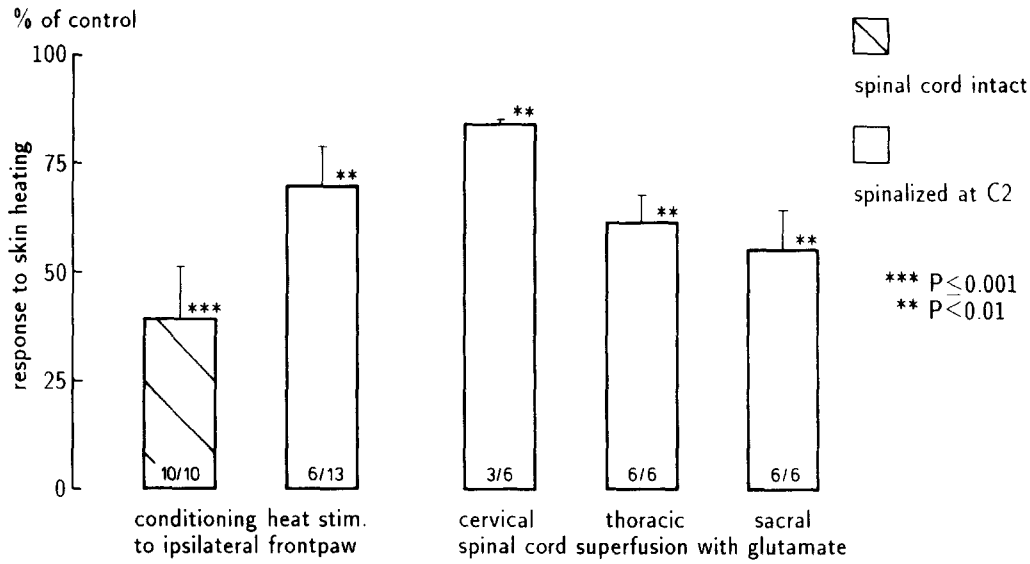

Fig. 16. Propriospinal inhibition in spinal dorsal horn. Each bar represents mean heat-evoked responses during conditioning stimulation in percent of control responses immediately prior to conditioning stimulation. Vertical lines indicate 1 SEM. Only neurons which were inhibited during conditioning stimulation are included. The incidence of inhibited neurons is given at the bottom of each bar. Wilcoxson rank-sum test for paired data was used for statistical comparison. All results were obtained in animals with a high cervical spinalization, except for the 10 neurons which were recorded with the cord intact (hatched bar on the left).

white and gray matter. Electrical stimulation was applied through another barrel of the same pipette (100 μA, 100 Hz). The distribution of antinociceptive stimulation sites in spinalized or intact rats is shown in Fig. 17. Electrical stimulation was effective both rostral and caudal to the recording site in the dorsal columns and in the ventral funiculi. Inhibition also could be evoked by electrical stimulation throughout the dorsal horn and in the medial part of lamina VIII. Glutamate microinjections were most effective bilaterally in the superficial dorsal horn and ipsilaterally in the lower thoracic cord close to lamina VIII. Table 2 illustrates that incidence of inhibitory sites and efficacy of propriospinal inhibition is similar to the inhibition produced by stimulation in the PAG. Electrical stimulation was effective at numerous sites throughout the dorsal and ventral horns. At these sites, long descending or ascending fibers were most likely not activated, as almost all spinal fibers travel within the spinal cord white matter (Chung and Coggeshall, 1983). Thus, low-level electrical stimulation in the gray matter of the spinal cord probably excited dendrites, cell bodies and axons of spinal neurons which originate at or close to the site of stimulation or fibers which terminate at that segmental level. Sites at which electrical stimulation but not glutamate microinjection was effective do not necessarily indicate that fibers of passage are involved, as glutamate microinjections may reveal a high incidence (up to 50%) of false negative results (Sandkühler *et al.*, 1991b). This is due probably to a depolarization block of some neurons. At sites where electrical or chemical stimulation was effective in the intact but not in spinalized animals, ascending neurons may originate which trigger supraspinal descending antinociceptive systems. These ascending spinal neurons could mediate diffuse noxious inhibitory controls, i.e. an inhibition of spinal nociceptive neurons by conditioning, heterosegmental noxious stimulation. This form of inhibition requires a supraspinal loop (Le Bars *et al.*, 1979b).

2.4.2. *Descending Inhibition Via Propriospinal Neurons*

To test the hypothesis that propriospinal antinociceptive neurons mediate descending inhibition from the brainstem, we have blocked neurons originating in lower thoracic cord segments without affecting impulse conduction in fibers of passage (Sandkühler *et al.*, 1991a). This was achieved by superfusion of spinal cord segments with the excitotoxin kainic acid at 5 mM. To test selectivity of kainic acid blocks, we recorded field potentials which were evoked by electrical stimulation in dorsal columns. These field potentials represent postsynaptic responses of a population of dorsal horn cells. These postsynaptic responses are depressed to 14.1 ± 9.1% of controls 30 min after onset of kainic acid superfusion at the recording segment. The same superfusions did not, however, impair impulse conduction in fibers of passage in the dorsal columns. This was verified by superfusion of spinal cord dorsum with kainic acid at 5 mM between the stimulation site and the recording site in the dorsal columns. Apparently, kainic acid selectively has blocked or destroyed neurons originating from the superfused cord segment. This conclusion is supported by other electrophysiological (Curtis and Malik, 1985) and histological data (Coyle *et al.*, 1978) showing that nerve fibers and nerve terminals are relatively insensitive to the toxic effect of kainic acid compared to cell bodies.

When activating descending inhibition by microinjection of bicuculline into the PAG, noxious heat-evoked responses in the lumbar spinal dorsal horn were depressed to 18.2 ± 6.0% of controls ($n = 8$) when synaptic transmission in the spinal cord was intact (superfusion with artificial cerebrospinal fluid). When the lower thoracic cord was superfused with kainic acid at 5 mM, however, depression of the same neurons from the same sites in the PAG was to 60.0 ± 6.5% of control (Fig. 18). This reduction in efficacy is statistically significant ($P < 0.001$; Wilcoxon signed rank test). In two other neurons, kainic acid superfusions did not impair descending inhibition.

Taken together, the identification of propriospinal antinociceptive neurons by stimulation in the lower thoracic, cervical and sacral spinal cord, induction of c-Fos in spinal dorsal horn neurons by stimulation in the NRM and the blockage of descending inhibition by kainic acid superfusions of the lower thoracic spinal cord provide converging evidence that:

1. Propriospinal antinociceptive neurons may constitute a third component of endogenous antinociception (in addition to segmental and supraspinal descending inhibition).
2. Propriospinal antinociceptive neurons mediate a significant portion of descending inhibition from the brainstem.
3. Propriospinal antinociception can be activated by heterosegmental, conditioning noxious stimulation.

Table 2. Effect of Focal Heterosegmental Stimulation on Noxious Heat-evoked Responses in the Dorsal Horn of the Lower Lumbar Cord

Stimulation site	Electrical stimulation	Glutamate injection
	Upper lumbar cord	
Dorsal columns	59.1 ± 8.8 (3/3)	— —
Dorsal horn	45.5 ± 9.8 (6/11)	59.7 ± 19.9 (4/9)
Ventral horn	61.8 ± 9.8 (5/10)	—
Ventral funiculus	78.5 (1/2)	—
	Lower thoracic cord	
Dorsal columns	38.0 ± 8.0 (7/13)	— (0/5)
Dorsal horn	52.0 ± 8.0 (8/16)	77.7 ± 1.3 (4/7)
Ventral horn	56.4 ± 7.0 (4/8)	54.7 ± 1.5 (2/2)
Ventral funiculus	76.3 ± 0.8 (2/8)	— (0/1)
	Midbrain	
Periaqueductal gray*	52.7 ± 3.5 (9/10)	69.4 ± 6.1 (4/10)

Mean (± SEM) noxious heat-evoked responses of nociceptive lumbar spinal dorsal horn neurons during electrical stimulation or immediately after microinjection of glutamate at sites in upper lumbar, lower thoracic or midbrain are expressed as percent of control values prior to conditioning stimulation. The number of inhibitory sites and the total number of stimulation sites are given in brackets.

* Sandkühler *et al.* (1988).

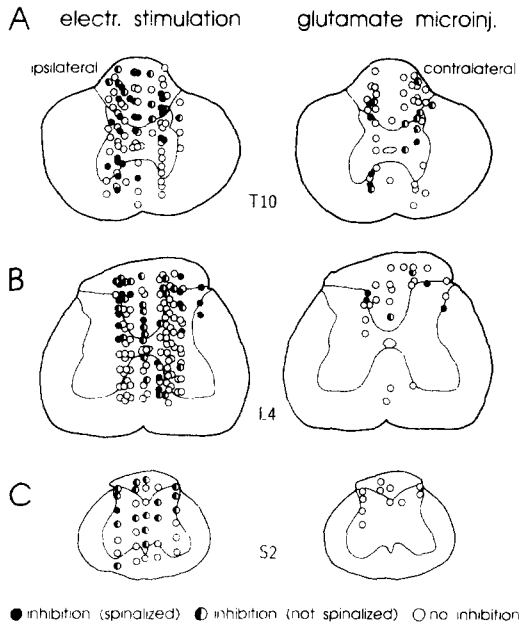

Fig. 17. Distribution of all stimulation sites in the lower thoracic, lumbar and sacral spinal cord to activate propriospinal inhibition. Electrical stimulation consisted of 0.1 msec cathodal pulses of 100 μA given at 100 Hz for 45 sec. Conditioning stimulation started 10 sec prior to the onset of heat stimuli. Sixty nanolitres of 1 M glutamate were microinjected. Recordings were made either in intact animals or in animals spinalized at the second cervical segment. Noxious heat-evoked responses were calculated as total number of action potentials in 25 sec. A reduction by more than 20% of control was considered effective inhibition.

3. DESCENDING MODULATION OF DISCHARGE PROPERTIES

The code which is used by the nociceptive system to transmit and process information about noxious, potentially tissue-damaging events is not known. Parameters such as total number of action potentials over a period of several seconds, mean discharge rates, or maximal discharge frequency are often used as convenient, simple parameters in electrophysiological experiments and may correlate well with behavioral parameters of nociception (e.g. Carstens and Douglass, 1995). It seems unlikely, however, that these parameters are most relevant for encoding neuronal information. The time constant of the decay of synaptic potentials is usually in the millisecond rather than in the second range and behavioral responses often are elicited before the maximal discharge frequency in a particular neuron is reached. Measuring mean discharge rates may not yield the full information transmitted. Stochastic, oscillating or clustered discharges with the same mean discharge rate may have very different effects on transmitter release (Iverfeldt *et al.*, 1989), temporal summation of postsynaptic potentials, long-term changes of synaptic strength (Dunwiddie and Lynch, 1978) and second-messenger mediated effects (Li and Goldbeter, 1992). The temporal correlation of discharges of different neurons may be relevant for an assembly coding of sensory information (Singer, 1993) and also, of course, for the spatial summation of converging neurons. Possibly, different codes are used to encode acute noxious stimuli versus chronic pain. Only recently, some insights into the impact of

endogenous modulatory systems on these discharge properties of nociceptive neurons have been gained.

It is well known that discharge rates of sensory neurons in the spinal dorsal horn can be modulated by descending systems originating at various brainstem sites. Some of these descending systems may be active tonically under the given experimental conditions and the effect of tonic descending modulation often is studied by reversible cold block spinalization (Wall, 1967; Dickhaus *et al.*, 1985) or sometimes by irreversible transection of the spinal cord rostral to the recording site.

3.1. Descending Modulation of Nonlinear Dynamics of Discharges in Spinal Dorsal Horn

Many dorsal horn neurons may display background activity which has been considered to be 'stochastic noise' (Steedman and Zachary, 1990). Indeed, some of the mechanisms underlying spike generation are apparently stochastic processes, as revealed by the open and closing times of ion channels (Colquhoun and Hawkes, 1981) and peak amplitudes of unitary post-synaptic potentials (Stern *et al.*, 1992). The degrees of freedom in neuronal discharges also may be increased by up to 10^4 synapses contacting one neuron. Recently, powerful analytical tools which are based on the ergodic theory of nonlinear dynamics (Eckman and Ruelle, 1985) were employed to describe discharge patterns either graphically in form of a phase-space portrait (Takens *et al.*, 1985) (see Fig. 19) or quantitatively as the correlation dimension D_2 (Grassberger and Procaccia, 1983). Irregular discharge patterns can be generated by simple, purely deterministic functions. Their dynamic is represented by an attractor with a dimension D, which provides the number of degrees of freedom m, and which is therefore a quantitative measure for the complexity of a dynamic system. White noise would then reveal $D = \infty$, strictly periodic discharges would reveal $D = 0$. Attractors with non-integer dimensions are called 'fractals'. We have adopted the Grassberger–Procaccia algorithm (Grassberger and Procaccia, 1983) to determine the correlation dimension D_2 for point processes (i.e. for the occurrence times of the action potentials Θ_i or interspike-intervals ($I_i = \Theta_{i+1} - \Theta_i$) or higher deviations ($I' = I_{i+1} - I_i$). For various reasons discussed in Debus and Sandkühler (1996), we suggest that I' is the most suitable variable to determine D_2.

Intuitively, one might expect that removal of any input from a spinal neuron, e.g. by spinalization, should reduce the degrees of freedom of its discharge. However, we have found the contrary when comparing the D_2 correlation dimension of background activity of 22 multireceptive neurons and four low threshold neurons before and after spinalization (Debus and Sandkühler, 1996). With the spinal cord intact, 17 (73%) of the neurons displayed background activity with D_2 values lower than five and the corresponding phase-space portraits revealed characteristic patterns (Fig. 20). The remaining seven neurons had 'high' D_2 values larger than 10 before and during spinalization. In the majority of neurons

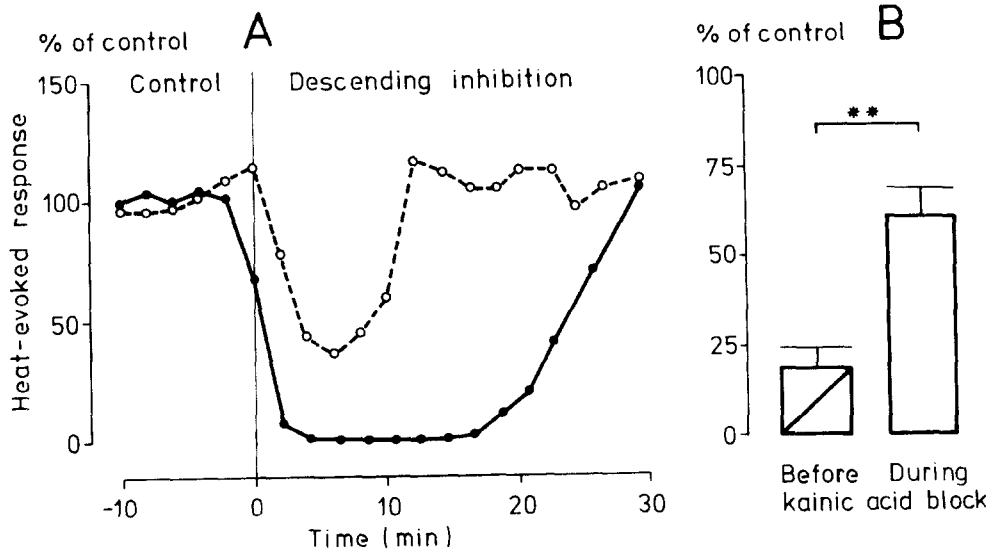

Fig. 18. Stimulation-produced descending inhibition of noxious heat-evoked responses in the lumbar spinal dorsal horn are reduced during kainic acid block of synaptic transmission in lower thoracic spinal cord. In (A), heat-evoked responses of one neuron are plotted vs time. At time zero, 200 pmol bicuculline was microinjected into the ventrolateral PAG. With the spinal cord intact, bicuculline microinjection produced a complete inhibition of noxious heat-evoked responses 3–18 min after injection (filled circles, solid line) Thirty minutes after onset of superfusion of the lower thoracic cord with kainic acid, the same injection of bicuculline produced only partial and transient depression of heat-evoked responses of the same neuron (open circles, dotted line). In (B), mean heat-evoked responses of eight neurons were recorded 2–4 min after the injection of bicuculline into the PAG. Responses are expressed as percent of control responses. The efficacy of descending inhibition was lowered significantly during kainic acid block of the lower thoracic cord (open bar) compared to the descending inhibition with the spinal cord intact (hatched bar). In two other neurons, descending inhibition was not affected during kainic acid superfusions.

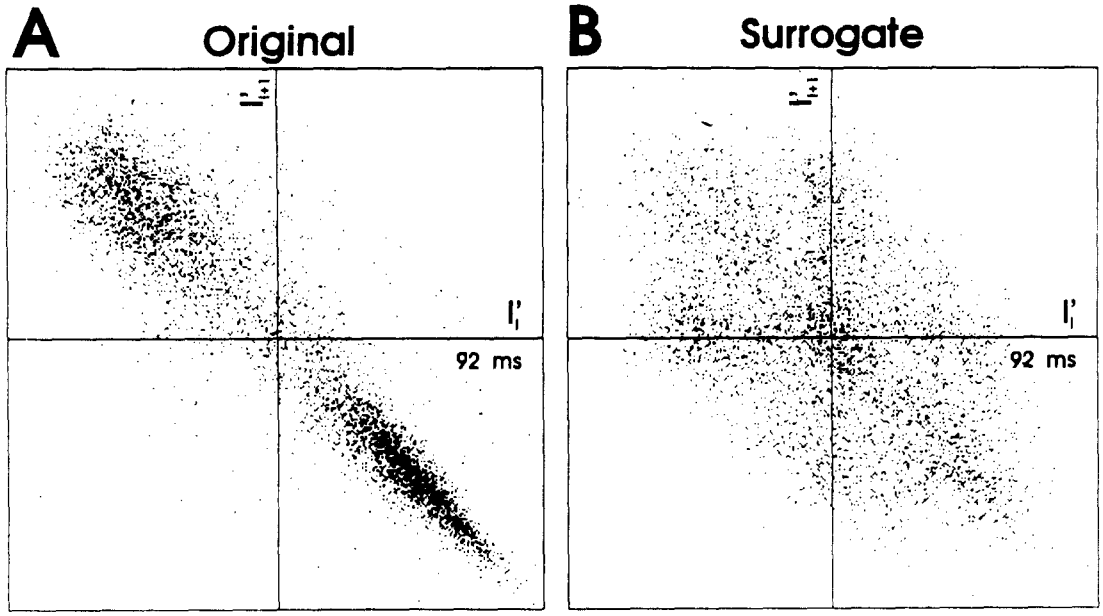

Fig. 19. Phase-space portrait of background activity of one multireceptive spinal dorsal horn neuron. The difference between two consecutive interspike intervals (I_i) is plotted on the abcissa. The difference between the following interspike intervals (I_{i+1})is plotted on the ordinate. In (A), the phase-space portrait of the original discharge is displayed. A surrogate of this discharge was achieved by randomizing all interspike intervals. The phase-space portrait of this surrogate is shown in (B). The D_2 correlation dimension of the original discharge is 0.96, the correlation dimension of the surrogate is 'high'.

(13 out of 19; 68%) with previously low D_2 values spinalization led to a significant increase in D_2 values and in a loss of order in phace-space portraits (Fig. 20). This increase was apparent irrespective of functional classification and lamina location of the neurons.

Thus, it appears that tonic descending systems may maintain a high order in the discharges of some sensory spinal dorsal horn neurons. The three neurons which displayed no change in the correlation dimension of background activity could have a simple intrinsic dynamic which is expressed independently from suprasinal systems, as can be found in pacemaker neurons. Recent results suggest indeed that pacemaker neurons exist in the spinal dorsal horn (Jiang *et al.*, 1995). Alternatively, some neurons could be part of a local neuronal network, which generates a simple dynamic independently from the brain.

3.2. Rhythmicity in Spinal Dorsal Horn Neurons

The most simple discharge pattern which leads to a low correlation dimension would be a harmonic oscillation. In a sample of 223 spinal dorsal horn neurons, we have identified 99 neurons which displayed oscillating background activity (Sandkühler and Eblen-Zajjur, 1994) (see Fig. 21 for examples). Fundamental spectral frequencies were assessed quantitatively by fourier transformation of the delay values from 0 to 5000 msec of the autocorrelation function. None of the 99 oscillating neurons had more than one significant peak in the autospectrum, as revealed by Fischer's κ-test ($P < 0.05$). Typically, the mode of the fundamental frequency was stable over time. The distribution of fundamental spectral frequencies of background activity of all oscillating neurons presented a bimodal shape, with the first mode centered around 2 Hz and the second mode centered around 10 Hz (Fig. 22). Oscillating multireceptive neurons and low threshold neurons were recorded in all laminae of the dorsal horn. During reversible cold-block spinalization at the thoraco-cervical junction, 24 out of 36 (66.6%) oscillating neurons retained their rhythmicity (Figs 22 and 23), 18 of which did not change their fundamental spectral frequency during spinalization. Three neurons switched to higher and three neurons switched to lower spectral frequencies. Twelve out of the 36 previously oscillating neurons lost their rhythmic discharge patterns during spinalization. With only one exception, all 31 neurons with non-oscillating background activity with the spinal cord remained non-rhythmic during spinalization. The observation that about one-third of oscillating neurons lose their rhythmicity during spinalization suggests that supraspinal generators of rhythmicity may exist. In fact, evidence has been provided that oscillations in sympathetic nerve discharges, at least in part, are generated by supraspinal pacemakers located in the hypothalamus and in the medial thalamic nuclei (Varner *et al.*, 1988). Raphe-spinal neurons also may display broad frequency bands in their discharges (Gebber *et al.*, 1990). Thus, some oscillating neurons in the dorsal horn may have supraspinal generators located for example in the raphe nuclei. Alternatively, cellular or spinal generators of rhythmicity may be modulated by tonic

descending systems, e.g. by shifting the membrane potential into a range in which oscillations are expressed (Jiang *et al.*, 1995). For the majority of neurons which did not lose their rhythmicity during spinalization, supraspinal generators clearly are not necessary. This is in agreement with the results from intracellular recordings of deep dorsal horn neurons in the rat (Jiang *et al.*, 1995).

3.2.1. *Possible Biological Meaning of Oscillations in Neuronal Discharges*

The length of muscle fibers and the degree of skin stretching may be monotonically and almost linearly correlated with the principle mode of the power spectrum of the discharges of sensory nerve fibers and this relationship may be maintained in second-order neurons of the cuneate nucleus (Surmeier and Towe, 1987). Thus, in these sensory systems, the spectral frequency of discharges may encode information about stimulus intensity with high accuracy. It is highly unlikely that this is also the case for the discharges of nociceptive neurons, since noxious skin heating almost always depressed oscillations which were present in background activity. For lateral geniculate neurons, it was shown that while discharging rhythmically the neuron may act as a bandpass filter limiting the transmission of information to the frequency domain of the oscillation, thereby suppressing the information transfer. Switching to the transfer mode was characterized by non-rhythmic discharges (Surmeier and Towe, 1987).

Rhythmicity also may play a role in neuronal plasticity and long-term effects of transynaptic stimulation. The synaptic strength may be altered for long periods of time, depending upon the patterns of synaptic use (Dunwiddie and Lynch, 1978). Regular, low-frequency stimulation may result in long-term depression of synaptic transmission (Sandkühler and Randić, 1996; Bernard and Wheal, 1995) and discharges with clustered action potentials (e.g. induced by tetanic stimulation) may lead to long-term potentiation of synaptic transmission (Randić *et al.*, 1993; Dunwiddie and Lynch, 1978) (see Section 4).

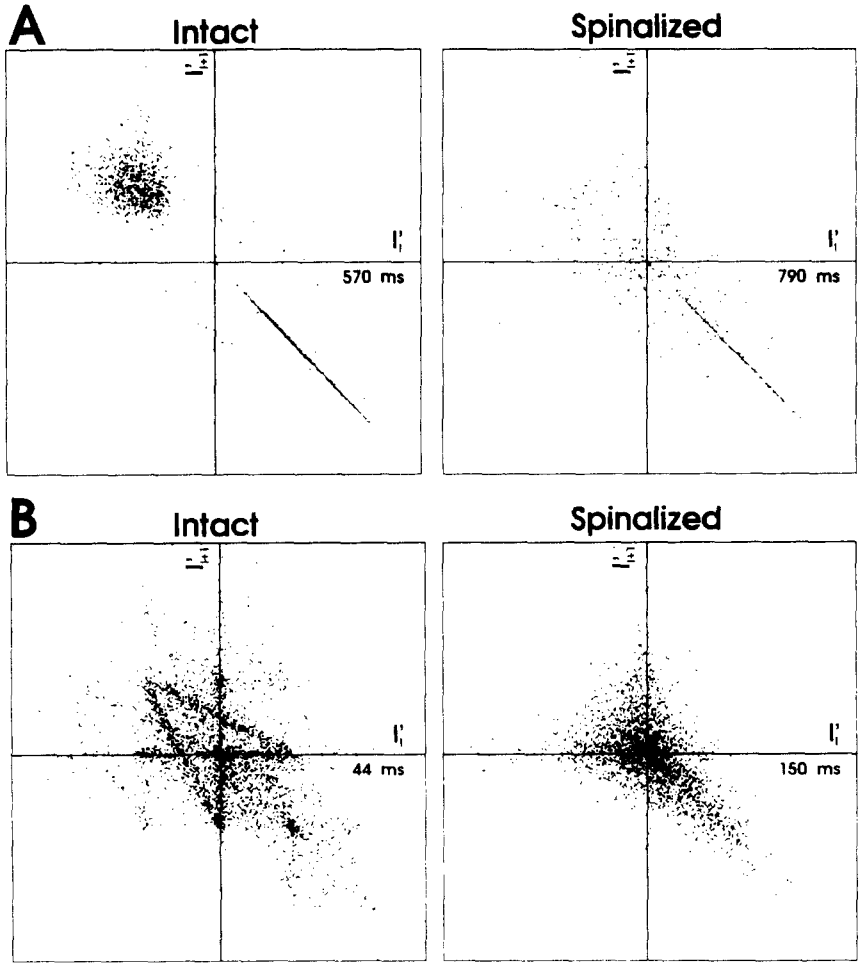

Fig. 20. Phase-space portraits of background activity of two different multireceptive neurons are shown with the spinal cord intact (Intact) and during reversible cold-block spinalization (spinalized). Both neurons displayed high order in the phase-space portraits with the spinal cord intact and, correspondingly, a low correlation dimension. This high order was lost during spinalization.

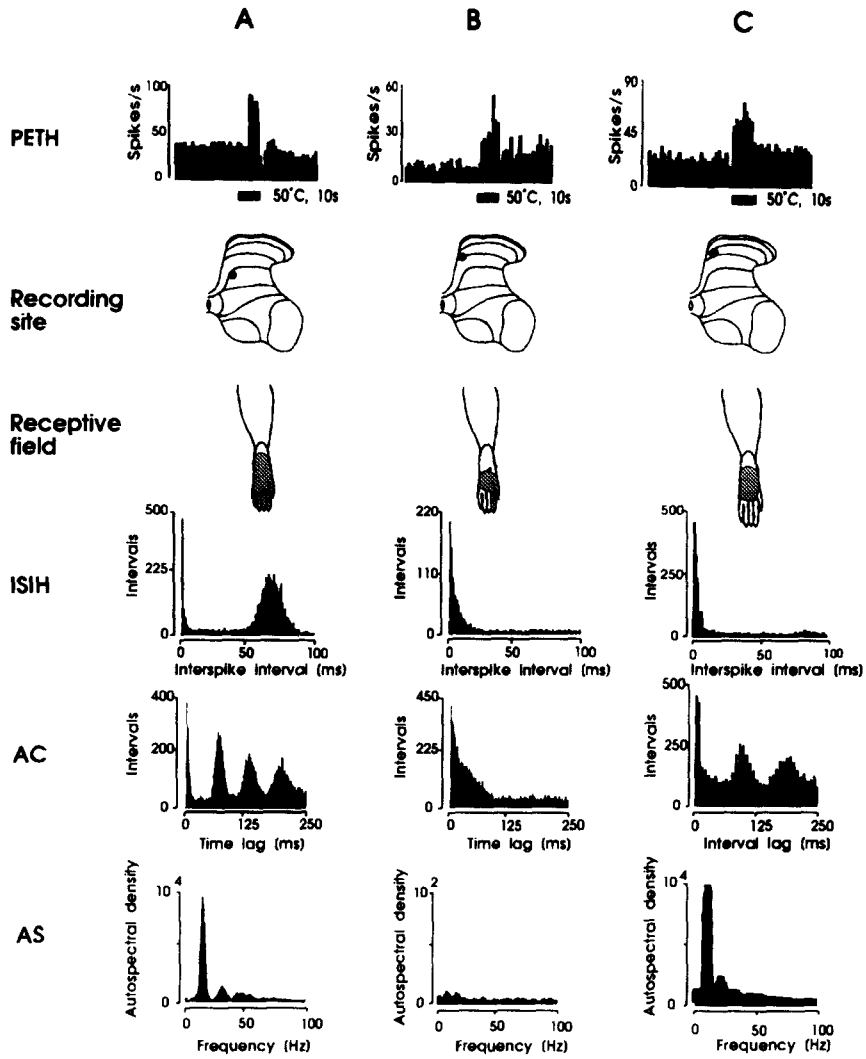

Fig. 21. Discharge properties of multireceptive lumbar spinal dorsal horn neurons. All neurons responded to noxious radiant skin heating as can be seen in the peri-event time histograms (PETH). Cutaneous mechanoreceptive fields were located on the glaborous skin at the ipsilateral hindpaw. Interspike interval histograms (ISIH) and the autocorrelation function (AC) and the autospectra (AS) of the background activity of the three neurons are shown. All recordings were made in animals with the spinal cord intact.

3.3. Descending Excitation and Inhibition are Simultaneously Active

Stimulation at brainstem sites may activate a large number of neurons throughout the diencephalon and brainstem, some of which give rise to pathways descending bilaterally in the lateral funiculi of the spinal cord. These descending systems may convey tonic inhibitory and/or tonic excitatory controls of sensory neurons in the spinal dorsal horn. It has been suggested that the various descending systems also would have distinct targets in the spinal cord with respect to the lamina location and the functional characteristics of the neurons modulated. Wall (1967) has found a lamina-specific modulation of spinal neurons by descending systems. The size of cutaneous receptive fields of nociceptive specific neurons may be reduced tonically by descending pathways, while multireceptive neurons may be either inhibited or excited (Laird and Cervero, 1990). Neurons in lamina V which receive afferents from the splanchnic nerve may be mainly subject to tonic descending inhibition, while neurons in lamina VIII receive a predominately tonic descending excitation (Tattersall *et al.*, 1986). These results suggest that tonic descending inhibition and excitation might be simultaneously active. To prove this conclusion directly, we have made simultaneous recordings from two to five neurons in the spinal dorsal horn (Sandkühler *et al.*, 1995) (Fig. 24). Multineuron recordings were made through a single electrode and action potentials were assigned to individual neurons by cluster analysis of their principal components (Sarna *et al.*, 1988). Only those recordings with clearly distinguishable action potentials were analyzed further. Surprisingly, cutaneous receptive fields of pairs of low threshold or pairs of multireceptive neurons which were recorded simultaneously at the same site in the spinal dorsal horn often did not overlap, or did so only minimally. This was the case in 19 out of 37 (51.4%) pairs of neurons.

Pairs of neurons with relatively stable levels of background activities (1–100 impulses/sec) were chosen to determine the effect of spinalization on discharge rates. In 23 out of 50 (46%) pairs of neurons, spinalization produced qualitatively different effects on the level of background activities, i.e. rate increased, decreased and/or remained unchanged. For 23 out of 50 (46%) additional pairs of neurons, spinalization produced quantitatively different effects i.e. background activity was changed in the same direction but to a very different degree (Fig. 25). We have also calculated the coefficient of dispersion (CD = variance/$\bar{X}$) of interspike intervals as a quantitative measure of clustered action potentials (burst-like discharges) (Cocatre-Zilgien and Delcomyn, 1992). Twenty-three out of 57 (40.4%) neurons displayed an increased tendency for burst-like discharges during spinalization. Twenty-nine out of 57 (50.9%) neurons displayed a decreased burst activity and only three out of 57 (5.3%) neurons did not change their discharge patterns. Neurons which were recorded simultaneously at the same site changed their discharge patterns in opposite direction. Thus, discharges of neurons which were recorded simultaneously at the same site, i.e. under identical experimental conditions, often were differentially affected by spinalization. No general 'tone' of descending controls exist, providing further evidence for the existence of multiple, parallel and possibly independent descending modulatory systems.

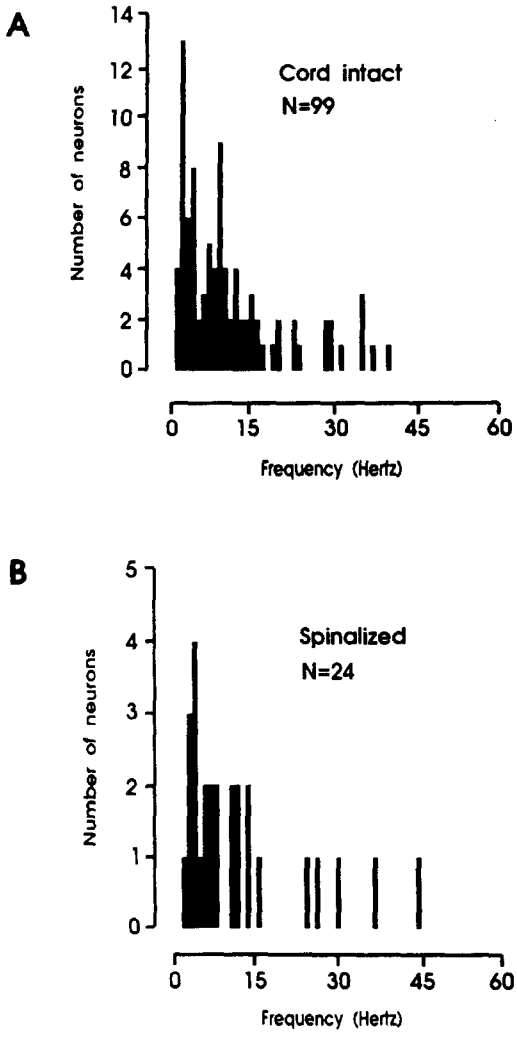

Fig. 22. Oscillating background activity recorded from spinal dorsal horn neurons in animals with the spinal cord intact (A) or during reversible cold-block spinalizaton (B). The figure illustrates the distribution of fundamental spectral frequencies of background discharges of all rhythmic neurons. Fundamental spectral frequencies were distributed in a bimodal fashion with higher incidences of neurons in two spectral bands, centered around 2 Hz and 10 Hz.

Tonic descending inhibition of nociceptive neurons has been studied intensively, as descending inhibition is a major mechanism of endogenous antinociception. Tonic descending inhibition also may, in addition, improve sensory discrimination, by reducing the size of cutaneous mechanoreceptive fields (Laird and Cervero, 1990) and by reducing the level of background activity, thereby reducing the signal-to-noise ratio, if the background activity is considered to be purely stochastic noise (Steedman and Zachary, 1990); see, however, more recent results summarized in Section 3.1. If noxious stimulation activates descending inhibition, it may improve sensory discrimination by inhibiting nociceptive neurons in somatotopically inadequate spinal cord segments. This mechanism has been termed 'diffuse noxious inhibitory control' (Le Bars *et al.*, 1979a; Le Bars *et al.*, 1979b). It is conceiveable to assume that diffuse noxious inhibitory control may contribute to the tonic descending inhibition which is present in the acute preparation.

Descending excitation has only recently been studied in greater detail (Zhuo and Gebhart, 1992; McMahon and Wall, 1988; Fields *et al.*, 1995). It has been suggested that descending inhibition is mediated via spinal inhibitory interneurons which are excited by descending pathways (Fields *et al.*, 1991). The induction of c-Fos in neurons of the spinal dorsal horn by stimulation in the ventral medial medulla is in line with this hypothesis as well as the observation that propriospinal neurons exist which may inhibit nociceptive spinal dorsal horn neurons. A serial system with intersegmental, inhibitory interneurons implies that descending excitation and inhibition must be present simultaneously. Also, this was indeed the case when recording simultaneously from neurons which change the level of their background activity in opposite direction during spinalization.

4. PLASTICITY AND ENDOGENOUS ANTINOCICEPTION

The depression of nociceptive responses of single cells or in behaving animals by endogenous antinociceptive systems has attracted much attention. Almost all studies have investigated immediate, short-term effects on phasic or on tonic nociceptive responses. Virtually nothing is known about long-term changes of endogenous antinociceptive systems and about a possible impact of these neuronal systems on plastic changes of nociception.

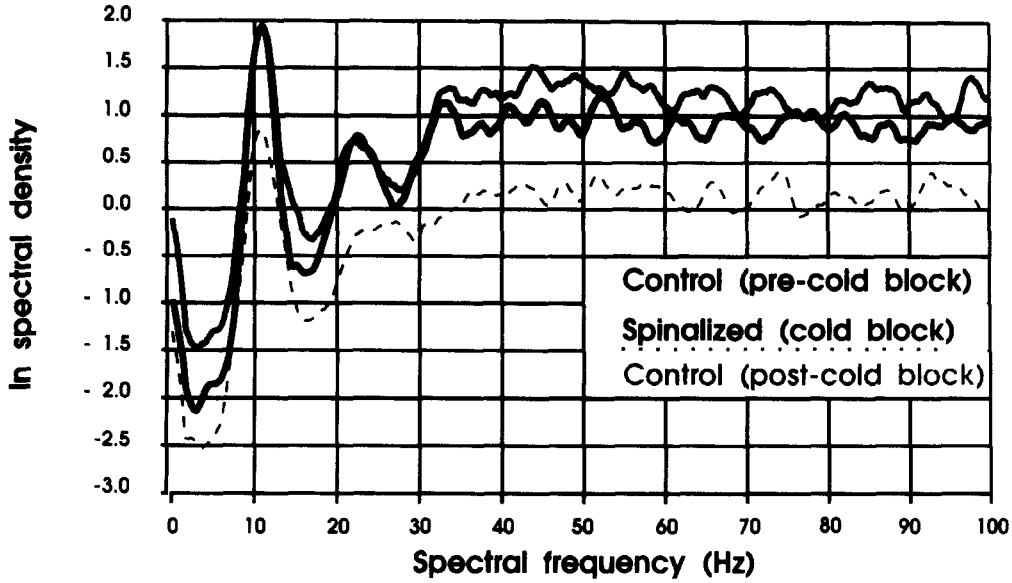

Fig. 23. Spectral density function of background activity of one multireceptive spinal dorsal horn neuron with a fundamental frequency around 12 Hz. This fundamental spectral frequency was not different before, during or after reversible cold-block spinalization, indicating that the generators of this rhythmicity are not located supraspinally.

4.1. Possible Plasticity of Descending Modulatory Systems

Evidence has been provided that tonic descending inhibition of nociceptive spinal dorsal horn neurons may increase during the development of acute arthritis in the cat (Schaible *et al.*, 1991). Descending adrenergic controls also may increase during development of carrageenan inflammation as spinal blockage of α_2-adrenoceptors facilitate nociceptive responses of spinal dorsal horn neurons in inflammatory but not in normal states (Stanfa and Dickenson, 1994). This may indicate plasticity of endogenous antinociceptive systems or, alternatively, may be due to an increased activity in a negative feedback loop which consists of nociceptive spinal dorsal horn neurons with ascending projection to antinociceptive neurons in the brainstem (Bouhassira *et al.*, 1995) including the NRM (Oliveras *et al.*, 1990). An increased ongoing activity in spinal nociceptive neurons during peripheral trauma of joint or skin would than be expected also to increase descending inhibition which is apparently tonically active, under the given experimental conditions.

4.2. Tonic Descending Modulation of Plasticity in Spinal Dorsal Horn

During strong activation of nociceptors, a mixture of classical neurotransmitters and neuropeptides are released into the spinal dorsal horn (see reviews by Besson and Chaouch, 1987; Levine *et al.*, 1993). At least some of these neuromediators spread extrasynaptically to sites remote from their release (Duggan *et al.*, 1990). This extrasynaptic spread of neuroactive substances has been termed 'volume transmission' (Agnati *et al.*, 1986) and cannot, of course, mediate the punctate fast synaptic transmission but may be involved in late and long-lasting changes of an ensemble of neurons (Sandkühler *et al.*, 1994; Liu and Sandkühler, 1995a). Recently, it has been shown that high-frequency bursts in afferent nerve fibers may induce a long-term potentiation of primary afferent neurotransmission in Aδ-fibers of a spinal cord slice preparation (Randić *et al.*, 1993) and also may potentiate spinal C-fiber-evoked field potentials *in vivo* (Liu and Sandkühler, 1995b). The co-activation of NMDA and tachykinin receptors by glutamate, substance P and neurokinin A is required for induction — but not maintenance — of long-term potentiation of C-fiber-evoked field potentials in the superficial spinal dorsal horn following tetanic stimulation of afferent nerves. We have shown recently that intact tonic descending systems may prevent induction of long-term potentiation of spinal C-fiber evoked potentials by skin inflammation or nerve injury (Liu and Sandkühler, 1996). In this study, C-fiber-evoked field potentials were recorded in superficial layers of the lumbar spinal dorsal horn. Potentials were elicited by electrical stimulation of sural nerve (5–8 V, pulse width 0.5 msec) in 24 rats which were anesthetized with urethane. In five rats, strong inflammation of one hind-paw was induced by noxious radiant skin heating (60°C, given from four to six times for 30 sec at 60 sec intervals). This inflammation never induced a long-term potentiation of C-fiber-evoked field potentials with the spinal cord intact. In another group of animals, the spinal cord was transcended at the third cervical level. This caused an increase in the mean amplitude of C-fiber-evoked field potentials in all five rats tested (to $210 \pm 25\%$ of control responses prior to spinalization). In five spinalized rats, conditioning noxious skin heating induced strong inflammation and long-term potentiation of C-fiber-evoked field potentials to $183 \pm 21.4\%$ of control responses after spinalization. This potentiation lasted for at least 4 hr

and was not affected by trans-section of the sural nerve distal to the site of electrical stimulation. Subcutaneous formalin injection into a hind-paw induced long-term potentiation (to 205 ± 15.3%) in all five spinalized rats tested for at least 5 hr. Noxious mechanical skin stimulation at a hind-paw with an artery clamp (10 times for 5 sec, at 10 sec intervals) also induced long-term potentiation of C-fiber-evoked field potentials (to 210 ± 20% of control) which lasted for 1–4 hr in four spinal rats tested. Nerve injury by squeezing the sural nerve with forceps (four times) distal to the stimulation electrode long-term potentiation in all five spinal rats tested (to 250 ± 35% of control). This potentiation lasted for 2–4 hr. In rats with the spinal cord intact, the same conditioning stimulation never induced long-term potentiation. Thus, supraspinal descending systems do not only modify discharge rates, discharge patterns and the correlation dimension of discharges of spinal dorsal horn neurons, they may prevent also long-lasting potentiation of synaptic transmission between primary afferent C-fibers and higher order neurons in the spinal dorsal horn. The mechanism of this supraspinal control of plasticity of spinal nociception is presently not known. Apparently the release of substance P, which is required for induction of LTP, is not altered by supraspinal descending systems (Duggan *et al.*, 1988). Intact descending pathways in the dorsolateral funiculus may rather lead to an increase in substance P content in the superficial spinal dorsal horn following noxious stimulation (Zhang *et al.*, 1993). Descending inhibitory pathways may exert their effects postsynaptically by hyperpolarizing nociceptive spinal dorsal horn neurons and long-term potentiation is less likely induced by afferent stimulation in neurons which are hyperpolarized (Randić *et al.*, 1993). This may explain our observation that long-term potentiation of C-fiber evoked field potentials is induced more easily with descending pathways blocked. Hyperpolarization of some nociceptive spinal dorsal horn neurons by descending pathways from the NRM also would explain that electrical stimulation in the NRM may depress noxious heat-evoked FOS protein immunoreactivity in the spinal dorsal horn of anesthetized animals (Jones and Light, 1990). If expression of c-Fos protein in nociceptive spinal dorsal horn neurons is considered a marker for plastic changes of spinal nociception, then this result would support our notion that intact descending pathways may prevent long-term changes of nociception. However, the use of immediate-early gene expression in spinal dorsal horn as a reliable marker

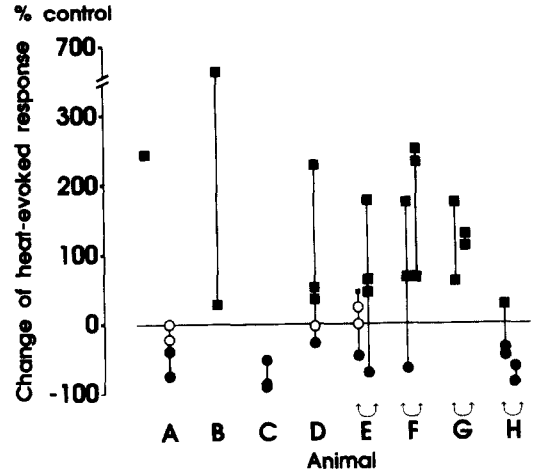

Fig. 25. Spinalization differentially affects noxious heat-evoked responses of simultaneously recorded multireceptive spinal dorsal horn neurons. Each data point indicates the percent change of noxious heat-evoked response of one neuron during spinalization compared to control responses with the spinal cord intact. Responses from neurons which were recorded simultaneously are connected by a vertical line. In animals (E)–(H), two consecutive reversible spinalizations were performed. Thus two groups of data points are connected by vertical lines in each animal. Open circles represent heat-evoked responses which were not changed during spinalization; squares indicate significantly enhanced responses; filled circles indicate significantly reduced responses.

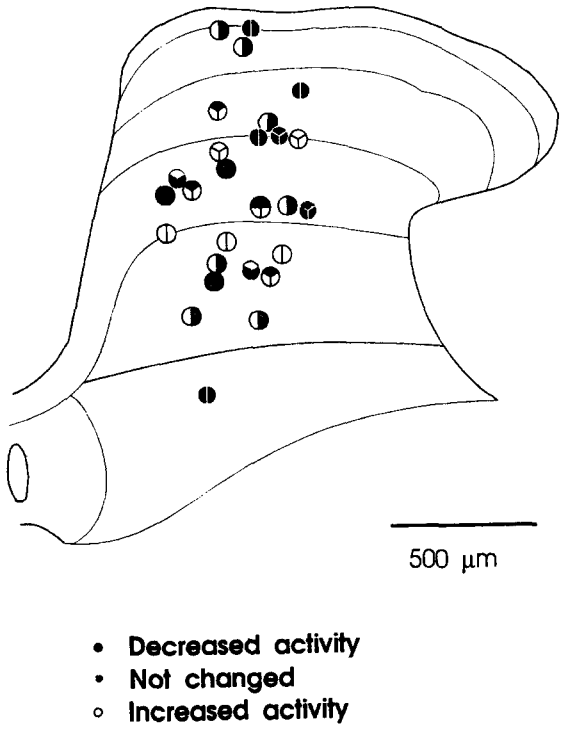

Fig. 24. Differential effects of spinalization on background activity of simultaneously recorded neurons in lumbar spinal cord. Schematic summary of all histologically verified recording sites which are superimposed on a representative transverse section through the 4th lumbar segment of spinal cord. Each circle indicates one recording site. The number of slices corresponds to the number of simultaneously recorded neurons at that site. Neurons which displayed a decreased background activity during spinalization are indicated by black slices, neurons which did not significantly change background activity are indicated by hatched slices and neurons which increased their background activity during spinalization are indicated by open slices. The Mann–Whitney *U*-test was used for statistical comparison of mean background activity before and during spinalization.

of plastic changes in nociception was challenged recently, as behavioral parameters of spinal nociception were not altered 1–14 days after even massive expression of c-Fos (Sandkühler *et al.*, 1996).

Results from previous studies suggest that at least some of the presently used general anesthetics such as barbiturates (Collins *et al.*, 1990) and halothane (Herrero and Headley, 1995) may preferentially depress activity in polysynaptic, e.g. in descending inhibitory, pathways. Correspondingly, the spinally mediated nociceptive tail-flick reflex generally has shorter latencies in lightly pentobarbital anesthetized rats as compared to awake, drug-free animals (Sandkühler and Gebhart, 1984b; Gebhart and Ossipov, 1986). Light pentobarbital anesthesia also may diminish the antinociceptive potency of morphine administered intracranially but not intrathecally in the rat (Ossipov and Gebhart, 1984). In single cell recordings from spinal dorsal horn neurons in awake cats, Collins and co-workers have shown that pentobarbital (20 mg/kg, i.v.) may unmask responses to noxious stimuli (Collins *et al.*, 1990). In awake sheep, it was reported recently that cutaneous receptive fields of spinal dorsal horn neurons are significantly larger in all classes of neurons recorded under halothane anesthesia compared to awake sheep. (Herrero and Headley, 1995). Thus, it might be speculated that some forms of general anesthesia currently used in humans and animals may reduce descending inhibition and thereby favor long-term increases in nociception.

4.3. Robust Long-Term Depression of Primary Afferent Neurotransmission

It has long been known that impulses in primary afferent A-fibers may depress transmission of nociceptive information in the spinal dorsal horn of the same segment (e.g. Handwerker *et al.*, 1975). This form of segmental antinociception is different, therefore, from heterosegmental, propriospinal and from supraspinal descending inhibition.

In their gate-control theory, Melzack and Wall (1965) propose that excitation of inhibitory interneurons in substantia gelatinosa mediate afferent-induced segmental antinociception. This does not, however, readily explain the fact that some form of segmental antinociception may outlast the duration of conditioning stimulation by hours or even days. This long-lasting analgesia now is being used for the treatment of certain kinds of pain in humans (Lindblom and Meyerson, 1976; Urban and Nashold, 1978). Randić and co-workers have shown that high frequency stimulation of dorsal roots may produce either long-term depression or long-term potentiation of primary afferent neurotransmission in the superficial spinal dorsal horn in a transverse slice preparation of the young rat. Recently, we have identified a robust long-term depression of Aδ-fiber-evoked mono- or polysynaptic excitatory postsynaptic potentials in the superficial spinal dorsal horn by low frequency stimulation of afferent A-fibers. Conventional intracellular recordings were made from neurons in the superficial spinal dorsal horn in a transverse slice preparation of young (18–27 days) rat spinal cords with long dorsal roots attached. To recruit afferent A-fibers, bipolar electrical stimulation of IVth or Vth lumbar dorsal roots was used (0.5–8 V pulses of 0.1 msec duration).

Only neurons which displayed stable mono- or polysynaptic excitatory postsynaptic potentials were included in this study. Conditioning stimulation of afferent roots consisted of 900 pulses (10 V, 0.1 msec) given at 1 Hz. As a quantitative parameter of synaptic strength, the peak amplitude of Aδ-fiber-evoked excitatory postsynaptic potentials was measured. In all eight neurons tested, low frequency conditioning stimulation decreased synaptic responses (to a mean of 40.8 ± 10.3% of control). This inhibition was not reversible throughout the recording period of up to 2 hr. Conditioning low frequency stimulation of both A- and C-fibers (25 V, 0.5 msec) was as effective as A-fiber stimulation alone. Mean synaptic responses of three neurons were depressed to 38.7 ± 14.1% of control. In the presence of the $GABA_A$ receptor antagonist bicuculline (5 μM) and glycine receptor antagonist strychnine (2 μM), the efficacy of long-term depression was not affected. Mean synaptic responses were depressed to 45.5 ± 6.0% in six neurons tested. Blockage of NMDA receptors by bath application of D-APV at 50 μM reduced or abolished long-term depression. In the presence of extracellular calyculin A, a potent, membrane-permeable inhibitor of protein phosphatases 1 and 2A, long-term depression appeared to be even somewhat more effective. The EPSP amplitudes were depressed to 15.0 ± 6.0% of control in four neurons tested.

Thus, a strong and long-lasting depression of primary afferent neurotransmission in Aδ-fibers, many of which may be nociceptors, can be produced by conditioning low frequency stimulation of afferent A-fibers. This long-term depression of synaptic transmission may underlie segmental antinociception.

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