Differential effects of spinalization on discharge patterns and discharge rates of simultaneously recorded nociceptive and non-nociceptive spinal dorsal horn neurons

J. Sandkühler a,*, A. Eblen-Zajjur a, Q.-G. Fu a and C. Forster b

a II. Physiologisches Institut, Universität Heidelberg, 69120 Heidelberg (Germany) and b Institut für Physiologie und Biokybernetik, Universität Erlangen, 91054 Erlangen (Germany)

(Received 18 November 1993, revision received 18 February 1994, accepted 13 April 1994)

Summary

Recordings were made simultaneously from 2–5 neurons at the same site in the lumbar spinal dorsal horn of pentobarbital-anesthetized rats. Neurons were classified as low-threshold (LT) or multireceptive (MR) according to their responses to non-noxious mechanical or noxious radiant heat stimuli of the skin. At the same recording sites neurons could be encountered which belong to different classes and/or which had mechanoreceptive fields which did not overlap. Cold blocks of the upper or lower thoracic cord or transsections of the upper cervical cord were made to evaluate the effects of spinalization on both the rate and pattern of background activity and/or noxious heat-evoked responses of different dorsal horn neurons under identical experimental conditions. At 24 of 27 recording sites, spinalization had qualitatively or quantitatively different effects on the rate of background activity of simultaneously recorded neurons. Interspike interval (ISI) means of background activity were significantly reduced in 29 of 65 (44.6%) neurons, prolonged in 23 of 65 (35.4%) neurons, or unchanged in 13 of 65 (20%) neurons. MR neurons displayed a significantly higher incidence of decreased background activity 17 of 45 (37.8%) and a lower incidence of increased background activity (18 of 45, 40%) during spinalization than the LT neurons from which 1 of 12 (8.3%) decreased and 8 of 12 (66.6%) increased background activity. Almost all (95.4%) neurons changed their discharge patterns after spinalization. At 9 of 27 recording sites, the discharge patterns of simultaneously recorded neurons were affected differently by spinalization as revealed by the coefficient of dispersion of the interspike intervals (ISI), indicating changes in the tendency to discharge action potential in clusters (bursts). At the same recording sites the level of noxious heat-evoked responses of simultaneously recorded MR neurons was also differentially affected by spinalization. Nociceptive responses were significantly enhanced in 19 of 37 (51.4%) neurons (137.8 ± 142.6% of control, mean ± SD), reduced in 13 of 37 neurons (35.1%) (by 58.9 ± 20.9%) and/or unchanged in 5 of 37 (13.5%) neurons. It is concluded that no general ‘tone’ of descending antinociception exists and that tonic descending excitatory and inhibitory systems may be active simultaneously modulating both the level and pattern of neuronal discharges.

Key words: Tonic descending inhibition; Tonic descending excitation; Endogenous analgesia; Multiple single-neuron recordings; (Rat)

Introduction

Recordings from single neurons in the spinal dorsal horn have provided important insights into the spinal mechanisms of nociception and antinociception. It is now well established that the excitability of spinal nociceptive neurons is subject to strong descending controls which may be tonically active under the given experimental conditions. Some of the supraspinal sources of tonic descending modulation of discharge rates have been located to the lateral reticular nucleus in the ventrolateral medulla of cats (Morton and Duggan, 1988) but not rats (Janss and Gebhart, 1988a), and
to the nucleus raphe magnus and adjacent reticular formation of rats (Cho and Basbaum, 1991) but not cats (Gebhart et al., 1983). Apparently, multiple descending spinal pathways exist bilaterally in the lateral funiculi (Carlton et al., 1985; Sandkühler et al. 1987a,b; Janss and Gebhart, 1988b) which convey tonic inhibitory and/or tonic excitatory controls of spinal dorsal horn neurons. It was proposed that the various descending systems would also have different targets in the spinal cord with respect to the laminar location and the functional properties of the neurons modulated. In an early work Wall (1967) has shown that spinalization affects excitability of spinal dorsal horn neurons in a lamina specific fashion. Laird and Cervero (1990) reported that the size of cutaneous receptive fields of nociceptive specific neurons in the deep dorsal horn is tonically reduced by descending pathways, and that multireceptive (MR) neurons in the deep dorsal horn were either inhibited or excited. Tattersall et al., (1986) found that the responses of spinal dorsal horn neurons (mainly in laminae V) to electrical stimulation of the splanchnic nerve were mainly subject to tonic descending inhibition, while responses of neurons in ventral horn (mainly lamina VIII) were predominantly enhanced by tonic descending systems. For some of their neurons the level of background activity and the magnitude of evoked response was differentially affected by spinalization.

It is, however, not possible to decide on the basis of single cell recordings alone to what extent the inevitable and possibly undetected differences in the experimental conditions account for differential effects of spinalization on the discharge rates of neurons recorded in different experiments. Further, virtually nothing is known about the impact of tonic descending systems on the patterns of neuronal discharges in the spinal dorsal horn.

Here, we have recorded simultaneously from two to five neurons at the same site in the lumbar spinal dorsal horn and found, that spinalization rostral to the recording site may simultaneously reduce the level of background activity and/or noxious heat-evoked responses in one neuron and increase activity in another, showing that tonic descending excitatory and inhibitory systems are simultaneously active. It is further shown that tonic descending system may modulate the patterns of discharges of spinal dorsal horn neurons and that spinalization may differentially affect discharge patterns of simultaneously recorded neurons, with or without changes in their discharge rates.

Materials and methods

Surgery and anesthesia

Experiments were performed on 22 male Sprague-Dawley rats under deep pentobarbital anesthesia (60 mg/kg, i.p. initially, 15–20 mg/kg/h, i.v. for maintenance). The surgical level of anesthesia was verified by the absence of flexor, withdrawal and corneal reflexes and later in the course of the experiments by stable mean arterial blood pressure and a constant heart rate during noxious skin heating. Butylscopolamium bromide (Buscopan) was given subcutaneously at a dose of 10 mg at the beginning of the experiment to reduce secretions into the respiratory tract. One external jugular vein was cannulated for continuous infusion of a glucose-tyrode solution that also contained pentobarbital sodium. One common carotid artery was cannulated to continuously monitor mean arterial blood pressure, which ranged between 80 and 100 mm Hg under control conditions (spinal cord intact). Colorectal temperature was kept constant at 37.5 ± 0.5°C (mean ± SD) by a feed-back-controlled heating blanket underneath the ventral surface of the animals. A laminectomy was performed from the 11th thoracic to the 4th lumbar vertebra to expose the lumbar enlargement of the spinal cord for multiple single-neuron recordings. A second laminectomy at the 1st thoracic or the 1st cervical vertebra was made for reversible cold-block spinalization. The dura matter was incised longitudinally to allow implantation of bilateral surface electrodes for antidromic stimulation of ascending axons. The vertebral column was suspended in a horizontal position by means of 2 vertebral clamps fixed to mid-thoracic and lower lumbar vertebrae to a metal frame. The head of the animal was fixed in a horizontal position in a stereotaxic frame. The left sural nerve was dissected free for bipolar stimulation with platinum hook-electrodes and left in continuity. All exposed nervous tissue was covered by warm (36°C) paraffin oil. The left hindpaw was fixed pad upwards in a holder with paraffin wax to allow noxious radiant skin heating of the glabrous skin.

Recording and stimulation

Extracellular multi-neuron recordings were made with tungsten microelectrodes (4–5 MΩ impedance at 1 kHz, A-M System). Electrode tracers were made vertically into the lumbar spinal dorsal horn in steps of 5 μm with an electronically controlled microstepping motor. Light mechanical skin probing at the left hindpaw was used as a search stimulus to identify also neurons with low background activity. Neuronal discharges were digitized by an AD converter card DT2821 at 32 kHz. The principal-component method based on waveform shape (Salganikoff et al. 1988) was used for discrimination of the action potentials in multi-neuron recordings. The quality of action potential sorting was tested on-line by simultaneous window discrimination of the action potentials. The original multi-neuron signal was stored on FM tape and the digitized signal was stored on hard disk for off-line analysis. Only discharges of those neurons which could be clearly differentiated by the morphology of their action potentials were included in this study. The first 20–40 min of each recording period were not analyzed to allow stabilization of background activity which was verified by tests for stationarity.

Whole side-by-side bipolar electrical stimulation (0.5 msec, 2–3 V) at the upper cervical cord was applied to identify antidromic responses in the lumbar dorsal horn, using the conventional criteria: constant latency, response to high-frequency stimulation (at 333 Hz) and collision of antidromic and orthodromic action potentials. The sural nerve was electrically stimulated to recruit Aβ and Aδ fibers (0.1 msec pulse, 2.5 V) or A and C fibers (0.5 msec pulses, 25 V). Noxious radiant heat stimuli were applied to the glabrous food pad by a feed-back controlled quartz-halogen lamp with a heating focus of 16 mm². Standard heat stimuli consisted of 50°C pulses given for 10 sec in intervals of not less than 2 min resulting in stable control responses. In some experiments more than 100 heat stimuli were applied to the same skin area without producing any detectable signs of sensitization of nociceptors or inflammation of the heated skin area throughout the course of the experiments. This is probably due to the use of a feedback-controlled radiant heat source which produces a stable surface temperature of 50°C and an intracutaneous temperature of 43–44°C (Beck et al. 1974).
Responses to tapping, brushing and squeezing the skin were tested and the size of cutaneous mechanoreceptive fields were determined by responses to skin probing with calibrated von Frey hairs (6.7 g). The location and the shape of the receptive fields were reconstructed on paper and the receptive field size was determined with the weighing method.

Reversible spinalization at the cervico-thoracic cord

For reversible spinalization a thermode (0°C at the surface of the thermode) was placed directly on the spinal cord dorsum at the cervico-thoracic junction or at the lower thoracic cord. The efficacy of the cold block was always verified by a drop in mean arterial blood pressure by 40–50% of its previous value. In some experiments a transection at the upper cervical cord was performed under local anesthesia (infiltration with 2% lidocaine) for permanent spinalization.

Histology

At the end of the experiments, electrolytic lesions were made at the recording sites through the recording electrodes (30–40 μA, 20–25 sec). The rats were killed by an overdose of pentobarbital and transcardially perfused with phosphate-buffered saline followed by paraformaldehyde (4%). The spinal cords were removed and cut in a cryostat in 50 μm coronal sections which were stained with cresyl violet. The recording sites were identified and reconstructed on a schema of the lumbar cord.

Data analysis

Original recordings were stored on FM tape and analyzed off-line. After discrimination of action potentials, individual neuronal discharges were evaluated by peristimulus time histogram (PETH), bin width 1 sec and interspike interval histogram (ISIH), bin width 1 msec. For all discharges the arithmetic mean (X) and the standard deviation (SD) of the ISIs were determined. The coefficient of variation (CV = SD/X) was calculated and used to evaluate the variability of the ISIs. The coefficient of dispersion (CD = Variance/X) was also calculated to evaluate the tendency of the neurons to discharge action potentials in clusters (bursts) (Cocatre-Zilgien and Delcomyn 1992). Heat-evoked responses were calculated as total number of impulses in 15 sec beginning with the onset of the heat stimulus and corrected for background activity. Mean of 7 consecutive heat-evoked responses recorded immediately before spinalization served as controls for comparison with 7 consecutive responses recorded at the end of the spinal cold block or when

![Graphical representation of data analysis](Image)

Fig. 1. Differential effect of spinalization on background activity of 3 neurons recorded simultaneously at the same site the lumbar spinal dorsal horn during cold block of spinal segments C6 to ThE. The continuously recorded mean arterial blood pressure (A) was reversibly reduced from 87 mm Hg to 50 mm Hg. Peristimulus time histograms (PETH) bin width 1 sec, of the background activity of 3 MR neurons are shown in B, C and D, respectively. Representative shapes of action potentials of the 3 neurons are presented in E, F, and G. Note the inflection on the initial slope of the spikes (arrows) suggestive of cell body activation. The histologically verified recording site is shown in H. Note that the cutaneous excitatory mechanoreceptive fields of the 3 neurons represented in I, J, and K did not or only partially overlap.
arterial blood pressure and level of heat-evoked responses reached a stable plateau after traumatic spinalization. A trend test was used to verify stationarity of the responses (Wang and Vagnucci 1989). The Mann-Whitney U test (Soto et al. 1980) was used for statistical comparisons; P < 0.05 was considered significant.

Results

In 22 animals, the background activity (10 animals) or noxious heat-evoked responses (12 animals) were recorded before and during reversible cold block of the upper or lower thoracic spinal cord. In 4 animals the upper cervical cord was transected later in the course of the experiments. During spinalization, mean arterial blood pressure was always significantly reduced from an average of 89.8 ± 6.4 mm Hg (mean ± SD) to 48.7 ± 8.5 mm Hg (see Fig. 1 for an example).

Using the method described by Gochin et al. (1991) we estimate the diameter of the recording area of our microelectrodes to be about 100 μm (range: 80–120 μm). This value is the mean of the difference of the distances travelled by the microelectrode between the appearance and disappearance of the neuronal action potentials during the recording.

Inflections seen on the initial slope of action potentials corresponding to the activation of the initial segment of the axon or indicating soma-dendritic activation were observed in 94.7% of the action potentials, suggesting that the vast majority of recordings were made from neuronal cell bodies (Lipski 1981; Barman and Gebber 1992) (see Fig. 1).

Most of the neurons (24 of 29, 82.7%) could not be activated antidromically from the 2nd to 4th cervical segments. Neurons responding to light mechanical skin probing (tapping, brushing and hair movement) and to electrical stimulation of Aβ fibers but not to noxious skin heating, pinching or with a long latency to electrical nerve stimulation at C-fiber intensity were classified as low-threshold (LT) neurons. Neurons responding additionally to noxious skin stimuli and with a long latency (> 80 msec) to electrical nerve stimulation at C-fiber strength were classified as MR (wide-dynamic-range) neurons.

Cutaneous receptive fields of simultaneously recorded neurons

In 19 of 37 (51.4%) pairs of neurons which were recorded simultaneously at the same site in the spinal dorsal horn and which belonged to the same class (i.e., 2 LT neurons or 2 MR) the cutaneous mechanoreceptive fields did not or only minimally overlap (see Fig. 11–K for examples). The incidence of non-overlapping cutaneous receptive fields was even higher for pairs of neurons which belonged to different classes (6 of 8 pairs, 75%).

Effect of spinalization on background activity

Level of background activity. Background activity was recorded from 65 neurons in 10 animals at 27 sites in the form of 17 pairs, 9 triplets and 1 quadruplet, resulting in 50 possible combinations of pairs of neurons recorded at the same site and located in laminae I–VI (see Fig. 2). Neurons were selected on the basis of a stable and relatively high (1–100 imp/sec) level of background discharges.

In 23 of 50 (46%) pairs of neurons recorded at 14 different sites, spinalization produced qualitatively different effects on the level of background activity, i.e., discharge rates were decreased, increased and/or not changed (see Figs. 1–3). For 23 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. For 4 of 50 (8%) pairs of neurons spinalization failed to induce changes in the level of their background activity. In 2 ketamine-anesthetized animals spinalization also produced qualitatively different effects on discharge rates of simultaneously recorded pairs of spinal dorsal horn neurons (results not shown).
TABLE I
DIFFERENTIAL EFFECT OF SPINALIZATION ON THE RATE OF BACKGROUND ACTIVITY OF SPINAL DORSAL HORN NEURONS

Results are reported as number of neurons with a particular response to spinalization vs. number of neurons tested. The corresponding incidences are indicated in parentheses. LT, low-threshold neurons; MR, multireceptive neurons. Depth of the recording sites is expressed in μm from the cord dorsum. The responses to 10-min cold-block spinalization were classified based on the Mann-Whitney U non-parametric analysis of neuronal discharge rates.

<table>
<thead>
<tr>
<th>Discharge rate during cold block</th>
<th>Incidence (%)</th>
<th>Incidence (%)</th>
<th>Incidence (%)</th>
<th>Depth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT</td>
<td>MR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td>23/65 (35.4)</td>
<td>1/12 (8.3) **</td>
<td>17/45 (37.8)</td>
<td>593.4 ± 332.8</td>
</tr>
<tr>
<td>Unchanged</td>
<td>13/65 (20.0)</td>
<td>3/12 (25.0)</td>
<td>10/45 (22.2)</td>
<td>648.7 ± 318.9</td>
</tr>
<tr>
<td>Increased</td>
<td>29/65 (44.6)</td>
<td>8/12 (66.7) *</td>
<td>18/45 (40.0)</td>
<td>611.6 ± 255.6</td>
</tr>
</tbody>
</table>

* P < 0.01.
** P < 0.001.

MR neurons presented a significantly higher incidence of decreased background activity (37.8%) during spinalization than LT neurons (8.3%), and the incidence of increased background activity was higher for LT neurons (66.7%) than for MR neurons (40%) (see Table I).

The most frequent combinations in our sample were pairs of neurons with 1 neuron which increased and another neuron which decreased their activity during spinalization and pairs of neurons which both increased their background activity. These incidences match with the theoretical probability of combinations calculated after their general incidence.

During spinalization, the CV of the background activity of 33 of 65 (50.7%) neurons increased but decreased in 29 of 65 (44.6%) neurons. The CV did not change by more than 10% in 3 of 65 (4.6%) neurons (see Table II). An increase in the CV was most common for LT neurons (incidence 66.7%) and for those neurons which had an increased discharge rate during spinalization. In contrast, a decrease of the CV was most often found for neurons which decreased their discharge rates during spinalization (see Table II and Fig. 4).

Patterns of background activity. The magnitude of the coefficient of dispersion of the ISI was used to evaluate the tendency of a neuron to discharge action potentials in clusters (bursts). Twenty-three from 57 (40.4%) fully characterized neurons increased their CD values during spinalization, 29 of 57 (50.9%) neurons presented a decrease and 3 of 57 (5.3%) neurons did not change this parameter during spinalization. A complete inhibition of background activity was produced in 2 other neurons (see Table III). LT and MR neurons presented similar incidences of decreased or increased CD values. From a total of 65 neurons, decreased CD values were most common for those neurons which increased their discharge rates during spinalization.

TABLE II
EFFECTS OF SPINALIZATION ON THE VARIABILITY OF THE ISIs OF THE DISCHARGES OF SPINAL DORSAL HORN NEURONS AS EVALUATED BY THE COEFFICIENT OF VARIATION (CV)

The coefficient of variation of the ISIs was calculated as \( CV = SD/X \); incidences are indicated in parentheses. Five neurons were not fully tested for classification and therefore are not included here. Three neurons were nociceptive specific; 2 other neurons were completely inhibited.

<table>
<thead>
<tr>
<th>Class of neuron</th>
<th>Discharge rate</th>
<th>Incidence</th>
<th>Increased CV</th>
<th>Decreased CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-threshold</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 12)</td>
<td>Decreased</td>
<td>1 (8.3%)</td>
<td>1 (8.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Unchanged</td>
<td>3 (25.0%)</td>
<td>2 (16.7%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>8 (66.7%)</td>
<td>5 (41.7%)</td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td>Multireceptive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 45)</td>
<td>Decreased</td>
<td>17 (37.8%)</td>
<td>9 (22.5%)</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td></td>
<td>Unchanged</td>
<td>10 (22.2%)</td>
<td>5 (12.5%)</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>18 (40.0%)</td>
<td>6 (15.0%)</td>
<td>12 (30.0%)</td>
</tr>
<tr>
<td>All neurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 65)</td>
<td>Decreased</td>
<td>23 (35.4%)</td>
<td>14 (60.9%)</td>
<td>6 (26.1%)</td>
</tr>
<tr>
<td></td>
<td>Unchanged</td>
<td>13 (20.0%)</td>
<td>8 (61.5%)</td>
<td>5 (38.5%)</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>29 (44.6%)</td>
<td>11 (37.9%)</td>
<td>18 (62.1%)</td>
</tr>
</tbody>
</table>

* P < 0.01.
contrast, neurons which decreased their discharge rates displayed a higher incidence of increased CD values (see Table III).

**Effects of spinalization on noxious heat-evoked responses of multireceptive neurons**

Heat-evoked responses of 40 spinal dorsal horn neurons were recorded in 12 other animals. The background activity of these neurons ranged between 0 and 8 imp/sec. Mean discharge rates of control heat-evoked responses were at least 7 times higher than level of background activity. During spinalization noxious heat-evoked responses were significantly enhanced in 19 cases by 137.8 ± 142.6% of control (Mann-Whitney U test; mean control responses: 117.3 ± 68.2 imp/15 sec, see Figs. 5 and 6) but in 13 cases responses were significantly reduced by 58.4 ± 20.9% of controls (193.2 ± 220 imp/15 sec). Responses did not change significantly in 5 cases (-6.8 ± 21.4% of control). The increase in the noxious heat-evoked responses was positively correlated with the depth of the recording sites ($r_s = +0.73$, linear function, $P < 0.01$).

The change of heat-evoked responses during spinalization was not correlated with the level of control heat-evoked responses with the cord intact ($r_s = -0.1532$, $P > 0.05$, Spearman rank correlation coefficient, Fig. 6B). In 5 of 8 experiments (animals A, D, E, F and H in Fig. 6A) qualitatively different effects of spinalization on noxious heat-evoked responses of simultaneously recorded dorsal horn neurons were observed. Cold-block spinalization and transection generally produced qualitatively the same effects sometimes with different time courses (see Fig. 5). The effects of cold-block spinalization were fully reversible in most neurons (see Figs. 1 and 5). In 3 animals propranolol (20 mg/kg) was slowly infused intravenously and reduced mean arterial blood pressure to 45–65 mm Hg without affecting noxious heat-evoked response of 3 MR spinal dorsal horn neurons.

**Discussion**

The present results have shown that spinalization rostral to the recording site in the lumbar spinal dorsal horn affects either discharge rates or discharge pat-
Intact Blocked Intact Transsected

Blood Pressure

0

Time (min)

100

Fig. 5. Differential effect of spinalization on noxious heat-evoked responses in the lumbar spinal dorsal horn. Results from 1 animal are shown. Top: mean arterial blood pressure was measured in 2-min intervals and plotted on the ordinate (mm Hg) versus time of the experiment (min). Individual responses of 3 different neurons with overlapping cutaneous receptive fields to standard noxious skin heating were recorded simultaneously in 2-min intervals and plotted as total number of impulses in 15 sec versus time (filled circles). Vertical lines delineate intervals of cold blocking the lower thoracic cord (Blocked), absence of blockage (Intact) and surgical transection at the upper cervical cord (Transsected). With a delay of some minutes cold blocking reduced mean arterial blood pressure and the nociceptive responses of neuron 1 but enhanced responses of neurons 2 and 3. All effects had similar time course and were fully reversible except for the depression of neuron 1. Note that traumatic spinalization at C3 produced a triphasic blood pressure response in this animal and enhanced responses of all 3 neurons. The results from this animal are shown in Fig. 6 (animal F).

Properties of dorsal horn neurons recorded simultaneously at the same site

Neurons were recorded simultaneously at the same site in lamina I–VI of the spinal dorsal horn in pairs, triplets or quadruplets, only rarely in groups of 5 neurons. It is reasonable to assume that the generators of the action potentials recorded were located within a distance of not more than 60 μm from the tip of the electrode since this is within the range of appearance and disappearance of the recorded signal (Gochin et al. 1991). It can, of course, not be excluded that we have recorded from large dendrites in some of the experiments; we do, however, believe that the vast majority of our recordings were made from cell bodies rather than from fibers of passage, based upon the morphology of the action potentials. Thus, we conclude that in most cases action potentials were recorded from cell bodies of neurons which were located not further than 60 μm from the tip of the recording electrode and not more than 120 μm from each other.

If these assumptions are correct, then our results suggest that in the rat spinal dorsal horn sensory neurons with similar afferent input are not strictly grouped together in clusters. Neurons which belong to different functional classes (LT, MR) and neurons with completely separate cutaneous mechanoreceptive fields

TABLE III

EFFECTS OF SPINALIZATION ON THE TENDENCY TO DISCHARGE ACTION POTENTIALS IN BURSTS AS EVALUATED BY THE COEFFICIENT OF DISPERSION (CD) OF THE ISI OF BACKGROUND ACTIVITY OF SPINAL DORSAL HORN NEURONS

The coefficient of dispersion was calculated as \( CD = \frac{\text{Variance}}{\bar{X}} \). Five neurons were not fully tested for classification. Three neurons were nociceptive specific. Background activity of 2 other neurons was completely inhibited during spinalization, thus no CD could be determined.

<table>
<thead>
<tr>
<th>Class of neuron</th>
<th>Incidence</th>
<th>Discharge rate</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Increased</td>
</tr>
<tr>
<td>Low-threshold</td>
<td>12</td>
<td>Decreased</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unchanged</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased</td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased</td>
<td>8 (18.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unchanged</td>
<td>3 (7.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased</td>
<td>5 (11.6%)</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>Decreased</td>
<td>12 (52.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unchanged</td>
<td>4 (30.8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased</td>
<td>8 (27.6%)</td>
</tr>
</tbody>
</table>

* \( P < 0.01 \).
were recorded simultaneously at the same site. Brown et al. (1980) have convincingly shown for cat spinocervical tract neurons that overlapping cutaneous receptive fields are always associated with interdigitating dendritic trees. Neurons with cell bodies which were up to 600 μm apart in rostro-caudal direction always had overlapping receptive fields and interdigitating dendritic trees. They also found neuronal pairs which were not more than 100 μm apart in medio-lateral direction with completely separate dendritic trees and terminal boutons (Woolf and King 1987). Local axon collaterals of some MR spinal dorsal horn neurons with en-passage and terminal boutons (Woolf and King 1987) provide a morphological substrate for such a mechanism.

General effects of spinalization

Spinalization was always accompanied by a drop in mean arterial blood pressure from about 90 to 50 mm Hg. An insufficient perfusion of the spinal cord at the recording site is not a plausible explanation for any of the changes in discharge rates and discharge patterns observed as it was shown (Holtz et al. 1988) that autoregulation maintains sufficient blood flow through the rat spinal cord with little changes unless the systemic blood pressure falls below 45 mm Hg. Further, the time course of changes in mean arterial blood pressure often did not correspond to the changes in discharge rates which returned to pre-block values faster (e.g., Fig. 5 neurons 2 and 3; Fig. 1 neuron B) or slower (e.g., Fig. 5 neuron 1; see also Fig. 1 neuron D with a slow rise in discharge rate during cold block) than mean arterial blood pressure. Finally, reduction of mean arterial blood pressure by i.v. propranolol never produced significant changes in discharges of spinal dorsal horn neurons.
Differential effects of spinalization on discharge rates

From single-cell recordings in the spinal dorsal horn it is known that spinalization may enhance, reduce or have no effect on the level of background activity of MR or LT neurons (Wall 1967; Tattersall et al. 1986; McMahon and Wall 1988; Laird and Cervero 1990) and may also have qualitatively different effects on the magnitude of nociceptive responses of MR neurons (Handwerker et al. 1975). Tonic descending inhibition of nociceptive neurons in the spinal dorsal horn has attracted much attention, as descending inhibition is a major mechanism of endogenous antinociception and may modulate the intensity coding of spinal dorsal horn neurons for noxious stimuli (Dickhaus et al. 1985). Tonic descending inhibition may, however, also improve sensory discrimination, e.g., by reducing the size of cutaneous mechanoreceptive fields (Laird and Cervero 1990), 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). If descending inhibition is activated by noxious stimuli, it may improve sensory discrimination by reducing excitability of MR neurons in somatotopically inadequate spinal cord segments. This mechanism which has been termed 'diffuse noxious inhibitory control' (Le Bars et al. 1979a, b) may well contribute also to tonic descending inhibition in the acute preparation.

Tonic descending excitation is much less well studied and has previously never been observed together with tonic descending inhibition. It has been proposed that neurons which are excited by descending pathways could be inhibitory propriospinal neurons (Sandkühl er et al. 1991a, 1993) or neurons which activate a supraspinal inhibitory loop (Cervero and Wlostencroft 1984; McMahon and Wall 1988). Thus, these neurons would be part of a serial inhibitory system. McMahon and Wall (1988) concluded from their work that lamina I neurons could mediate descending inhibition of neurons in deeper laminae and we (Sandkühl er et al. 1993) have provided evidence that part of the descending inhibition is mediated by propriospinal neurons originating bilaterally from circumscribed areas of the spinal cord gray matter, mainly from laminae I, II, VIII. Such a serial system with intercalated inhibitory interneurons implies that descending excitation and descending inhibition must be simultaneously present. The present study has indeed identified pairs of neurons which changed the level of their background activity in opposite directions during spinalization.

The fact that descending excitation and inhibition are simultaneously present may also indicate that some sensory channels are selectively and differentially controlled by descending systems improving the signal-to-noise ratio and/or spatial discrimination in some, but not in other, sensory systems, thereby functioning as a gate which selectively controls the transmission of information (e.g., about a noxious skin stimulus) through some spinal dorsal horn neurons. Since most neurons encountered could not be antidromically activated from the second cervical level it seems unlikely that the information is simply directed to spinal or supraspinal targets. Possibly, those nociceptive neurons which were differentially affected by spinalization could also project to different spinal targets sites mediating different functional consequences of spinal nociception. This is of course a likely assumption for those neurons which were recorded in different laminae of the spinal dorsal horn and which may be affected differentially by spinalization (Dubuisson and Wall 1980; Tattersall et al. 1986; McMahon and Wall 1988) but it seems also reasonable for some of the neurons which were recorded simultaneously at the same site in the present study. Some of the neurons belonged to different functional classes, i.e., they were LT or MR neurons and the level of background activities of pairs of neurons of different classes was very often affected differentially by spinalization: the vast majority of LT neurons was found to be subject to tonic descending inhibition, whereas the level of background activity of MR neurons was equally often subject to descending excitation or inhibition. Thus, tonic descending systems may improve the signal-to-noise ratio in most LT and some MR neurons.

Pairs of neurons which belong to the same functional class, e.g., 2 MR neurons, may very well also be involved in separate sensory functions, as we have shown recently that the discharges of some pairs of neurons which belong to the same functional class and which were recorded simultaneously at the same site in the spinal dorsal horn may not be causally related in time, as revealed by perfectly flat cross-correlation of their discharges (Sandkühl er et al. 1994). Flat cross-correlation suggests that the neurons do not have any suprathreshold common input and that the neurons are not interconnected serially.

Differential effects of spinalization on discharge patterns

Background activity of sensory neurons in the spinal dorsal horn has often been considered to be random noise which affects the signal-to-noise ratio. This assumption implies that information is transmitted using a mean code mode. An increase in the CV of the ISIIs would then indicate a reduced capacity to transmit information. This is not necessarily the case if a pattern code mode is used and indeed detailed examination of the ISI distributions and autocorrelation analysis have shown that the background activity of spinal dorsal horn neurons may have characteristic features which may be related to functional properties of the neurons (Surmeier et al. 1989).
Burst-like discharges with periods of short ISIs may have an important impact on information transmission not only resulting in higher temporal summation at the postsynaptic membrane, but probably also resulting in differential release of neuroactive substances at the presynaptic terminal (Cazalis et al. 1985). If the coefficient of dispersion of the ISI is an useful quantitative parameter for the tendency to discharge action potentials in clusters (bursts) (Cocatre-Zilgien and Delcomyn 1992), then an increase in the CD may be interpreted as a change in discharge pattern which favors synaptic transmission. Here, we have shown that tonic descending pathways may not change discharge rates of some of the spinal dorsal horn neurons but may prevent some of the neurons from discharging in bursts. Thus, descending inhibition which is classically evaluated by a decrease in discharge rate could also be interpreted as a change in discharge pattern from a clustered to a more uniform distribution of ISIs. If burst-like discharges identified in the present study are not generated solely by cellular properties but in the neuronal network of the spinal cord, then an increase in the tendency to discharge action potentials in clusters would also indicate changes in the functional neuronal connectivity.

Recently we have shown that inflammation of the skin and the extrasynaptic spread of substance P in the recording segment may also produce changes in the CD and in the non-linear dynamics (phase space portrait and correlation dimension D₂) of spinal dorsal horn neuronal discharges (Sandkühler 1993; Sandkühler et al. 1994). Taken together these results suggest that processing of nociceptive information at the level of the spinal dorsal horn may not only be modulated by changes in mean discharge rates, but also by more subtle changes in discharge patterns.

Neuronal system(s) mediating tonic descending controls of spinal dorsal horn neurons

It is a consistent finding in the present study that under identical experimental conditions the discharges of neurons which were recorded at the same site of the spinal dorsal horn may be differentially modulated by tonic descending systems. This does not necessarily mean that different descending systems are operating. The diversity and specific distribution of postsynaptic receptors for neurotransmitters is consistent with the hypothesis that a single modulatory system may have very different effects on different spinal dorsal horn neurons. Electrophysiological data also demonstrate that iontophoretic application of neurotransmitter candidates may have very different effects on the discharge rates and patterns of neurons recorded sequentially in the same or in different animals (Jones et al. 1990). Even if a single descending modulatory system would be responsible for the changes in discharge rates and patterns, multiple neurotransmitters could be involved (Sandkühler and Zimmermann 1988), as co-existence of neurotransmitter candidates has been established in many of the descending pathways investigated so far (Millhorn et al. 1988, 1989). There is, of course, also convincing evidence that spinalization will interrupt a variety of different descending pathways, some of which may be tonically active under the given experimental conditions. Although the supraspinal origin of tonic descending modulation has not been identified with certainty, the results by Morton and Duggan (1988) suggest that in the cat, but not in the rat (Janss and Gebhart 1988a), the lateral reticular nucleus may be involved. Numerous additional brain-stem sites are known to be involved in descending modulation (see review by Besson and Chaouch 1987; Gebhart 1988) via different pathways in white (Jones and Gebhart 1987; Sandkühler et al. 1987a,b) and gray matter (Sandkühler et al. 1991a) of the spinal cord. It remains to be shown that the various descending pathways may differentially innervate neurons that are located in close proximity to each other.

Acknowledgements

We thank Gabriele Eilber and Manfred Böhm for excellent technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SA 435) and the Deutscher Akademischer Austauschdienst.

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