Responses of Rat Sacral Spinal Neurons to Mechanical and Noxious Thermal Stimulation of the Tail

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Douglass, Diana K. and E. Carstens. Responses of rat sacral spinal neurons to mechanical and noxious thermal stimulation of the tail. J. Neurophysiol. 77: 611–620, 1997. In this study we investigated the receptive field properties, responses to mechanical and thermal stimuli, and sensitivity to systemic administration of pentobarbital sodium and morphine of single neurons recorded in the sacral spinal cords of pentobarbital-anesthetized rats. Fifty-three neurons responded to innocuous mechanical stimulation of the tail. Of 45 neurons that were additionally tested with noxious thermal stimulation, 62% responded and were classified as wide-dynamic-range or multireceptive neurons. Recording sites were located mainly in the middle layers of the S2–S4 dorsal horn. Mechanosensitive receptive fields on the tail varied widely in size (range 0.14–35 cm², mean 10.33 cm²) and form, and were in nearly all cases bilateral. Most neurons responded with a high-frequency discharge followed by a more slowly adapting response to pressure stimuli delivered with von Frey hairs. Responses (maximal frequency and total number of impulses) increased in a graded manner to pressure stimuli ranging from 1.2 to 447 g. For neurons responsive to noxious heating of the tail, responses increased in a linear manner over the range of 38–54°C and often leveled off at higher temperatures. Of nine neurons tested with both graded von Frey and noxious heat stimuli, mean responses (maximal frequency and total number of impulses) evoked by the strongest pressure stimuli were larger than those evoked by the most intense heat stimuli, but the difference was not statistically significant. Responses to repeated 48°C stimuli were significantly attenuated within 8 min after systemic administration of morphine (1 or 2 mg/kg ip), reaching maximal suppression (to 37.3%; N = 13) after 18 min, with recovery following systemic naloxone. After morphine (1 and 2 mg/kg ip), the slope of the population stimulus-response function for noxious heat was reduced (51.8%), and the threshold was increased (by 4°C). Responses to noxious heat were significantly depressed (to a mean of 54%; N = 10) by supplemental administration of pentobarbital (mean 17 mg/kg over 5 min). On the basis of similarities between the present data and previous behavioral measures of tail flick stimulus-response functions and their modulation, it is suggested that some of the present neurons might function as interneurons in the tail flick reflex arc.

INTRODUCTION

The rodent tail flick reflex is a widely used pain assay, yet until fairly recently relatively little was known about the underlying spinal mechanisms. The tail flick is a spinal reflex that usually involves a ballistic movement elicited in response to noxious stimulation of the tail (Danne-man et al. 1994; Irwin et al. 1951; Ness and Gebhart 1986). The afferent limb of the reflex arc consists of nociceptors innervating the tail (Fleischer et al. 1983; Handwerker et al. 1987; Neckar and Hellon 1978), whose afferent fibers enter over S4–C0 spinal segments (Grossman et al. 1982). The efferent limb consists of motoneurons located in segments L4–C0 (Grossman et al. 1982) innervating the three sets of back muscles that control tail movements (Brink and Pfaff 1980; Cargill 1983; Grossman et al. 1982). The interneuronal pathway linking nociceptors to tail flick motoneurons is thought to be polysynaptic ( Jasmin et al. 1996).

In a few previous studies researchers have examined sacral spinal neurons that receive afferent input from the tail and that might function as interneurons (Cervero et al. 1988; Laird and Cervero 1989; Mitchell and Hellon 1977; Neckar and Hellon 1978). Such neurons in the sacral dorsal horn responded to mechanical stimuli (Cervero et al. 1988; Laird and Cervero 1989) or to noxious thermal stimulation by immersion of the tail in hot water (Mitchell and Hellon 1977; Neckar and Hellon 1978). However, in most behavioral studies the tail flick reflex is elicited by restricted radiant thermal stimuli. We previously investigated the responses of sacral neurons in anesthetized rats to spatially restricted noxious radiant thermal stimulation of the tail and the ability of midbrain stimulation to suppress these responses (Carstens and Douglass 1995). Neuronal responses increased in parallel with the magnitude (force vector) of tail flicks elicited by the same thermal stimuli in conscious rats (Carstens and Douglass 1995; Carstens and Wilson 1993). Neuronal responses and tail flicks were elicited at mean thresholds of ~36 and 40°C, respectively, and linearly increased up to 48–54°C, at which point the stimulus-response function usually saturated. These data are consistent with the hypothesis that such sacral dorsal horn neurons might function as interneurons in the tail flick reflex arc.

In the present study we wished to more fully investigate the functional characteristics of sacral neurons with afferent input from the tail, focusing on receptive field properties, responses to mechanical and noxious thermal stimuli, and sensitivity of neuronal responses to barbiturate anesthesia. We additionally investigated whether sacral neurons are suppressed by the opioid analgesic morphine in a manner consistent with opioid suppression of the tail flick reflex. An abstract of part of this work has previously appeared (Douglass and Carstens 1993).

METHODS

Surgery

Experiments were performed with 33 adult male Sprague-Dawley rats (350–500 g). Surgical anesthesia was induced with pentobarbital (Nembutal, 65 mg/kg ip). During recording, anesthesia was maintained by continuous infusion of undiluted pentobarbital (65 mg/ml) through a catheter in the jugular vein at a

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rate (5–15 mg·kg\(^{-1}\)·h\(^{-1}\)) sufficient to block all nociceptive limb withdrawal reflexes; the corneal and pinna reflexes were present (see below). Core body temperature was monitored and maintained near 37.5°C with a circulating heating pad and lamps. A laminectomy exposed the sacral spinal cord for single-unit recording. The animal was fixed in a stereotaxic frame and the spinal column was stabilized by vertebral clamps. An agar pool was formed over the laminectomy and filled with warm saline to keep the cord moist and minimize movement artifacts.

**Recording**

Extracellular single-unit activity was measured with a tungsten microelectrode (F. Haer, ~10 MΩ) inserted into the sacral cord with a hydraulic microdrive (D. Kopf). Microelectrode signals were amplified and displayed conventionally and fed to a computer programmed to construct peristimulus time histograms (binwidth 1 s). To avoid traumatizing the tail skin, we used innocuous mechanical stimulation of tail skin as a search stimulus. This biased us against finding high-threshold neurons, but we were readily able to find wide-dynamic-range (WDR) neurons.

**Characterization of neurons**

When a mechanically responsive neuron was isolated, its cutaneous excitatory receptive field was first carefully mapped with the use of the tip of a forceps. The field in which lightly tapping with the forceps evoked a discernible response was outlined with a marker pen. After the animal was killed at the conclusion of the experiment, the skin of the tail was slit longitudinally along the ventral midline, removed, and flattened. The skin and receptive field perimeters were traced onto transparencies and receptive field areas were measured by computerized planimetry.

After mapping the receptive field, in most cases we tested whether the neuron responded to a noxious thermal stimulus. Some neurons were further tested with graded pressure and/or graded noxious thermal stimuli. Graded pressure stimuli were applied with the use of von Frey hairs with bending forces ranging from 1.2 to 447 g. Each von Frey hair tested was fixed on a mechanical arm such that its tip could be lowered and held in place against the center of the receptive field with a micromanipulator. With this method we sought to reduce variations in stimulus pressure that might occur when the von Frey hair was applied manually. Pressure-evoked responses were quantified by measuring the maximal frequency and the total number of impulses during a 15-s stimulus period.

Noxious heat stimuli were delivered to the tail with a feedback-controlled quartz-halogen lamp. Heat pulses (rise time 500 ms) of 5 s in duration and ranging from 38 to 58°C (usually in ascending order) were applied at 2-min interstimulus intervals. A constant adapting temperature of 35°C was maintained between stimuli to rule out any possible effect of spontaneous changes in tail temperature (Hole and Tjølsen 1983). Responses to heat were quantified by measuring the maximal frequency and the total number of impulses during the 10-s period beginning with the onset of the heat stimulus. Stimulus-response functions for graded pressure and noxious heat were subjected to a one-way analysis of variance (ANOVA) with significance accepted if \( P < 0.05 \), as well as to linear regression analysis (and higher-order polynomial curve fitting) to determine correlation coefficients. Effects of cooling were also tested by placing a brass cylinder (cooled to ~0°C in a freezer) onto the tail receptive field and comparing the unit’s response with that evoked by an identical cylinder at room temperature.

**Morphine**

To assess the effects of morphine, responses to three to four standard 48°C heat stimuli were recorded, after which an ascending series of graded noxious heat pulses (38–58°C) was given to establish the unit’s control stimulus-response function. Morphine sulfate was then administered intraperitoneally in doses of 0.5 mg/kg (\( N = 5 \)), 1 mg/kg (\( N = 7 \)), or 2 mg/kg (\( N = 6 \)). Only one dose was tested per animal. Responses to the standard 48°C heat stimulus were recorded every 2 min, after which (at 20 min postmorphine) the stimulus-response function was run again. Finally, naloxone (1–2 mg/kg ip) was administered and responses to the 48°C heat stimulus were recorded for another 18 min, followed by a final stimulus-response function (at 20 min postnaloxone).

Data were pooled by morphine dose and subjected to repeated-measures ANOVA to determine whether mean postmorphine stimulus-response functions differed significantly (\( P < 0.05 \)) from control or postnaloxone conditions.

**Pentobarbital**

To study the effect of the anesthetic on neuronal responses to noxious heating, the rate of pentobarbital infusion was stepped up higher for a brief period. Responses to repeated application of a standard heat stimulus (48°C, 5 s in duration) at 2-min interstimulus intervals were recorded before and during the increased rate of infusion. The increase in the rate of infusion varied (range 52–627 mg·kg\(^{-1}\)·h\(^{-1}\)) depending on the initial rate (range 5–15 mg·kg\(^{-1}\)·h\(^{-1}\)), and the duration of increased infusion also varied (range 2–12 min) because the infusion was stopped as soon as responses were observed to be depressed. Mean responses before and during the increase in infusion rate were compared by paired \( t \)-test with \( P < 0.05 \) accepted as significant.

**Histology**

At the conclusion of the experiment, an electrolytic lesion was made at the final recording site and the rat was killed by overdose of pentobarbital. The spinal cord was fixed in 10% buffered Formalin, after which 40-µm frozen sections were cut and slide mounted. Lesion sites were identified under the light microscope and drawn with a camera lucida.

**RESULTS**

**Unit sample**

A total of 53 neurons responsive to mechanical stimulation of the tail was recorded in 32 rats. The majority exhibited either no (62%) or a very low level (<1 Hz, 20%) of spontaneous activity, whereas 18% had spontaneous firing rates of 3–20 Hz. Most units were also tested with a noxious radiant thermal stimulus (48°C) delivered to the tail; the majority (28 of 45, 62%) that responded were classified as WDR neurons. The mechanosensitive neurons that were unresponsive to noxious heat were classified as nonnociceptive, although other types of noxious stimuli (e.g., chemicals) were not tested. Two additional neurons responded to changes in tail angle and two responded only to movement of tail bristles. Figure 1 shows histologically recovered recording sites for 33 neurons, which were mainly in the midsinal horn.

A typical example of the common WDR neuron is shown in Fig. 2. This neuron had a mechanical receptive field along the dorsal surface of the tail bilaterally, and was located in the midsinal horn (Fig. 2A). It gave increasing responses to graded noxious heat stimuli (Fig. 2A) and also to pressure stimuli applied with graded von Frey hairs (Fig. 2B). None of the neurons tested responded to tail cooling or to innocu-
There was no significant difference in receptive field areas for WDR (12.3 ± 8.2 cm², mean ± SD, N = 28) compared with nonnociceptive neurons (10.5 ± 9.5 cm², mean ± SD, N = 15). Most neurons (82%) exhibited little or no ongoing activity, so we were not able to determine whether there were inhibitory receptive field areas.

Responses to graded pressure stimuli

Thirteen neurons were tested with a graded series of von Frey hairs delivered to the center of the receptive field. Figure 4 shows examples from three neurons. Responses of all neurons tested increased as a function of pressure. Of the 13 neurons, 11 gave a high-frequency response at the onset of the stimulus that adapted to a lower tonic discharge rate that was maintained for the duration of the pressure stimulus (e.g., Figs. 2B and 4A and B). Responses of the other two neurons rapidly adapted to the baseline firing rate during the maintained stimulus (Fig. 4C).

The mean maximal response frequency for the 13 neurons increased significantly as a function of stimulus pressure from an average threshold of 15 g (ANOVA, F = 4, P = 0.0001). In 9 of these 13 neurons we also obtained data with graded noxious heat. Mean responses to pressure and heat for these nine neurons are shown in Fig. 5. Figure 5A plots mean maximal frequency, which increased significantly (ANOVA, F = 2.6, P = 0.01) with stimulus pressure up to ~300 g and then appeared to plateau. Seven of these nine units displayed no spontaneous firing, whereas two fired spontaneously at 3–4 Hz. Linear regression analysis of the stimulus–response function yielded a correlation coefficient \( r^2 = 0.313 \) (\( r^2 = 0.373 \) when the data were fit by a 2° polynomial function). Figure 5C plots mean responses summed over the 15-s stimulus duration for the same neurons, and yields a stimulus–response function similar to that for maximal frequency.

Responses to graded noxious heat stimuli and comparison with pressure

Figure 5B plots the mean maximal frequency versus temperature of graded noxious heat stimuli for the same nine...
neurons that were also tested with pressure (see above). Mean response frequency increased significantly with temperature (ANOVA, $F = 2.5, P < 0.05$), and the stimulus-response function was fit best by linear regression ($r^2 = 0.2$). Mean responses summed over 10 s are plotted versus stimulus temperature for the same neurons in Fig. 5D. Again, mean responses increased significantly with temperature (ANOVA, $F = 2.9, P = 0.02$) and the stimulus-response function was best fit by linear regression ($r^2 = 0.23$). Although the mean extrapolated threshold appears to be $<38^\circ C$, the thresholds of individual neurons were usually $38-40^\circ C$ and no neuron responded to a $35^\circ C$ stimulus.

When pressure- and noxious heat-evoked responses are compared in the same neurons (Fig. 5, A and B), it is apparent that pressure stimuli evoked higher-frequency responses on average. The difference in mean responses evoked by the most intense pressure (447 g) and heat (58$^\circ C$) stimuli was, however, not statistically significant. A direct comparison of mean integrated responses to pressure (Fig. 5C) and heat (Fig. 5D) cannot be made because pressure-evoked responses were summed over the entire 15-s period of stimulation, whereas heat-evoked responses were summed over a 10-s period and heat stimuli were limited to 5 s. Nevertheless, it appears that pressure stimuli are able to evoke responses of a magnitude comparable with those evoked by noxious heat.

To test for possible habituation, we compared responses to the first and second heat stimulus delivered to previously unstimulated skin on the tail for the first neuron recorded in a given experiment. For 21 neurons, mean responses to the first and second heat stimuli were $436 \pm 60$ and $396 \pm 49$ (SD) impulses per 10 s, respectively. The difference was not statistically significant.

**Morphine**

Unit responses to repeated 48$^\circ C$ heat stimuli were depressed after systemic morphine (1 and 2 mg/kg) in a naloxone-reversible manner. Figure 6A shows an example of one neuron’s responses, which decreased to a minimum within 10 min after morphine and increased after systemic naloxone. Neuronal responses were depressed by $\approx 50\%$ in five of seven units at the 1-mg/kg dose and in four of six units at the 2-mg/kg dose. The mean time course and magnitude of depression was similar for the two doses (1 mg/kg: to 38.1% of baseline; 2 mg/kg: to 32% at 18 min postmorphine). For this reason, data for the two doses were pooled, and mean responses are graphed in Fig. 6B versus time relative to morphine and naloxone injection. The mean response was significantly attenuated 8 min after morphine, reaching a minimum (mean 37.3% of premorphine level) at 16 min. After naloxone, the mean responses increased to become significantly larger than the responses at 18 min postmorphine (Fig. 6B). After 0.5 mg/kg morphine, responses of three of five units were briefly attenuated, with recovery by 16 min postmorphine. Only the mean decrease at 8 min (to 66%, $N = 5$, $P = 0.023$) reached statistical significance.

Morphine at the 1- and 2-mg/kg doses reduced the slope, and increased thresholds, of stimulus-response functions for graded noxious heat in many, but not all, neurons. Data for the neuronal population are summarized in Fig. 7. After 0.5 mg/kg morphine, there was no change in the population stimulus-response function (Fig. 7A). After 1 mg/kg morphine, there was a depression of the stimulus-response function, which, however, failed to reach statistical significance. Responses of five of the seven neurons were markedly suppressed (Fig. 7B, ▲), whereas responses of two were not suppressed at all (Fig. 7B, △). Of the five neurons suppressed by morphine, responses of three were abolished at all temperatures, whereas responses of the other two were subtotally reduced. After the 2-mg/kg dose, the slope of the mean stimulus-response function was significantly reduced (Fig. 7C; $P = 0.0085$). The response of one neuron was unaffected, the response of one was totally abolished, and responses of four were subtotally reduced. Naloxone partially reversed the effects of morphine at the 1- and 2-mg/kg doses (Fig. 7, B and C, ○). Data from the 1- and 2-mg/kg groups are pooled in Fig. 7D. The reduction in the slope of the stimulus-response function...
function (51.8%) and the increase (4°C) in the mean threshold were both significant ($P < 0.001$). Responses after naloxone (Fig. 7D, ○) were significantly different from responses after morphine ($P = 0.0071$) but not from controls ($P = 0.86$). Recording sites for these experiments were in the intermediate laminae of the $S_2$–$S_4$ dorsal horn (see Fig. 1), with no obvious correlation between recording site and effect of morphine.

![Diagram](image)

**FIG. 4.** Examples of neuronal responses to graded von Frey pressure stimuli. A: PSTHs (binwidth: 1 s) of a neuron’s responses to von Frey stimuli at the indicated pressures. *Drawings at right:* receptive field (black) on tail. B and C: responses of 2 additional neurons (same format as in A). Time and frequency scales in C also apply to A and B.

![Diagram](image)

**FIG. 5.** Responses to graded pressure and noxious heat stimuli. A: mean maximal firing rates for 9 neurons vs. stimulus pressure. Error bars here and in subsequent figures: mean ± SE. B: responses (mean maximal firing rate) of same neurons in A vs. temperature of noxious thermal stimuli. C: as in A, plotting mean responses (total number of impulses per 15-s stimulus period) vs. pressure. D: as in B, plotting mean responses (total impulses per 10 s following heat onset) vs. temperature.
DISCUSSION

Receptive field properties

The present sacral dorsal horn neurons were mainly (62%) WDR type, with the remainder apparently nonnociceptive; our sampling procedure was biased against finding nociceptive-specific types. With the use of electrical stimulation of coccygeal nerves, Cervero et al. (Cervero et al. 1988; Laird and Cervero 1989) identified neurons of both the class 2 type (responsive to innocuous and noxious mechanical stimuli) and the class 3 type (responsive only to noxious mechanical stimuli) in the sacral cord; a small population of class 1 neurons (responsive only to innocuous mechanical stimuli) was also identified. Most class 2 neurons were in the middle layers of the dorsal horn, comparable with our present unit sample, whereas most class 3 neurons were in the superficial laminae (Cervero et al. 1988; Laird and Cervero 1989). Laird and Cervero (1989) reported variability in the size of the mostly bilateral receptive fields, similar to those seen in the present study, and the mean receptive field area of their class 2 neurons (8.9 cm²) (Laird and Cervero 1989) was comparable with that observed here for WDR neurons (10.3 cm²). Class 3 neurons had smaller receptive field areas (Cervero et al. 1988; Laird and Cervero 1989). The latter authors did not report on the thermal responsiveness of sacral neurons. In the present study, all thermally responsive WDR neurons necessarily responded to mechanical stimuli that were used to search for neurons; this method did not allow us to determine whether some neurons respond to thermal but not mechanical stimuli.

The bilateral organization and often large size of the receptive fields is interesting in regard to the tail flick reflex. Tail flicks usually involve tail dorsiflexion and movement in one preferred horizontal direction (Cargill 1983; Carstens and Wilson 1993). Electromyographic recordings revealed that the three sets of muscles responsible for tail movements are bilaterally active during the tail flick reflex (Cargill 1983). If some of the present WDR neurons served as reflex interneurons, then noxious stimulation at any point on the tail should excite interneurons, and thus tail flick motoneurons, bilaterally by virtue of the bilateral distribution of most neurons' receptive fields.

Responses to pressure

Most (11 of 13 or 85%) of the present neurons tested with graded von Frey hair stimuli responded with a biphasic response consisting of an initial discharge that quickly adapted to a lower level of maintained firing for the 15-s stimulus duration; the other 2 neurons exhibited more rapidly adapting responses. This biphasic response pattern is similar to that reported for responses of most class 2 and 3 sacral spinal neurons to noxious (4–8 N) constant-pressure stimulation of the tail (Cervero et al. 1988; Laird and Cervero 1989). The tonic response component of those latter neurons did not adapt during a long (2 min) stimulus period, and in many cases it was proportional to stimulus intensity (Cervero et al. 1988), suggesting that these neurons are capable of encoding mechanical intensity in the noxious range, as well as in the innocuous range as demonstrated here. The noxious pressure-evoked neuronal responses were likely to

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**FIG. 6.** Time course of suppression of responses to noxious tail heating by systemic morphine. A: neuron’s responses to 48°C stimuli vs. time relative to systemic administration of morphine and naloxone (↓). B: as in A, plotting mean responses of the neuronal population (N = 13) vs. time relative to morphine and naloxone administration (↓). Error bars: mean ± SE. Asterisks: responses significantly different compared with premorphine level (P < 0.05, paired t-test).

**Pentobarbital**

Neuronal responses to successive heat stimuli (48°C) were also depressed after supplemental administration of pentobarbital sodium. Figure 8A shows an example of a neuron’s responses to heat, which were reduced 10 min after a brief increase in the rate of pentobarbital infusion, with partial recovery after 20 min. Figure 8B plots the same neuron’s responses versus time. Figure 8C similarly plots mean responses for 10 neurons, and shows a significant decline (paired t-test, P < 0.05) to a mean of 54% of control when the rate of pentobarbital infusion was increased (5.6 ± 3.6 min, mean ± SD) to provide a mean dose of 17 mg/kg (range 8.6–34.4 mg/kg). Responses usually recovered within 10–40 min.
have been mediated by input from C fiber polymodal and A-delta mechanical nociceptor afferents (Handwerker et al. 1987), although curiously the afferent fibers exhibited considerably greater adaptation in their tonic discharge compared with the sacral neurons. In the present study we used lower-intensity pressure stimuli (1.2–447 g = 0.01–4.5 N). Responses to the most intense stimuli used might have been via activation of nociceptors, whereas responses to graded stimuli in the nonnoxious range were presumably mediated by slowly adapting (SA-I, SA-II) mechanoreceptors that also innervate tail skin (Fleischer et al. 1983; Handwerker et al. 1987).

Responses to noxious heat

A majority of neurons (62% of those tested) additionally responded to noxious heating of the tail. Both the maximal discharge frequency and total number of impulses over 10 s correlated positively with stimulus temperature. From a threshold near 38–39°C, response magnitude increased nearly linearly up to ~54°C and then began to plateau (Fig. 5D). This confirms our earlier data recorded from a separate population of noxious heat-sensitive sacral neurons (Carsens and Douglass 1995). Only one other group has examined responses of sacral neurons to noxious thermal stimulation of the tail (Mitchell and Hellon 1977; Neckar and Hellon 1978). Those authors found that responses increased from 42 to 46°C and then declined, to yield a bell-shaped stimulus-response function. However, there are significant differences in stimulus type and area between those studies and ours. In the other studies, the entire tail was immersed in hot water, whereas in the present study we used a restricted radiant heat stimulus similar to that commonly used in behavioral tail flick reflex tests. Additionally, those researchers recorded from class 3 (nociceptive specific) neurons, whereas we studied WDR (equivalent to class 2) neurons.

Our present results are consistent with those from our previous behavioral studies of the magnitude (force vector) of tail flick reflexes evoked by identical noxious radiant heat stimuli in conscious rats (Carstens and Douglass 1995; Carstens and Wilson 1993). From a threshold of 38–40°C, tail flick magnitude increased up to 48–54°C and often plateaued at higher temperatures. Mean responses of the presently recorded sacral neurons, as well as a separate population reported previously (Carstens and Douglass 1995), increased over a similar range of 38–54°C, plateauing at the higher stimulus levels (Fig. 5D). In most behavioral studies the tail flick reflex is a rapid, ballistic movement elicited by an uncontrolled, rapidly rising and ramping radiant thermal stimulus. The latter stimulus corresponds most closely with the largest (58°C) stimulus used presently, which evoked maximal neuronal responses and tail flick forces (Carstens and Wilson 1993).

The similarities in behavioral and neuronal stimulus-response functions, and their modulation (Carstens and Douglass 1995), provide support for the argument that such sacral neurons might function as interneurons in the tail flick reflex arc. However, this argument is clouded by our observation...
FIG. 8. Depression of noxious heat-evoked responses by pentobarbital. A: individual example showing PSTHs (binwidth: 1 s) of a neuron’s response to 48°C heat stimuli before (left), 10 min after (middle) and 20 min after (right) the infusion rate of pentobarbital was increased (to deliver 7.4 mg over 5 min). Drawings at right: receptive field and recording site, respectively. B: responses of neuron in A vs. time relative to pentobarbital administration (bars at top). C: as in B, plotting mean responses of 10 neurons to 48°C heat stimuli vs. time relative to pentobarbital administration. Error bars: mean ± SE. Asterisks: significantly different from mean response before pentobarbital infusion (P < 0.05, paired t-test).

that nonnoxious mechanical stimuli, which would not normally elicit a tail flick, are capable of evoking responses similar in magnitude to those evoked by noxious heat. Conceivably, further serial interneuronal processing may be needed to channel nociceptive, but not nonnociceptive, signals on to motoneurons for the initiation of the tail flick reflex.

Successive unit responses to repeated noxious heat stimuli (48°C) tended to decrease, but this was not statistically significant for the population. In a previous behavioral study, we observed a statistically significant reduction in the magnitude of successive tail flicks evoked by repetition of a weaker (44°C), but not a stronger (50°C), noxious heat stimulus (Carstens and Wilson 1993). In a related study we found that the successive responses of lumbar dorsal horn neurons to repeated noxious heat stimuli also declined only slightly (88%), whereas the magnitude of simultaneously recorded hindlimb flexor motor neurons, and limb withdrawal force, decreased significantly (Carstens and Campbell 1992). If noxious heat-sensitive sacral neurons function as interneurons early in the tail flick reflex arc, then the argument can be presented that their trend to decrement with repeated stimuli might contribute partly to, but cannot completely explain, habituation of the reflex. A more rigorous assessment of sacral neuron responses to repetitive stimuli is needed to determine whether they decrease in a manner consistent with the habituation seen in the tail flick reflex.

Comparison of responses to pressure versus noxious heat

The maximal responses evoked by mechanical pressure tended to be larger than those evoked by noxious heat (Fig. 5, A and B), although the difference was not statistically significant. None of the pressure stimuli were considered to be painful when applied to the volar surface of human forearms, and it was interesting that those at the upper end (126–447 g) evoked discharge frequencies comparable with those evoked by noxious heat. It was reported some time ago that nonnoxious repetitive mechanical stimuli evoked significantly larger responses than those evoked by noxious heat stimuli in rat lumbar dorsal horn “convergent” neurons (LeBars and Chitour 1983). These authors invoked the concept of “diffuse noxious inhibitory control” in an attempt to resolve the paradox that functionally different (i.e., tactile vs. painful) stimuli evoke responses of similar magnitude in a population of neurons that is widely considered to be important in signaling pain. According to this idea, normal behavior activates peripheral receptors that in turn produce ongoing discharge in convergent neurons. A noxious stimulus activates diffuse noxious inhibitory control, which serves to globally suppress all spinal neurons except those activated directly by the noxious stimulus. By reducing background firing, diffuse noxious inhibitory control enhances the contrast of the “pain” signal. Although our data do not afford any further insights into neural mechanisms that differentiate
nonnoxious from noxious inputs, they demonstrate that nonnoxious tactile and noxious thermal stimuli can evoke responses of comparable magnitude in sacral spinal neurons in a manner similar to that previously shown for lumbar dorsal horn neurons.

**Morphine**

Our results show that systemically administered morphine can suppress the responses of sacral spinal neurons to noxious heating of the tail, similar to the largely depressant action of opiates on nociceptive lumbar dorsal horn neurons reported in many previous studies (e.g., Dickinson and Sullivan 1986; Duggan et al. 1977; Einspahr and Piercy 1980; Gebhart et al. 1984; Kitahata et al. 1974; LeBars et al. 1975; Magnuson and Dickenson 1991; Ness and Gebhart 1989; Suberg et al. 1985; Toyooka et al. 1978). Although we did not presently assess effects of morphine on neuronal responses to nonnoxious stimuli, several of these previous studies reported a differential effect of opioids to suppress responses to noxious but not innocuous stimuli. Of particular relevance are reports that morphine (usually 1 mg/kg iv) usually resulted in a decrease in slope and increase in threshold by 3–4°C of neuronal stimulus-response functions for graded noxious heating (Duggan et al. 1977; Einspahr and Piercy 1980; Gebhart et al. 1984; Ness and Gebhart 1989; Toyooka et al. 1978), as presently observed. Morphine exerted a similar effect on stimulus-response functions of the RII reflex and concomitant pain ratings in humans (Willer 1985).

In our experiments, responses of more than half of the units were suppressed in a graded manner by morphine, whereas the remainder appeared to be affected in an all-or-none (quantal) manner, i.e., some units' responses were totally abolished at all stimulus temperatures, whereas the other units' responses were unaffected. This differential sensitivity to morphine might be due to differences in intraperitoneal drug absorption, although this argument is weakened by observations that direct spinal superfusion with morphine also had differential effects on nociceptive neurons (Suberg et al. 1985). The present results are consistent with previous behavioral studies showing both graded and quantal effects of morphine in the rat tail flick reflex assay (Carstens and Wilson 1993; Levine et al. 1980; Yoburn et al. 1985).

Opioids are thought to act via μ-receptors at both supraspinal and spinal levels to suppress nociceptive transmission (Besson and Chaouch 1987; Fields et al. 1991). We presently made no attempt to ascertain spinal versus supraspinal sites of action of systemic morphine. However, because morphine largely suppressed sacral neuronal responses, we suggest that a direct spinal inhibitory effect of systemic morphine outweighs any possible supraspinal action that might lead to descending facilitation of dorsal horn neuronal responsiveness (Carstens et al. 1988; Dickenson and LeBars 1987a,b).

**Pentobarbital**

The present data are consistent with previous reports that pentobarbital depressed spinal nociceptive neuronal responses (Paik et al. 1989; Sandkühler et al. 1987; Wall 1967). Lumbar dorsal horn neurons appear to be more resistant to suppression by comparable doses of pentobarbital (up to 30 mg/kg iv), which, however, were sufficient to depress concomittant flexor reflexes (Carstens and Campbell 1992; Paik et al. 1989). Pentobarbital at cumulative doses of 5–24.5 mg/kg iv significantly suppressed responses of cat lumbar dorsal horn neurons to noxious heat in a dose-dependent manner (Sandkühler et al. 1987). Our results suggest that in the rat, sacral neurons are more sensitive to pentobarbital than lumbar dorsal horn neurons, and indicate the importance of maintaining an appropriate level of anesthesia when using barbiturates to observe nociceptive responses in sacral tail neurons.

Comparable doses of pentobarbital have also been reported to facilitate the rat tail flick reflex (Sandkühler and Gebhart 1984), to enhance C fiber inputs to spinothalamic tract neurons (Hori et al. 1984), and to “unmask” nociceptive inputs onto low-threshold neurons recorded in cats (Collins et al. 1990). In our experiments, heat stimuli were administered every 2 min and it is conceivable that we may have missed an early facilitatory effect of pentobarbital at the beginning of the infusion period, which was then presumably overridden by the depressant effect that was observed.

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