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Expansion of formalin-evoked Fos-immunoreactivity in rats with a spinal cord injury

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Abstract

Peripheral tissue injury as well as spinal cord injury (SCI) may lead to sensitization of dorsal horn neurons and alterations in nociceptive processing. Thus, peripheral injuries experienced by SCI patients, even if not initially perceived, could result in a persistent and widespread activation of dorsal horn neurons and emerge as chronic pain with interventional repair or modest recovery from SCI. To visualize the spinal neuron response to peripheral tissue injury following complete SCI in rats, the neural transcription factor Fos was quantitated in the spinal cord. Two weeks following either a complete transection of the spinal cord at the level of T8 or a sham surgery (laminectomy), rats were injected with formalin into the left hind paw. Sham-operated rats demonstrated biphasic hind paw pain-related behavior following formalin injection, but transected rats displayed fewer behaviors in the second (tonic) phase. Stereological analysis of the sham group revealed that the extent of formalin-induced Fos expression was within the lumbar dorsal horn, with numerous Fos-like immunoreactive profiles in the ipsilateral dorsal horn and some contralateral immunoreactive profiles. In contrast, the level of Fos-like immunoreactivity in the transected group was significantly elevated and expanded in range compared to the sham group, with increases observed in the normal laminar distribution regions, as well as multisegmentally through sacral levels and increases in the contralateral dorsal horn segments. The data demonstrate that widespread activation of spinal, especially dorsal horn, neurons following peripheral insult can occur in the injured spinal cord, despite reduced pain responsiveness, and suggests that exaggerated pain may emerge as spinal recovery or repair progresses.

Keywords

central pain; spinal cord injury; dorsal horn; immediate early genes

1. Introduction

Peripheral tissue injury leads to increased sensitivity of spinal dorsal horn neurons to cutaneous stimulation (Dubner and Ruda, 1992; Coderre et al., 1993). Intense peripheral nerve activity accompanying injury leads to increased spontaneous firing and metabolic activity of spinal nociceptive neurons. In addition, sprouting of myelinated peripheral nerve central terminals into laminae containing nociceptive neurons has been reported following injury (Woolf et al.,

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1992). The behavioral consequences of injury-evoked plasticity may underlie un-evoked pain and cutaneous hypersensitivity, both of which persist long after the initial injury.

Injury to the CNS also leads to persistent pain (Eide, 1998). Spinal cord injury (SCI) results in a dramatic loss of descending inhibitory pathways originating from the brainstem which may lead to increased activity of dorsal horn nociceptive neurons. Abnormal spontaneous activity and exaggerated responses of nociceptive neurons to peripheral stimuli have been observed both below and above the level of the lesion (Wang et al., 2005). There are potential therapies that are currently being developed that aim to reestablish motor and sensory function in SCI patients (Kapfhammer, 1997). However, many of these encourage sprouting, which may, in addition, lead to increased spontaneous pain and hypersensitivity (Hofstetter et al., 2005). Thus, as normal axonal connections are made, SCI patients may experience emerging pain and further be discouraged from continuing with the therapy. Also, accidental or surgical injuries below the level of the spinal lesion may lead to further maladaptive changes in the spinal cord, even in the absence of acute pain perception, which can further complicate treatment of SCI. It is crucial that these potential neurological complications be understood and addressed prior to the commencement of restorative clinical interventions for SCI.

The goal of the current study was to evaluate dorsal horn neuronal responsiveness following a peripheral insult in rats with complete spinal transection. The presence of the peptide product of the nuclear transcription gene *c-fos* was used as a marker for neural activity. Hind paw injection of formalin was used as the noxious insult, as it leads to robust expression of Fos in the ipsilateral dorsal horn in spinally intact rats, normally limited to those lumbar spinal segments that receive hind paw (sciatic nerve) afferents (Presley et al., 1990; Hirakawa and Kawata, 1993).

2. Methods

All procedures were reviewed and approved by the University of Miami Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Animals*. Female Sprague-Dawley rats (200–225 g upon arrival; Charles River, MA) were housed two per cage and had free access to food and water. Upon arrival, rats were allowed 5–7 days to acclimate to the facility, which was on a 12:12 light-dark cycle.

2.1. Spinal cord transection

Rats were anesthetized with a subcutaneous injection of ketamine-xylazine-acepromazine mixture (31.25, 6.25, and 1.25 mg/kg, respectively). The back was shaved and the skin swabbed with iodine solution. A laminectomy was performed to expose thoracic spinal cord. The spinal cord was completely transected with fine micro-dissection scissors and a 2 mm gap was created at T8 in one group of rats ($n = 16$). The gap was visually confirmed and a piece of Gelfoam was placed within the gap to control hemorrhaging. Control (sham-operated) rats received a laminectomy only ($n = 14$). The wounds were sutured closed with 4-0 vicryl and the skin sealed with surgical staples. The bladders were expressed daily until spontaneous voiding returned.

2.2. Formalin Test

Two weeks following sham or transection surgery, 50 μ l of 5% formalin in saline was subcutaneously injected into the plantar left hind paw. One minute observations for formalin-evoked hind paw licking and flinching were made immediately after injection and at five-minute intervals thereafter. Rats were tested for 65 minutes. Pain-related behaviors are observed in two phases following formalin injection, the acute phase (phase 1, 0–5 min) and the tonic phase (phase 2, 15–65 min).

2.3. Fos Immunocytochemistry

Eight rats from each group were randomly selected for perfusion following formalin testing. Two hours after formalin injection, rats were deeply anesthetized with pentobarbital (60 mg/kg, intraperitoneal) and transcardially perfused with saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH = 7.4). Although not the main focus of the study, 2 additional control groups were inspected for comparison: intact rats and spinally transected rats receiving unilateral intraplantar saline injections (n = 3/group). All spinal cords were removed and post-fixed for 24 hours at 4° C in 4% paraformaldehyde. Spinal cord lumbar and sacral segments were embedded in paraffin, and sequential serial sections were collected following stereology rules of unbiased sampling (Howard and Reed, 1998). Spinal cords were sectioned at 30 µm on a rotary microtome (Leica RM 2135), and collected at intervals of 240 µm. Sections were treated in xylenes and re-hydrated in descending concentrations of alcohols. Tissues were incubated with rabbit anti-Fos primary antibody (1:5000; Oncogene Science, MA) overnight at 4° C, followed by a biotinylated goat anti-rabbit IgG (1:200; Vector Labs, CA) for two hours at room temperature. Immunostaining was revealed by an avidin-biotin-peroxidase complex reaction with diaminobenzidine as the chromagen. Sections from the control and transected groups were simultaneously immunostained.

2.4. Stereological cell counting

All analyses were done with the aid of the Axiophot (Zeiss) research microscope, furnished with fully motorized 3D LEP stage, Optronix cooled video camera and Stereo Investigator software (Micro Brightfield, Inc., VT). For lumbar segments (L4-L5) receiving sciatic nerve innervation, laminae I-II and III-IV and V ipsilateral and contralateral to formalin injection were outlined for quantification as described in previous studies in our laboratory and others (Presley et al., 1990; Abbadie et al., 1994; Siegan et al., 2002) as follows: the ventral border of the superficial dorsal horn (laminae I and II) and reticular part of lamina V are readily distinguishable morphologically. The ventral border of the nucleus proprius was delineated by a line drawn from the dorsal and lateral reticular border of lamina V tangentially across to the medial border of the dorsal horn. The ventral border of the neck of the dorsal horn was delineated by a parallel line drawn from the ventral border of the dorsal columns laterally to the border of the dorsal horn. To perform cell counts in each lamina, volume, optical fractionator and optical dissector were selected from the probes menu. Sampling for the cell number estimation was based on the assumption that the cell number (N) in the outlined structure was equal to the number of nuclear profiles (n) multiplied by the section thickness (T) divided by section thickness plus the mean profile diameter (D) (Coggeshall and Lekan, 1996). Dimensions of the probes were designed based on the preliminary population estimate from one of the animals from each group. Thirteen sections were analyzed from each animal, with the precision of 5–7%. Regions were optically fractionated and Fos-like immunoreactive (IR) nuclei were counted with a grid size of 100 µm² (10 µm × 10 µm). Investigators were blinded to all treatment groups.

3.4. Statistics

Data are presented as mean ± S.E.M. Statistical analysis of Fos-IR and behavior between rats with either a sham or transection surgery was done using a one-way ANOVA, with a Dunnett's test for post-hoc comparison (SigmaStat, v. 3.11). The level of significance was taken at $p < 0.05$.

3. Results

3.1. Pain-related behaviors following formalin injection

Following hind paw formalin injection, sham-operated rats displayed typical pain-related behaviors in the acute (phase 1, 0–5 min) and tonic (phase 2, 15–65 min) phases. Spinally-transected rats displayed the same total number of behaviors in the acute phase, but displayed less total pain-related behaviors in the tonic phase (Fig. 1; $p < 0.05$ vs. sham).

3.2. Fos-like immunoreactive profiles

Two hours after hind paw injection of formalin, numerous Fos-IR nuclei were observed in the dorsal horn of spinal cord, particularly on the side ipsilateral to formalin injection (Fig. 2, 3). The total number of Fos-IR profiles was significantly increased in T8-transected rats over sham-operated rats ($p < 0.05$). In the transected rats, at lumbar levels receiving hind paw innervation (L4-L5), there were a total of $33,286 \pm 1,896$ Fos-IR profiles in the ipsilateral and $4,592 \pm 623$ Fos-IR profiles in the contralateral spinal cord. In contrast, there was a total of $19,437 \pm 1,998$ Fos-IR profiles in the ipsilateral and a total of $2,080 \pm 484$ Fos-IR profiles in the contralateral spinal cord from rats with a sham surgery.

In general, the increase in Fos-IR profiles in T8-transected rats was observed in all laminae of the dorsal horn in lumbar segments (Fig. 4). The number of Fos-IR profiles in ipsilateral laminae III-IV and V were significantly higher than in sham-operated rats (Fig. 4; $p < 0.05$). In contralateral laminae I-II and V, significantly more Fos-IR was observed compared with sham-operated rats ($p < 0.05$). Although not quantified, there appears to be an increase in Fos-IR profiles in ventral horn lamina (VII-VIII) in transected rats, whereas there are few or no Fos-IR profiles in intact rats, at every level of spinal cord observed (Fig. 2). Although background cytoplasmic staining was present within motor neurons (lamina IX), specific nuclear Fos-IR was not observed within these neurons in either transected or sham-operated rats.

In a segment of the spinal cord that does not receive sciatic nerve innervation, the sacral (S1-S2) spinal cord, total Fos-IR profiles was significantly higher in T8-transected rats compared with sham-operated rats (Fig. 5; $p < 0.05$). In transected rats, the total number of Fos-IR profiles ipsilateral to formalin was $5,332 \pm 520$ and $2,071 \pm 786$ contralateral to formalin. In contrast, the total number of Fos-IR profiles ipsilateral to formalin was $2,166 \pm 722$ and 10 ± 10 contralateral to formalin in sham-operated animals.

Although not a primary focus of the study, lumbar (L4-L5) and sacral spinal cords from rats receiving an intraplantar saline injection instead of formalin were also inspected ($n = 3$). In sham-operated rats, intraplantar saline resulted in a total of 886 ± 289 Fos-IR profiles at lumbar levels L4-L5, ipsilateral to the saline injection. Fos-IR labeling elsewhere (contralateral and at sacral spinal levels) was below detectability. In transected rats, Fos-IR profiles were apparent following intraplantar saline injections, although not nearly to the extent following formalin injection ($p > 0.05$). In transected rats, a total of $2,446 \pm 1,458$ Fos-IR profiles were observed in spinal cord ipsilateral to hind paw saline injection and $1,311 \pm 851$ Fos-IR were found in the contralateral side. Ipsilateral (but not contralateral) sacral Fos-IR was also detected in these rats (857 ± 571).

4. Discussion

The current study confirms previous findings of robust Fos-IR expression in the spinal cord dorsal horn ipsilateral to hind paw formalin injection and also demonstrates significant Fos-IR in the contralateral spinal cord. In rats with a mid-thoracic spinal transection, the number of Fos-IR profiles is greatly increased both contralaterally as well as ipsilaterally in the spinal

cord following formalin injection, compared to sham-operated rats. The overall increased neuronal activity suggested by Fos expression, however, did not directly correspond to increased pain-related behavior, since spinally transected rats displayed fewer formalin-evoked behaviors than sham-operated rats. The results suggest that a vigorous activation of spinal neurons, indicated by Fos expression, accompanies peripheral injury in animals with a transected spinal cord, much more so than in animals with an intact spinal cord. The observation that hind paw saline injection increased Fos expression in spinally transected suggests tonic neural hypersensitivity in these rats. Nevertheless, this neural hypersensitivity was much lower compared with the marked response to hind paw formalin injection. Thus, the data suggests that spinal dorsal horn neurons below the level of the lesion in SCI patients are extremely sensitive to peripheral stimulation, particularly to an injurious stimulus.

The Fos peptide, a product of the *c-fos* immediate early proto-onco gene, has been shown to be rapidly upregulated and persist for several hours following acute peripheral injury in the spinal cord and brain areas (Hunt et al., 1987; Dubner and Ruda, 1992). Fos has been shown to be increased in dorsal horn neurons that express inhibitory amino acids and induces the expression of several pain modulating peptides (Todd et al., 1994; Hughes and Draganow, 1995). Thus, Fos expression has been used as a CNS marker signaling the initiation of persistent injury-induced hypersensitivity. Although the presence of Fos in the spinal dorsal horn suggests activation of the molecular processes related to nociception, Fos expression alone does not indicate whether there is a net increase or decrease in nociception (Yashpal et al., 1998). In fact, even with significantly decreased Fos expression with lumbar intrathecal injection of an antisense oligodeoxynucleotide to *c-fos*, hind paw responses to formalin have been reported to be either increased or decreased compared to vehicle-treated rats (Hunter et al., 1995; Hou et al., 1997; Ibrahim et al., 2001). The actual role of Fos in the initiation of injury-induced hypersensitivity remains to be elucidated.

It should be noted that the increased Fos expression in spinally transected rats is due to formalin injection and not due to an additional increase in Fos which might already be present in transected rats. A modest Fos expression has been observed about two hours following spinal cord injury (acute “spinal shock”) (Ruggiero et al., 1997). However, a significant increase over basal Fos expression was not observed two weeks following spinal transection (without formalin injection; data not shown) and no significant increase of Fos-IR was observed in rats 30 days after a spinal transection (Yeziarski, 2000; Landrum et al., 2002).

The increased Fos expression in transected rats compared with sham-operated rats, particularly in the contralateral spinal cord, may be due to decreased spinal inhibition and/or increased spinal excitation. Terminals from serotonergic and catecholamine containing brainstem neurons are found bilaterally in the spinal dorsal horn and synapse with primary afferent central terminals and nociceptive neurons, attenuating pain perception (Sagen et al., 1983). If the descending terminals are removed, as would be expected following complete spinal transection, presynaptic inhibition would be lost and peripheral stimulation results in greatly enhanced response of dorsal horn neurons (Zhang et al., 1994b). Thus, the increased injury-evoked Fos expression in transected rats is due, at least in part, to a loss of presynaptic inhibition by bulbospinal descending monoaminergic systems. That Fos-IR profiles were found bilaterally (as well as in ventral horn lamina) in rats with a spinal transection is consistent with the bilateral distribution of bulbospinal terminals.

A second potential source of increased Fos-IR is increased basal activity of dorsal horn neurons following spinal transection. Spinal cord injury leads to increased tissue levels of excitatory amino acids and inflammatory mediators which alter dorsal horn neuron responses to cutaneous stimulation, as observed by prolonged activity following a brief stimulus and sensitivity to stimuli outside of the normal receptive field (Yeziarski, 2000; McAdoo et al., 2005). These

physiological changes are found in neurons distal to the injury site. Numerous Fos-IR profiles were found in spinal segments outside of those normally activated by unilateral intraplantar formalin injection, indicative of an expansion of excitability both multi-segmentally and contralaterally. These findings suggest that not only a loss of inhibition but an increase in excitotoxicity lead to widespread excitability of dorsal horn neurons below the level of the SCI.

It is of interest that increased Fos-IR in spinally transected animals was not paralleled by increased behavioral pain responses to formalin injection. Other laboratories have reported that preventing Fos expression in the dorsal horn leads to increased formalin-evoked behaviors (Hunter et al., 1995; Ibrahim et al., 2001). These observations are indirectly supported by the current data. As mentioned earlier, the mere presence of spinal Fos is not entirely predictive of the final nociceptive outcome. The opposite is also true, in that the absence of nociception does not necessarily indicate the total suppression of Fos expression (Presley et al., 1990). Alternatively, it is possible that reduced behavioral indicators of pain are related to motor interference brought about by the spinal cord injury itself. In the current study both hind paw licking and flinching were recorded. A spinal transection may have decreased the number of hind paw licking, a supraspinally mediated behavior, thereby decreasing the total number of pain-related behaviors. However, such a selective decrease in a particular behavior was not observed in the present study. Spinal transection and transections through various brain regions did not diminish acute and tonic phase licking and flinching responses to hind paw formalin injection (Matthies and Franklin, 1992;Coderre et al., 1994). Despite the absence of increased overt pain symptoms, the increased levels and segmental expansion of Fos-IR following spinal transection may be indicative of exaggerated activity in spinal nociceptive processing.

Intuitively, one would have expected more pain-related behaviors because an enhancement of noxious stimulus-evoked motor function (spasticity) is observed following SCI (Marsala et al., 2005). Although Zhang et al. lesioned the dorsolateral funiculus and demonstrated increased dorsal horn Fos-IR following hind paw formalin injection, they did not present any behavioral data (Zhang et al., 1994b). Thus, our study is one of a few to show an inverse relationship between Fos expression and a nociception-related behavioral measure (Hunter et al., 1995; Ibrahim et al., 2001).

It is possible that the decreased formalin-evoked responses in transected rats may be due to increased activity of inhibitory neurons. In addition to excitatory interneurons and supraspinally projecting nociceptive neurons, one other population of spinal dorsal horn neurons that expresses Fos-IR following noxious peripheral stimulation is inhibitory GABAergic neurons (Todd et al., 1994). On one hand, SCI leads to decreased spinal dorsal horn GABA (Zhang et al., 1994a). On the other hand, peripheral inflammation leads to increased GABA expression in spinal dorsal horn (Castro-Lopes et al., 1992). It is unknown if hind paw formalin injection leads to an acute increase in inhibitory amino acids or other antinociceptive neuroactive substances in SCI rats, but such an increase could explain the decreased phase 2 formalin-evoked behaviors observed in the spinally transected rats. There was an increase in Fos-IR in ventral horn laminae, such as laminae VII, that contain interneurons, as well as supraspinally projecting nociceptive neurons, which may have taken the place of the dorsal horn inhibitory neurons lost following SCI (Barber et al., 1982; Harrison et al., 1984; Presley et al., 1990; Gogas et al., 1991; Willis and Coggeshall, 1991). Without further characterization of the ventral horn neurons, however, the relevance of these Fos-IR neurons to the behavioral outcome in the current study is unclear.

Whether there is either a complete loss or a significant attenuation of GABAergic tone following SCI, this suggests that supplementing it might be an effective analgesic treatment. In fact, drugs that increase inhibitory tone (e.g. intrathecal baclofen) effectively ameliorate SCI

pain (Finnerup and Jensen, 2004). Further clarification of the changes in dorsal horn neurochemistry following SCI may lead to the development of better analgesic treatments.

It is curious that a rat with a complete SCI is still able to respond to hind paw formalin injection. Following formalin injection, rats with a thoracic spinal transection exhibited biphasic pain-related behavior, albeit significantly less than that of sham-operated rats (Fig. 1). “Complex” or “integrated” behaviors such as licking are observed even in decerebrate rats (Grill and Norgren, 1978; Matthies and Franklin, 1992). A biphasic pattern was also observed in the rate of discharge of lumbar dorsal horn neurons following formalin injection in rats with an acute transection of the thoracic spinal cord (Pitcher and Henry, 2002). Taken together, these data point out that the spinally transected rat can exhibit robust pain-related behavior following an acute below-lesion tissue injury. Despite the surgical removal of significant segments of the spinal cord, patients have described spontaneous and evoked pain as originating below the level of the SCI (Melzack and Loeser, 1978). The mechanism that mediates these pains below a SCI could be a result of injury-induced neuro-plasticity.

The presence of spontaneous pain below the transected spinal cord could be due to the emergence of “pattern generators” in brain regions that modulate nociception (Melzack and Loeser, 1978). For example, a persistent functional reorganization of the somatosensory cortex has been observed in SCI rats with below-level pain (Morrow et al., 2000). Such “generators” may also be found in the spinal cord caudal to the lesion (Wang et al., 2005). Cortical activation has been recorded following stimulation of a region below the level of a “complete injury”, which suggests the presence of some surviving ascending projection axons or the existence of an alternate “sensory” pathway that bypasses the injury (Ioannides et al., 2002). Presently, the evidence supporting the existence of such a “neural bridge” following complete transection is rather weak based on behavioral and histological data (Basso et al., 1996). The current data do indicate the presence of an expanded pool of excitable (“generator”) neurons, supporting the concept of increased spinal plasticity following SCI.

As mentioned previously, it is possible that the increase in Fos-IR may be due to increased activity of spinal inhibitory neurons, thus diminishing the behavioral response to an acute peripheral injury, but having no effect on the ongoing spontaneous pain. A patient reported a lack of sensation from the upper thorax down following the removal of spinal cord segment T8-T9 (Melzack and Loeser, 1978). However, below-level spontaneous pain was present. Several years after the surgery, the patient fractured his right femur. Despite significant preexisting spontaneous pain, no pain due to the fracture was reported. Other reports demonstrated that SCI patients had higher heat pain thresholds in below-lesion areas with spontaneous pain than in areas without pain and above-lesion pain-free areas (Defrin et al., 2001). Although the degree of spontaneous pain in the SCI rat is not known, the behavioral response to formalin in SCI rats in the current study tends to parallel these clinical findings. The SCI rat model may be used to model the effect of various treatments on spinal dorsal horn neurons, with the possibility of unmasking untoward sensory-motor effects, prior to use in humans.

In summary, the increased Fos expression in rats with a spinal transection was accompanied by fewer formalin-evoked pain-related behaviors compared to intact formalin-injected rats. The results point out that despite an apparently diminished level of overt pain behaviors, segments of the spinal cord dorsal horn are significantly activated in spinally transected animals following peripheral injury. At this point the clinical relevance of increased Fos expression in SCI animals is not entirely clear. However, the data suggest that persistent alterations in cutaneous sensitivity may occur following SCI and may be further exacerbated during treatment aimed at restoring function.

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A.

B.

Figure 1.

Pain-related behaviors following formalin injection in the rat. Five percent formalin was injected into the left hind paw and the numbers of hind paw flinches and licks in one min. were counted up to 65 min post-formalin injection. **A.** Time course of behaviors following formalin injection every five minutes. Formalin was injected at time "0". **B.** Total number of pain-related behaviors in phase 1 (0–5 min) and phase 2 (15–65 min) are shown. Data are expressed as mean $\pm$ S.E.M. $n = 16$ transected, $n = 14$ sham-operated rats. * $p < 0.05$ vs. sham-operated rats.

Figure 2.

Formalin-evoked Fos-like immunoreactivity (IR) from rats with either a sham or transection surgery. Rats with either a spinal transection or sham surgery were perfused 2 hours following formalin injection into the left hind paw. Representative transverse spinal cord sections are shown. The left hand side of each photo is ipsilateral to the formalin injected hind paw. The spinal sections in the left column (**A, C, E, G**) were taken from the sham-operated rats and the sections in the right column (**B, D, F, H**) were taken from transected rats. Sections were taken from the level of lower thoracic (**A, B**), upper lumbar (**C, D**), lower lumbar (L4-L5) (**E, F**) and sacral (S1-S2) (**G, H**). In both groups, numerous Fos-IR profiles were observed in the ipsilateral spinal cord. In addition, in spinally transected rats, Fos-IR profiles were observed in the

contralateral spinal cord and appeared beyond the lower lumbar segment into thoracic and sacral segments. Scale bar = 1 mm

Figure 3.

Lumbar dorsal horn Fos-like immunoreactivity (IR) from rats with either a sham (**A**) or transection (**B**) surgery. Photomicrograph composites A and B are enlargements of the dorsal horn area of Figures 2E and 2F, respectively. The left hand side of each photo is ipsilateral to the formalin injected hind paw. **A.** In sham-operated rats, there is increased dorsal horn Fos-IR ipsilateral to formalin injection, compared to the contralateral dorsal horn. **B.** In spinally transected rats, dorsal horn Fos-IR ipsilateral to formalin injection is marked increased compared to the ipsilateral dorsal horn of sham-operated rats. In addition, the contralateral dorsal horn displays Fos-IR, which is markedly increased compared to the contralateral dorsal horn of sham-operated rats. Scale bar = 0.5 mm

Figure 4.

Total number of Fos-like immunoreactive (IR) profiles in the lumbar spinal cord from rats with either a spinal transection or sham surgery. Fos-IR profiles were counted in laminae I-II, III-IV and V of spinal segments L4-L5, which receive sciatic nerve central terminals. Data are the total mean ($\pm$ S.E.M.) immunoreactive profiles of each area. $N = 8$ rats/treatment group. * $p < 0.05$ vs. sham.

Figure 5.

Total number of Fos-like immunoreactive (IR) profiles in the sacral spinal cord from rats with either a spinal transection or sham surgery. Fos-IR profiles in S1-S2 spinal cord sections were counted throughout the dorsal horn. Data are the total mean ($\pm$ S.E.M.) IR profiles in S1-S2 spinal cord. N = 8 rats/treatment group. * $p < 0.05$ vs. sham.