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# Intrathecally administered c-*fos* antisense oligodeoxynucleotide decreases formalin-induced nociceptive behavior in adult rats

Wen-Yeong Hou [a], Bai-Chuang Shyu [d], Tzer-Ming Chen [b], Jai-Wei Lee [a], Jeng-Yung Shieh [c], Wei-Zen Sun [a,*]

[a] *Department of Anesthesiology, Medical College of the National Taiwan University, No. 7, Chung-Shan South Road, Taipei, Taiwan, ROC*
[b] *Department of Obstetrics and Gynecology, Medical College of the National Taiwan University, Taipei, Taiwan, ROC*
[c] *Department of Anatomy, Medical College of the National Taiwan University, Taipei, Taiwan, ROC*
[d] *Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC*

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**Abstract**

c-*fos* antisense strategy was applied as a pharmacological approach to characterize its dose-dependent role and reversibility in the reduction of formalin-induced hyperalgesia. Nociceptive behavioral responses (weighted score, flinching response, licking/biting) following formalin (50 μl 5%) injection were assessed in adult Wistar rats receiving different doses (50 nM, 250 nM) of intrathecally administered c-*fos* antisense oligodeoxynucleotides at different times prior to formalin injections. The treatments dose dependently decreased both Fos immunoreactivity expression in dorsal horn of rat lumbar spinal cord and all nociceptive measures in the tonic phase of the formalin test. c-Fos correlated well with weighted pain score and/or flinching responses, but not with licking/biting behavior. With the exception of a 48–120 h period required for licking/biting behavior to be restored to its normal status, the suppressive effect on c-*fos* expression and other nociceptive behaviors disappeared 48 h following c-*fos* antisense oligodeoxynucleotide treatment. The results suggest a pharmacological potential of c-*fos* antisense oligodeoxynucleotides in the central nervous system to block immediate-early genes and their resulting physiological consequence following noxious stimulus. © 1997 Elsevier Science B.V.

*Keywords:* c-*fos*; Formalin test; Hyperalgesia; (Intrathecal); Nociception; Antisense oligodeoxynucleotide

## 1. Introduction

The immediate-early gene, c-*fos*, is transiently induced in the central nervous system by a variety of stimuli. Fos-like protein is produced within the cortex and hippocampus following seizure activity (White and Gall, 1987; Munglani et al., 1989), within the hypothalamus following water deprivation (Sagar et al., 1988) and within the dorsal horn following peripheral stimulation (Hunt et al., 1987). c-Fos protein forms leucin-zipper heterodimers with members of the jun family as a transcriptional factor in vitro, which regulates the expression of the secondary response gene, suggesting a major role in neuronal activity (Naranjo et al., 1991). c-*fos* expression following peripheral inflammation has been shown to correlate well with behavioral hyperalgesia (Dubner and Ruda, 1992). Pretreatment with drugs like opioids (Gogas et al., 1991), NMDA receptor antagonists (Kehl et al., 1991) and nitric oxide synthase inhibitors (Chapman et al., 1995) can inhibit both c-*fos* expression and the noxious stimulus-evoked hyperalgesia. Furthermore, the time course of c-*fos* expression and behavioral hyperalgesia have also been shown to coincide (Draisci and Iadarola, 1989). c-*fos* expression has thus been widely accepted as a marker of neuronal activation, leading to its long-term change after peripheral stimulation (Bullitt, 1990). However, since a causal relationship between peripheral stimulation, immediate-early gene activation, and behavioral hyperalgesia is still lacking, direct evidence based on selective inhibition of c-*fos* expression is necessary to demonstrate the immediate-early gene activation leading to its physiological consequences.

* Corresponding author. Tel.: (886-2) 397-0800, ext. 5521; Fax: (886-2) 394-9681; e-mail: wzsun@ccms.ntu.edu.tw


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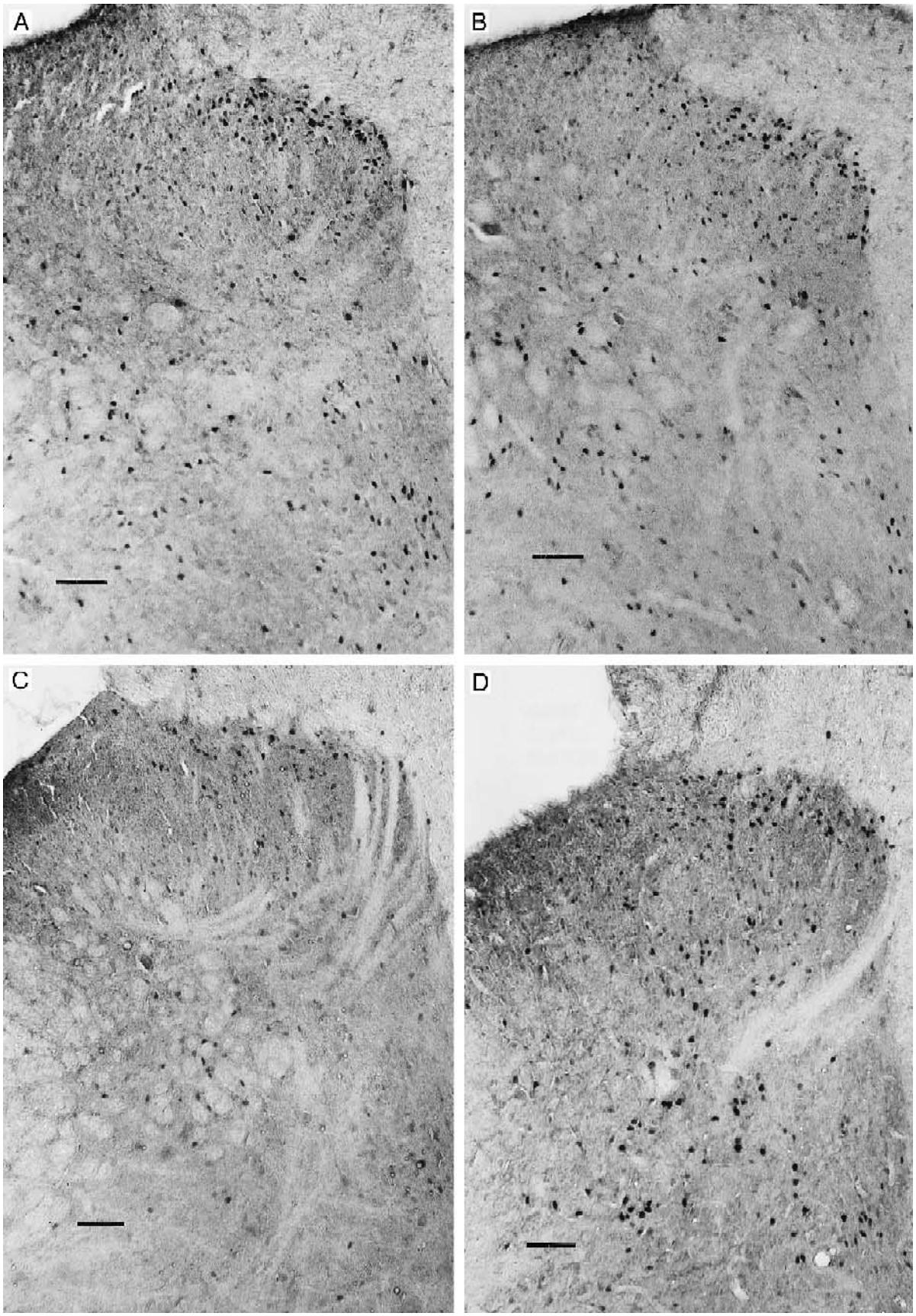
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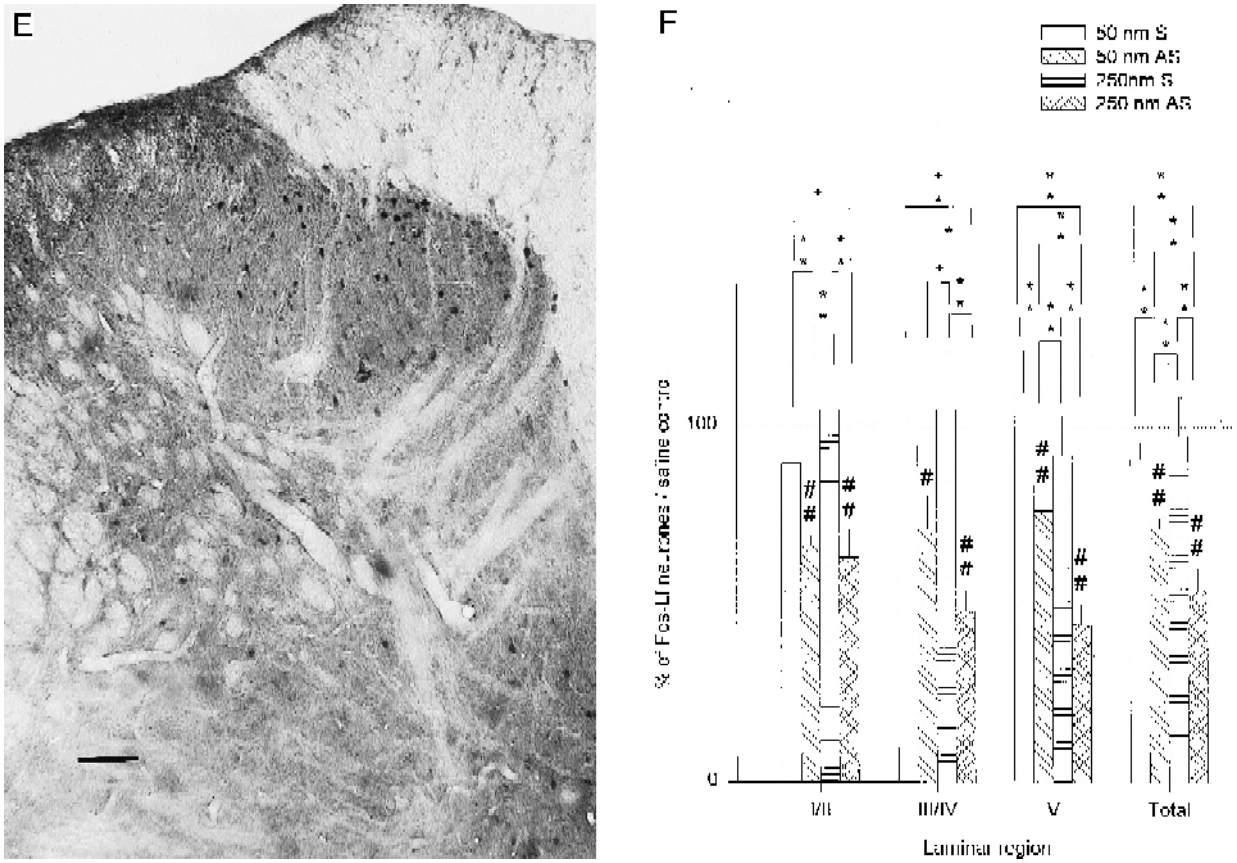


Fig. 1. Photomicrographs showing effects of pretreatment on the expression of Fos-labeled neurons following formalin injection with: (A) saline; (B) 50 nmol c-*fos* sense oligodeoxynucleotide; (C) 250 nmol c-*fos* sense oligodeoxynucleotides; (D) 50 nmol c-*fos* antisense oligodeoxynucleotides; (E) 250 nmol c-*fos* antisense oligodeoxynucleotides. Scale bar = 100 μm. (H) Histogram showing effects of c-*fos* antisense oligodeoxynucleotides on the expression of Fos-labeled neurons in each laminar region or in total laminae (mean ± S.E.M., percentage of saline control). Ratio of c-Fos-labeled neurons in experimental groups to saline control plotted at each lamina: I/II = superficial laminae; III/IV = nucleus proprius; V = neck of the dorsal horn. Significance is expressed by $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ taking saline control as reference, or $^{*}P < 0.05$, $^{**}P < 0.001$ comparing the experimental groups using one-way ANOVA and Student-Newman-Keuls post-hoc test. Note that (1) Fos-like immunoreactivity predominated in superficial laminae and neck of the dorsal horn; (2) c-*fos* antisense oligodeoxynucleotides dose-dependently decreased Fos protein expression following formalin injection.

The present study assessed the role of c-Fos protein in the development of central sensitization and tonic nociception following formalin injection. Injection of formalin into the hindpaw of rats can evoke a biphasic behavioral response (Dubuisson and Dennis, 1977) and induce laminar-specific distribution of c-Fos protein (Presley et al., 1990). It is suggested that neural activity generated during the early phase of the formalin response is capable of producing changes in central nervous system (CNS) function, which in turn influence processing during the late phase (Coderre and Melzack, 1992). Recently, antisense technology has provided a useful tool to inactivate genes both in vitro and vivo. Although inhibition of c-*fos* expression with antisense oligodeoxynucleotides has been successfully applied in previous studies (Kindy and Verma, 1988; Chiasson et al., 1992; Gillardon et al., 1994; Hooper et al., 1994; Hunter et al., 1995), the behavioral correlates assessing the amount and patterns of c-*fos* expression in adult rats have not been completely characterized. In the present study, varied doses of c-*fos* antisense pretreatments were administered intrathecally to adult rats to investigate whether the inhibition of c-*fos* expression decreases the formalin-induced behavioral hyperalgesia in a dose-dependent and time-reversible manner.

## 2. Materials and methods

The following studies were carried out under a protocol approved by the Animal Research Facility of the National Taiwan University Medical College.

Male Wistar rats weighing 250–350 g were used. All animals were anesthetized with pentobarbital (50 mg/kg i.p.) and a catheter (PE-10 tube) was introduced approximately 2.5–3 cm cranially via a laminectomy at the L5 vertebra with the tip of the catheter located near the lumbar enlargement of the spinal cord (Skilling et al., 1992). The catheter was then brought out of the neck of the animal and secured in situ. The rats were allowed to recover for a period of 5–7 days. The function and loca-

tion of the catheter were verified by intrathecal injection of 10 μl 2% xylocaine and post mortem examination. Only animals with functioning catheters and judged as neurologically normal were used for the experiments.

Oligodeoxynucleotides were synthesized by Quality System Incorporation (Taipei, Taiwan). The rats were randomly assigned to different groups: (1) c-*fos* antisense oligodeoxynucleotides (5′-GAA CAT CAT GGT CGT-3′, targeted at the codons immediately downstream of the initiation codon): moderate-dose group (50 nmol/10 μl saline, $n = 5$) and high-dose group (250 nmol/10 μl saline, $n = 5$). All treatments were administered 4 h prior to formalin injection. (2) Recovery periods (intervals): 48-h recovery group (250 nM c-*fos* antisense/10 μl saline 48 h before formalin test, $n = 5$) and 120-h recovery group (250 nM c-*fos* antisense/10 μl saline, 120 h prior to injection, $n = 4$). (3) c-*fos* sense oligonucleotides (5′-ACG ACC ATG ATG TTC-3′): 50-nM sense control (50 nmol oligodeoxynucleotides/10 μl saline, $n = 4$) and 250-nM sense group (250 nmol oligodeoxynucleotides/10 μl saline, $n = 5$) were used as control for each group of c-*fos* antisense oligodeoxynucleotides, while the saline group (0.9% saline 10 μl 4 h before scoring, $n = 5$) was used as control for recovery comparisons. All treatments were followed by 10 μl of saline injected via the chronic indwelling catheter.

Each formalin test began with a subcutaneous injection of 50 μl of 5% formalin into the plantar surface of the left hind paw using a 26-gauge needle. Immediately thereafter, each animal was placed in a $30 \times 30 \times 30$ cm Plexiglas box with a mirror below the floor at a 45° angle to allow an unobstructed view of the paws. The nociceptive responses were recorded by observing three different parameters: weighted scale, flinching response, and licking/biting, using a computer program designed by one of us (Shyu). The weighted pain score was assessed as described previously by computing the weighted average of the time spent in each of the following categories (Dubuisson and Dennis, 1977): 0, the injected paw is not favored; 1, the injected paw has little or no weight on it; 2, the injected paw is elevated and is not in contact with any surface; 3, the injected paw is licked, bitten or shaken. The time spent in each category was multiplied by the category weight, and calculated every minute and averaged as 5-min block during the 1-h test period, using the computer program. With the same program, the number of flinchings and time spent licking or biting per minute at 5-min intervals were recorded and averaged simultaneously. The observer responsible for the assessment was blind to the treatment of animals.

The animals were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) immediately after the formalin test and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The spinal cord was removed at the lumbar enlargement, postfixed, cryoprotected overnight in 30% sucrose in phosphate buffer. Frozen sections of 40 μm were cut and collected in phosphate buffer. The free-floating sections were treated with rabbit polyclonal anti-Fos antiserum (Santa Cruz Biotechnology, Cat. No. sc-52, epitope corresponding to amino acids 3–16, non-cross-reactive with Fos B, Fra-1 or Fra-2), diluted 1:750 for 48 h at 4°C, followed by biotinylated goat anti-rabbit IgG and a standard polymerization protocol with ABC reagent (Vectastain), and visualized with diaminobenzidine and nickel ammonium sulfate. All sections were mounted on gelatin-subbed slides, air-dried and coverslipped for light microscope inspection. c-Fos-labeled neurons were examined at the L4 spinal segments and across the lamina. The dorsal horn of each section was further divided into 3 regions under dark-field illumination (Molander et al., 1984): (1) the superficial lamina (lamina I/II), (2) the nucleus propius (laminae III/IV), (3) the neck (laminae V). Fos-labeled neurons which showed intense staining distinct from background were counted from the printed images with respect to each lamina. At least 10 sections were scanned for each segment, and three sections with the greatest number were selected. These three sections were averaged so that each animal had a mean value for regional or total c-Fos-labeled neurons.

All data are presented as means ± S.E.M. Statistical analysis was performed to compare the different groups of animals, using one-way analysis of variance (ANOVA) for the numbers of c-Fos-labeled neurons and two-way ANOVA for the different doses of animals and the laminar regions with the Student-Neuman-Keuls post-hoc test. Behavior scores transformed into areas under the curve (AUC), together with derived data at each 5-min time point, were analysed with one-way Kruskal-Wallis tests with Dunnett's test for post-hoc comparisons.

## 3. Results

### 3.1. Counting of c-Fos-labeled neurons

Numerous c-Fos-labeled neurons were observed in the dorsal horns ipsilateral to the injected paw in the saline control rats. Most of these neurons were observed in the medial region of the superficial lamina and the neck area, with fewer c-Fos-labeled neurons in the nucleus proprius as shown previously (Sun et al., 1996).

c-*fos* antisense pretreatment produced a significant inhibitory effect on the total number of Fos protein expression following formalin injection ($P < 0.005$, Fig. 1A–E; for significance of difference between groups, see Fig. 1F). Considering the total number of c-Fos-labeled neurons, the relative decrease in the number of c-Fos-labeled neurons was dose-dependent (71 and 54% of saline control, at 50 nmol and 250 nmol c-*fos* antisense oligodeoxynucleotides respectively). The effects were similar when c-Fos expression in the different laminae of the dorsal horn was

considered. Two-way ANOVA showed a significant main effect for different doses ($P < 0.0001$; for significance of difference between different groups, see Fig. 1), but not a significant main effect for laminar regions nor for dose × laminar region.

For recovery comparisons, no treatments for the 48-h

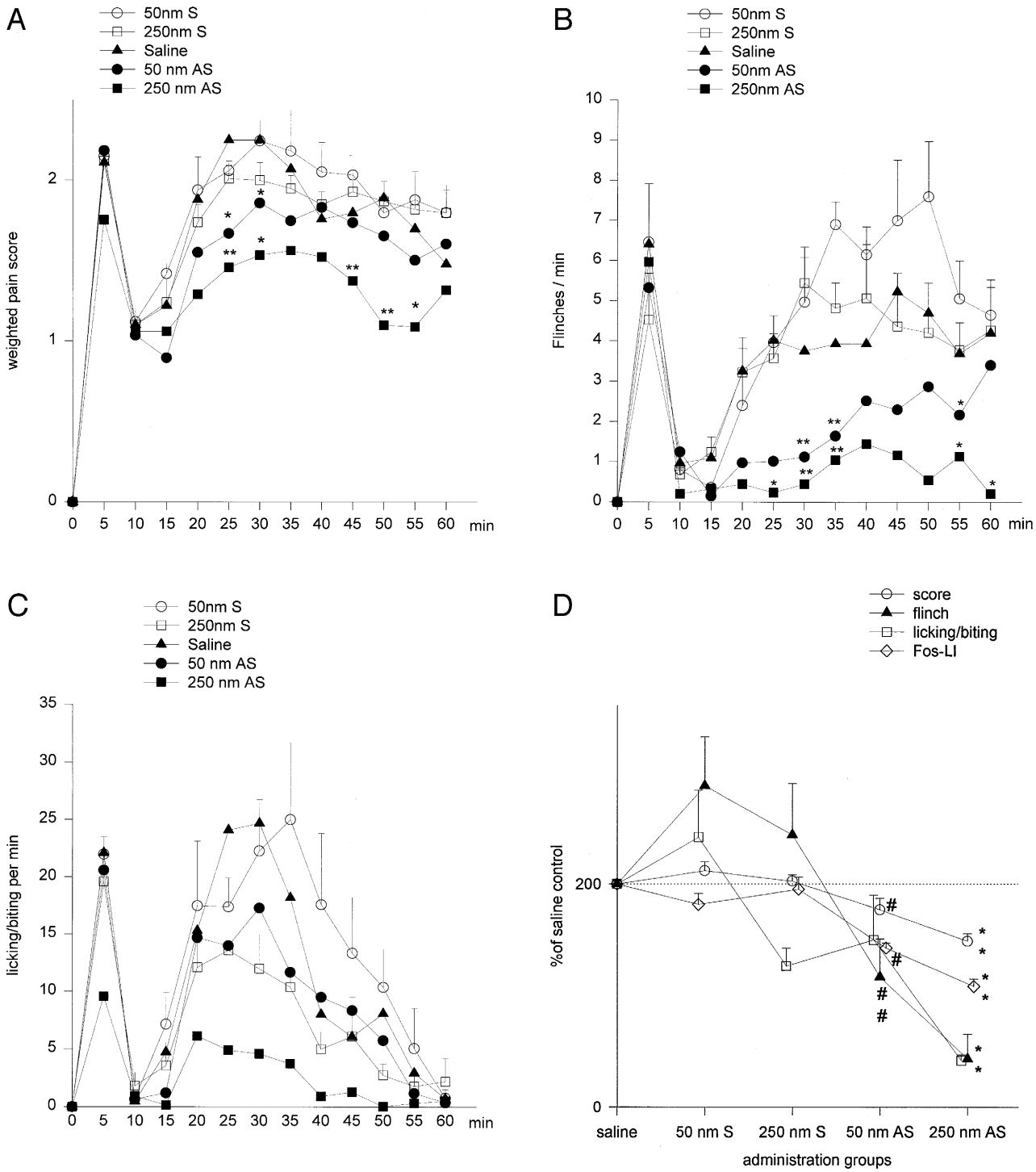


Fig. 2. Time-course of the effect of c-*fos* antisense oligodeoxynucleotide pretreatment on formalin (5%)-induced nociceptive behavior. (A) Weighted pain score; (B) flinching response; (C) licking/biting behavior (time spent licking/biting) versus minutes plotted as a function of time after injection of formalin; and histogram showing (D) comparisons for effect of c-*fos* antisense oligodeoxynucleotide pretreatment on formalin (5%)-induced nociceptive response (area under curve, AUC 5–60 min, mean ± S.E.M., percentage of saline control). Significance is expressed vs. sense control at the same concentration ($^{*}P < 0.05$, $^{**}P < 0.01$) as reference, using one-way ANOVA and Dunnett's post-hoc test. Note that (1) nociceptive responses in the acute phase among all experimental groups were indistinguishable; (2) c-*fos* antisense oligodeoxynucleotides dose dependently decreased all nociceptive scores (AUC 5–60 min) in the tonic phase; (3) the differences for licking/biting behavior were not significant vs. sense control as reference.

recovery or the 120-h recovery groups altered the numbers of c-Fos-labeled neurons.

### 3.2. Behavioral responses

Subcutaneous injections of 5% formalin produced two phases of nociceptive behavioral patterns which were similar to those described by Dubuisson and Dennis (1977). The first phase (acute phase) began immediately following formalin injection and lasted for 3–5 min, followed by a period of 10–15 min when the animals displayed very little nociceptive behavior. The second phase (tonic phase) started approximately 15–20 min following formalin injection and lasted for 20–40 min (Fig. 2A–C).

The nociceptive response in the acute phase was indistinguishable ($P > 0.05$) among all groups for nociceptive scoring with regard to weighted scale, flinching response, or licking/biting.

The AUC at the 5- to 60-min intervals were compared to assess the overall behavioral response to formalin in the tonic phase. The c-*fos* antisense oligodeoxynucleotides significantly decreased all nociceptive parameters for the treatment groups. Considering the nociceptive response for weighted pain score in the tonic phase, the relative decrease in AUC at 5–60 min among treatment groups was dose-dependent (88, 74% of saline control at 50, 250 nmol c-*fos* antisense oligodeoxynucleotides respectively; see Fig. 2D for significance of differences between groups). The dose-dependent suppression was similar when considering the flinching response (58, 21% of saline control at 50, 250 nmol c-*fos* antisense oligodeoxynucleotides respectively) or licking/biting behavior (75, 21% of saline control at 50, 250 nmol c-*fos* antisense oligodeoxynucleotides respectively) in the tonic phase.

At 48 h, but not 120 h, c-*fos* antisense oligodeoxynucleotide pretreatment significantly decreased the licking/biting response in the tonic phase ($P < 0.05$, see Fig. 4). The other nociceptive measures (weighted pain score and flinching response) did not differ from the saline control with either 48-h or 120-h c-*fos* antisense oligodeoxynucleotide pretreatment.

Table 1
Correlation between behavior assessment and dosage (negative correlation implies assessment decrease with dose) and c-Fos-labeled neurons (positive correlation implies assessment increase with Fos-like immunoreactivity (Fos-LI))

| Measure | Pain (AUC 5–60 min) | |
|---|---|---|
| | With dose | With Fos-LI |
| Score | −0.78 [c] | 0.72 [c] |
| Flinch | −0.66 [c] | 0.74 [c] |
| Licking/biting | −0.62 [a] | 0.36 |

Significance is expressed as [a] $P < 0.05$, [b] $P < 0.001$, [c] $P < 0.0001$. Note that (1) c-*fos* antisense oligodeoxynucleotides dose dependently decreased all nociceptive measures; (2) c-Fos-labeled neurons correlated well with weighted pain score and/or flinching response, but not with licking/biting behavior.

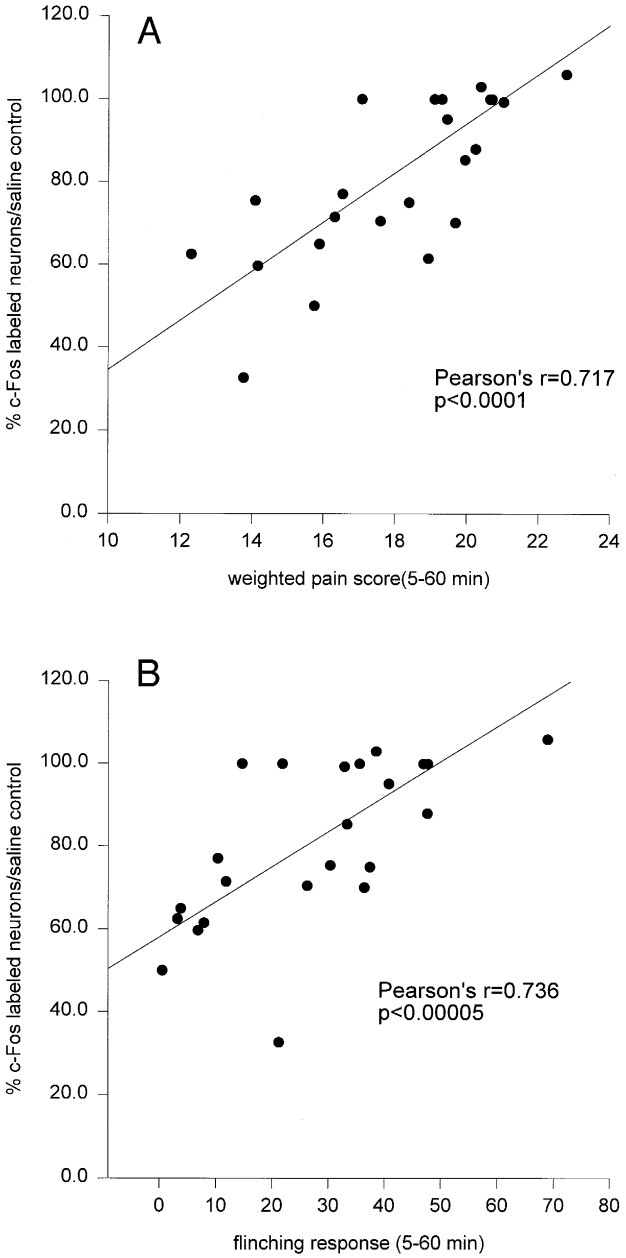


Fig. 3. Correlation between formalin-induced nociceptive behavior and spinal cord c-Fos expression. (A) Weighted pain scores, or (B) flinching responses are plotted against ratio of Fos-labelled neurons to saline control for each of the 5 intrathecal treatments used (saline, 50 nmol, 250 nmol c-*fos* sense oligodeoxynucleotides, 50 nmol, 250 nmol c-*fos* antisense oligodeoxynucleotides). Note that weighted pain score (area under curve, AUC 5–60 min, Pearson's $r = 0.717$, $P < 0.0001$) or flinching response (AUC 5–60 min, Pearson's $r = 0.736$, $P < 0.00005$) correlates significantly with the ratio of Fos-labeled neurons in experimental groups to saline control.

### 3.3. Correlation between dose and behavior response and correlation between Fos-like immunoreactivity and behavior responses

There were significant correlations between doses of c-*fos* antisense oligodeoxynucleotides and all nociceptive

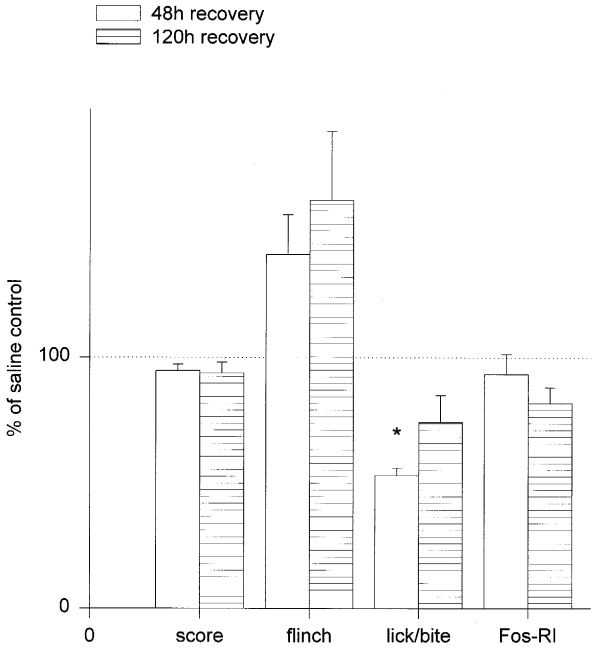


Fig. 4. Effects of 250 nmol intrathecal c-*fos* antisense oligodeoxynucleotides (48-h or 120-h pretreatment) on formalin-induced nociceptive behavior (area under curve, AUC 5–60 min) or Fos-like immunoreactivity expression. Ratio of AUC 5–60 min and/or Fos-like immunoreactivity for experimental groups to the saline control plotted against scoring method and/or c-Fos-labeled neurons. Significance is expressed as $^{*}P < 0.05$ taking saline control as reference using one-way ANOVA with Dunnett's post-hoc test. Note that (1) Fos-like immunoreactivity expression resumed its normal status 48 h and/or 120 h following c-*fos* antisense oligodeoxynucleotide pretreatment; (2) both weighted pain score and flinching response were identical to the saline control 48 h and/or 120 h following c-*fos* antisense oligodeoxynucleotide pretreatment; (3) it took 48–120 h for licking/biting behavior to return to its normal condition.

measures (Table 1). To determine if suppression of c-Fos could reflect the decrease in nociceptive behavior, the correlation between c-Fos and nociceptive behavior was examined. There was a significant positive correlation (Pearson's $r = 0.717$, $P < 0.0001$) between weighted pain score and spinal cord c-Fos evoked by formalin (Fig. 3A). This relationship was also significant (Pearson's $r = 0.736$, $P < 0.00005$) when flinching response and c-Fos were analyzed (Fig. 3B). Licking/biting responses and c-Fos, however, failed to show a significant correlation.

## 4. Discussion

These data show that the expression of the c-*fos* gene can be selectively suppressed by intrathecal administration of an antisense oligodeoxynucleotide and that the suppression is accompanied by decreased behavioral hyperalgesia in a dose-dependent and time-reversible manner.

The time of administration, composition and sequence of antisense c-*fos* oligodeoxynucleotides in the present study were based on results of previous investigations in which c-*fos* expression was successfully inhibited (Kindy and Verma, 1988; Chiasson et al., 1992; Gillardon et al., 1994; Hooper et al., 1994; Hunter et al., 1995). Although most investigators have reported successful inhibition with phosphororothioate oligodeoxynucleotides, the non-modified oligodeoxynucleotide was used in the present study. Phosphorothioate oligodeoxynucleotides, although more resistant to nuclease degradation, are also more slowly accumulated within cells and appear to possess significantly more non-specific toxicity (Necker and Whitesell, 1993). Phosphorothioate oligodeoxynucleotides may exert some of their toxicity through inactivation of various proteins essential for basic cellular function. They bind a variety of heparin-binding factors including platelet-derived growth factor and many members of the fibroblast growth factor family with a strong affinity and inhibit their biological activities. Since many such neurotrophic growth factors are essential for cellular subsistence, the phosphorothioate oligodeoxynucleotides might be expected to cause general neurotoxicity, resulting in a narrow range of effectiveness (Chiasson et al., 1994). Non-modified oligodeoxynucleotides, in contrast, have been shown to be tolerated at much higher doses. They are essentially stable for up to 24 h when incubated with rat cerebral spinal fluid (CSF), and have been found to penetrate brain tissue and neurons in rats (Wahlestedt, 1994). Furthermore, numerous studies have also demonstrated specific and effective attenuation of receptors with non-modified oligodeoxynucleotide delivered via intraventricular or intrathecal routes (Wahlestedt et al., 1993a,b; Standifer et al., 1995; Chien et al., 1994). With non-modified oligodeoxynucleotides, the concentration applied in the present study was higher than those in previous studies with phosphorothioate oligodeoxynucleotides (Kindy and Verma, 1988; Chiasson et al., 1992; Gillardon et al., 1994; Hooper et al., 1994; Hunter et al., 1995). We previously performed a pilot study in which the concentrations of oligodeoxynucleotides were tested to determine the appropriate dose. A dose-dependent suppressive relationship between c-Fos and antisense pretreatment confirmed that the dose regimen in the present study was effective. Two-way ANOVA revealed a significant main effect for dose but a non-significant main effect for laminar regions, indicating that the inhibitory effect of c-*fos* antisense oligodeoxynucleotides was not different among different laminae in the dorsal horn of the spinal cord. These results suggested that antisense oligodeoxynucleotides are dispersed among different laminae in the dorsal horn region. Furthermore, these results are comparable to the previous findings (Gillardon et al., 1994) that 5′-[$^{35}$S] end-labeled c-*fos* antisense oligodeoxynucleotides penetrate deeply into the spinal cord and distribute evenly below the superfused area.

The results also showed that the decreases in behavioral responses were significant only in the tonic phase but not the acute phase of the formalin test. Interestingly, formalin-induced hyperalgesia reached its maximum at 20–30

min, while c-Fos expression did so at 1–2 h (Presley et al., 1990). One might argue that the expression of the immediate early gene is too slow to participate in the development of central sensitization. We have no good explanations for the temporal discrepancy between behavioral hyperalgesia and c-*fos* expression at their maximum. However, it has been shown that distinct temporal phases of specific proteins are induced by the nature of the stimuli. The increase in c-Fos protein is readily detected within 15 min of growth factor treatment in vitro (Greenberg et al., 1985), within 30 min of C-fiber electric stimulation (Herdegen et al., 1991) or within 30 min of heat stimulation (Naranjo et al., 1991), while it is not observed until 60 min following carrageenin stimulation (Honore et al., 1995) or 2 weeks following adjuvant-induced arthritis (Abbadie and Besson, 1992). Formalin produced a more immediate and intense increase in the activity of C-fiber afferents relative to other inflammatories such as carrageenin (Heapy et al., 1987). This intense barrage is believed to induce a rapid increase in c-*fos* expression. In our laboratory, c-Fos protein is readily detected in the dorsal horn of spinal cord within 20 min of formalin injection (unpublished data). The fact that c-*fos* expression precedes the behavioral hyperalgesia following formalin injection, together with the results demonstrating a parallel decrease of tonic nociception and c-Fos caused by antisense pretreatment, suggests that c-Fos is involved very early in the development of central sensitization and plays a crucial role in the downstream regulation of signal transduction. Since pain signalling is rather complex, further effort is required to define how regulatory diversity and specificity are generated by different combinations of immediate-early gene coded protein that couple external stimuli to long-term changes.

The results, however, differ from those of Hunter et al. (1995) who reported an increase in the nociceptive responses during the tonic phase with c-*fos* antisense oligodeoxynucleotide pretreatment. The discrepancy may be a result of methodological differences in the modification of oligodeoxynucleotides, parameters used in scoring and/or the age of the experimental rats.

Licking/biting behavior, which was considered as a single parameter for scoring in the investigation by Hunter et al., has been shown to be a less robust measure when the correlation between nociceptive measures and c-*fos* expression was tested in our study (Table 1). The fact that licking/biting behavior showed a significant correlation with doses but not with the c-Fos protein implied that the decrease in licking/biting behavior is not specific for the inhibitory effect of Fos with c-*fos* antisense oligodeoxynucleotide treatment. It is possible that there are other pathways, sensitive to intrathecal oligodeoxynucleotides, involved in determining the expression of the licking/biting behavior. Therefore, it is not advisable to apply this scoring method exclusively in behavioral assessment when antisense strategy is used. Consistent with this observation is evidence (Wheeler-Aceto et al., 1991; Abbott et al., 1995) showing that licking/biting behavior is readily suppressed by compounds or non-analgesics which either stimulate or depress locomotor activity and by those which cause various stereotypic forms. Combination of behavioral parameters (weighted pain score or lifting plus licking) has therefore been suggested by Abbott et al. (1995) as being more consistent than, and superior to, any other single measure in the formalin test.

Differences arising from the age of the rats (26–30-day-old rats weighing 70–90 g in the study by Hunter et al. versus adult rats weighing 250–350 g in our study) may be another possible explanation. Fos protein is activated following peripheral stimulation and then forms a heterodimer with Jun. Jun binds to AP-1-like elements like preprodynorphin and preproenkephalin in the promoter region of its target gene (Iadorola et al., 1988; Noguchi et al., 1991). The increases in spinal dynorphin level following c-*fos* activation by peripheral inflammation are suggested to contribute to enhanced neuronal excitability in the superficial dorsal horn of adult rats via NMDA receptors (Iadorola et al., 1988; Massardier and Hunt, 1989). The physiological role of dynorphin in young rats may differ from that in adults. Evidence exists which suggests that κ-opioid receptor density is greatest in young (10- to 14-day) animals and decreases with age (Barr et al., 1986). The behavioral effects of κ-opioid receptor agonists in 5- to 20-day-old rats were also found to be markedly different from those observed in the adult (Jackson and Kitchen, 1989). Since dynorphin produced both opioid-mediated analgesic and NMDA-mediated facilitatory effects (Hylden et al., 1991; Caudle and Issac, 1988), it is possible that different behavioral responses to κ-opioid receptor alternations reflect a predominant interaction at different types of receptors in young rats and in adult rats. Further studies are required to elucidate the ontogenetic development of the nociceptive response to dynorphin.

The time intervals, 48 h and 120 h, used to test the recovery from the blocking effect in the spinal cord were based on the observation by Standifer et al. (1995), who found that approximately 1% of the intrathecally administered oligodeoxynucleotide was taken up by the spinal cord and about 0.1% was recovered intact 72 h later. The 48- h recovery pretreatment in the study had no effect on c-*fos* expression but decreased licking/biting behavior. It is possible that the level of intact c-*fos* antisense oligodeoxynucleotide in the spinal cord 48 h following administration in our investigation may not have been sufficient to yield a significant effect on Fos expression, but was sufficient to interfere with the licking/biting behavior. This observation provided further evidence to support the hypothesis that the decrease in licking/biting may specifically reflect the inhibitory effect of antisense pretreatment on c-Fos. The neuronal circuitry related to licking/biting recovers its normal response within 120 h when the oligodeoxynucleotide taken up by the spinal cord was further degraded, implying that the gene inactivation

and licking/biting interference by antisense oligodeoxynucleotides are reversible. Although further investigations are required to elucidate the efficacy and toxicity of oligodeoxynucleotides in the central nervous system, results of the present study suggest pharmacological prospects and clinical potential for antisense oligodeoxynucleotide strategy for the central nervous system to block immediate-early genes and the resulting physiological consequence following an acute insult.

In summary, we have demonstrated that c-*fos* antisense oligodeoxynucleotides selectively suppress in a dose-dependent and time-reversible manner the expression of c-*fos* in the spinal cord following formalin stimulation. The suppression of c-*fos* expression is paralleled by a decrease in behavioral hyperalgesia, suggesting its pharmacological potential for blocking immediate-early gene expression in the central nervous system and its resulting physiological consequence following noxious peripheral stimulation.

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