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# Effects of analgesic or antidepressant drugs on pain- or stress-evoked hippocampal and spinal neurokinin-1 (NK-1) receptor and brain-derived neurotrophic factor (BDNF) gene expression in the rat

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*Abbreviations:*

ANOVA = analysis of variance

AUC = area under the curve

BDNF = brain-derived neurotrophic factor

cAMP = cyclic adenosine 3', 5' – monophosphate

CFA = complete Freund's adjuvant

CNS = central nervous system

CRE = cAMP response element

CREB = cAMP response element binding protein

ERK = extracellular-signal related kinase

NK-1 = neurokinin-1

NSAID = non-steroidal anti-inflammatory drug

PKA = protein kinase A

SP = substance P

TCA = tricyclic antidepressant

TrkB = tyrosine kinase B

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**ABSTRACT**

Clinical studies show that people suffering from chronic pain are often also burdened by depression. Antidepressants are used to treat some types of chronic pain; however, little is known about their mechanisms of action. This study addressed the effects of a non-steroidal anti-inflammatory (NSAID) and a tricyclic antidepressant (TCA) drug on pain- and stress-evoked gene expression in the rat spinal cord dorsal horn and hippocampus. Rats were pretreated with either indomethacin or imipramine and then challenged with either intraplantar CFA or a bout of immobilization stress. Results showed that indomethacin significantly reduced nociception-related peripheral edema, hyperalgesia, and reversed the pain-evoked up-regulation of NK-1 receptor and BDNF gene expression in the spinal cord to levels not statistically different from controls. However, indomethacin did not protect against significant pain-induced down-regulation of these genes in the hippocampus by approximately 50%, suggesting that while analgesic drug treatment reduces nociceptive sensory activation in the spinal cord, it is insufficient to prevent the impact of pain on the hippocampus. Conversely, while imipramine did not provide significant behavioral analgesia, it significantly blocked both pain- and stress-evoked alterations in hippocampal and spinal NK-1 and BDNF gene expression. Thus, these results show that application of either analgesic or antidepressant drugs alone does not fully protect against both the behavioral and molecular effects of persistent pain on both “sensory” and “affective” processing within the CNS.

## INTRODUCTION

The primary drugs used for the management of chronic pain have traditionally been opioid receptor agonists and nonsteroidal anti-inflammatory drugs (NSAIDs), which modulate excitatory neurotransmission in the periphery as well as activation of ascending and descending pain pathways of the central nervous system (CNS). However, pain is characterized as a complex experience, dependent not only on the regulation of nociceptive sensory systems, but also on the activation of mechanisms that control mood (affect) in limbic brain areas such as the amygdala and the hippocampus. The relationship between pain and mood has been supported by numerous clinical studies indicating significant co-morbidity of chronic pain and various types of depressive illnesses (Ruoff, 1996; Fishbain et al., 1997). Beyond the assumption that pain is simply a form of stress, the physiological and molecular basis for the coexistence of pain and depression is still being investigated (Duric and McCarson, 2005).

The therapeutic effects of antidepressant drugs have been previously attributed to facilitation of central monoaminergic neurotransmission resulting in the attenuation and reversal of stress-induced pathophysiology occurring in the limbic system, particularly the hippocampus (Duman et al., 1997). Furthermore, the analgesic properties of antidepressants have been investigated in clinical settings. However, their efficacy as adjuvant therapeutics varies with the class of antidepressant used and the type of pain condition (Watson, 1994). Analgesic effects usually occur at lower doses and with an earlier onset than antidepressant effects (Sindrup et al., 1992; Lynch, 2001). Mechanistically, antidepressants are thought to produce analgesia by increasing the

activity of noradrenergic and serotonergic projections descending from the brain to the spinal cord to modulate the release of endogenous opioid peptides (Testa et al., 1987).

The tachykinin neuropeptide substance P (SP) and brain-derived neurotrophic factor (BDNF), as well as their preferred receptors, the neurokin-1 (NK-1) and tyrosine kinase B (trkB), respectively, are well-characterized neuromodulators of nociceptive sensory processing and important contributors to development and maintenance of hyperalgesia and the central sensitization associated with persistent pain (Henry, 1993; Bennett, 2001; Malcangio and Lessmann, 2003). Their involvement in the neurobiology of mood disorders has also been demonstrated (Nibuya et al., 1995; Duman et al., 1997; Kramer et al., 1998; McLean, 2005), suggesting that the effects of pain and stress may converge and activate similar neuronal pathways in the higher brain centers. Moreover, we have previously shown that both peripheral inflammatory pain and immobilization stress each have profound damaging effects on the limbic system, indicated by alterations in hippocampal volume and down-regulation of NK-1 receptor and BDNF gene expression (Duric and McCarson, 2005; Duric and McCarson, 2006).

Prior reports have indicated that administration of either an NSAID (indomethacin, a nonselective cyclooxygenase inhibitor), or a tricyclic antidepressant drug (imipramine, a nonselective monoamine reuptake inhibitor), produces dose-dependent antinociceptive effects in various rat models of inflammatory nociception (Yokogawa et al., 2002; Zarrindast and Sahebgharani, 2002; Nagakura et al., 2003; Bauerova et al., 2004; Zhang et al., 2004). However, the effects of analgesics and antidepressants on the coordination of sensory and emotional aspects of pain are still poorly understood. Therefore, this study addressed the effects of indomethacin and

imipramine pretreatments on pain- or stress-induced changes in NK-1 receptor and BDNF gene expression in “sensory” (spinal cord dorsal horn) versus “affective” (hippocampal) pain processing within the CNS.

## METHODS

### *Animal Housing and Handling*

Young adult male Sprague Dawley rats (Harlan Farms, Indianapolis, IN), used for all experiments, were age matched (7-8 weeks old) at the beginning of the drug and animal treatments. Rats were allowed at least one week of habituation before any treatments were applied. The maintenance of the rat colony and all the animal handling were performed in accordance with NIH laboratory care standards and approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Efforts were made to minimize animal suffering and to reduce the number of animals used in this study. Animals were housed (12 h light/dark cycle) in groups of three per cage with *ad libitum* access to food and water; they were mixed together so there was one member of each treatment group in every cage. All rats, including the sham control group, were handled identically to reduce stress-associated variablility.

### *Pain and Stress Treatments*

Rats (200-300 g) were subjected to intraperitoneal (i.p.) pretreatments with either a single dose of indomethacin (10, 20 or 30 mg/kg for 1hr) or repetitive imipramine doses (15 mg/kg for 21 days), and then challenged with either an inflammatory stimulus (subcutaneous injection of 50 μL of CFA; Sigma Chemical Co., St. Louis, MO) into the plantar aspect of the right hind paw or an acute (45 min) immobilization stress (Rodent restraint cones, Harvard Apparatus, Inc., Holliston, MA) (Figure 1). Sham-treated control animals were not exposed to pain or stress paradigms, but were administered (i.p.) vehicle (ethanol or saline) of the same volume as the drug injections. Otherwise, control

rats were handled identically to all other treatment groups. For each treatment condition (vehicle or drug), a sample size of four rats was used. For gene expression studies, a duplicate set of animals was added in order to avoid the potential effects of additional animal handling and stress associated with thermal and mechanical testing on the outcome of these experiments. Prior to the dissection of spinal and hippocampal tissues, both hind paws were removed and weighed to assess the effects of drug treatments on the nociception-related peripheral inflammation.

***Thermal and Mechanical Testing***

A Thermal Paw Analgesiometer (Department of Anesthesiology, University of California, San Diego, CA) was used to measure thermal withdrawal sensitivity. Rats were initially placed in Plexiglas chambers and allowed to acclimate for 15-20 min. A high-intensity light beam (CXL/CXR lamp bulb; 8V; 50W; Eiko, Tokaimura, Japan) was focused on the plantar surface of the hind paw as a noxious thermal stimulus; paw withdrawal latencies were measured with an automatic timer to the nearest 0.01 sec (Hargreaves et al., 1988; Dirig et al., 1997). The intensity of the light was set to 4.25 amperes in order to produce the baseline withdrawal latency of approximately 10 seconds. For quantification of mechanical sensitivity thresholds, von Frey monofilaments (Stoelting, Inc., Wood Dale, IL, USA) of graded bending forces (2.6 - 522 mN) were applied to the plantar aspects of the hind paw of unrestrained rats placed in an elevated Plexiglas chamber with a wire mesh grid bottom. Monofilaments were applied perpendicular to the hind paw surface with sufficient force to cause a slight bending of the filament in increasing order of intensity until the rat responded by vocalization or

withdrawal of the paw. Mechanical stimulation was repeated three times at 5-10 minute intervals, with randomization of order of testing for each paw (adapted from (Brennan et al., 1996) Monofilament thresholds were converted to grams of force using the manufacturer's table. Thermal and mechanical baseline measurements of both hind paws were taken for each animal prior to nociceptive inoculation with CFA. All behavioral measurements were conducted by an experimenter blind to animal treatments.

***Tissue dissection***

All rats were decapitated 24 h after receiving the pain or stress challenge. Immediately after decapitation, rat brains and spinal cords were removed. Rat brains were dissected along the saggital midline, followed by bilateral removal of the hippocampus. Spinal cord tissues were rapidly removed using hydraulic pressure (a forceful injection of ice-cold isotonic saline) applied to the caudal end of the vertebral canal with a 60 ml syringe and a 16-gauge needle. The lumbar portions ($L_I$-$L_{VI}$) of the vertebral column were then dissected. The dorsal horn regions were dissected by cutting the lumbar spinal cord along the saggital axis and dividing it into quarters. Only the ipsilateral side of the spinal cord was assayed. At the time of sacrifice, hind paws were also removed just above the tibio-tarsal joint and weighed to measure edema.

***Solution Hybridization – Nuclease Protection Assays***

The NK-1 receptor and BDNF (BDNF cDNA plasmid was graciously provided by Ronald Duman, Ph.D., Yale Medical Center) sense and antisense cRNA probes were generated by an *in vitro* run-off transcription reaction (McCarson and Krause, 1994;

Nibuya et al., 1995). Synthesis of the antisense $^{32}$P-labeled cRNA probes using [α-$^{32}$P]UTP (3000 Ci/mmol) was based on the protocol suggested by Promega (Madison, WI). Probes were purified through a NucAway spin column (Ambion, Austin, TX), and DNA template was subsequently digested using RQ1 DNase (Promega, Madison, WI). The total cellular RNAs were extracted from both hippocampal and spinal tissue samples using a rapid quanidinium isothiocyanate-phenol/chloroform extraction method and then assayed for NK1, BDNF or β-actin mRNAs using solution hybridization – nuclease protection assays. Approximately 2x10$^5$ d.p.m. of each specific $^{32}$P-labeled antisense cRNA probe was co-precipitated with 2-80 μg of total RNA, 100-1000 pg cRNA quantitation standards, or *E. coli* tRNA. The RNA-[$^{32}$P]RNA co-precipitates were each re-suspended in 10 μL of hybridization buffer (40 mM PIPES, pH 6.4, 400 mM NaCl, 1 mM EDTA, 80 % (v/v) deionized formamide) and incubated overnight at 45ºC. Samples were initially digested by nucleases A (4 μg/mL) and T1 (0.2 μg/mL) for 20 min at 37ºC, followed by an additional 15 min digestion at 37ºC with proteinase K (100 μg/sample). The digestion reaction products were precipitated, re-suspended, and electrophoresed on 6% polyacrylamide gels containing 7 M urea. Gels were fixed, dried, and exposed to phosphor plates over night. Molecular Dynamics PhosphorImager SF (Sunnyvale, CA) was used to generate and analyze the corresponding densitometric images. Specific mRNA amounts were determined by comparison to cRNA quantitation standards. Data values are reported as pg specific mRNA/ ng β-actin mRNA [mean ± S.E.M.]. Results are reported for the dorsal portion of the lumbar spinal cord ipsilateral to the CFA injection; hippocampal results are reported as a bilateral average. Results of previous studies have provided abundant evidence that afferent nociceptive information gains

access to the contralateral side of the spinal cord (McCarson and Krause, 1994; McCarson and Krause, 1996; Enna et al., 1998; McCarson and Enna, 1999; Allen et al., 2003; Allen and McCarson, 2005). Measurable alterations in gene expression are apparent in the contralateral cord and consistently appear similar to, but rarely as robust or consistent, as changes in the ipsilateral side. In previous studies of unilateral nociceptive activation of the hippocampus, changes in neurogenesis and BDNF expression were found to be fully bilateralized (Duric and McCarson, 2005; Duric and McCarson, 2006).

***Quantitation of Pain-Related Behavior***s

An additional set of animals was used to quantify pain-related behaviors during the first 26 min after CFA injection. Two groups of rats were compared; one that received CFA alone (n = 4), and another that received indomethacin (20 mg/kg) in addition to CFA (n = 4). Prior to CFA injection, rats were observed for 2 min to quantify baseline behaviors. Once the CFA was administered, each animal was placed in an individual cage and evaluated by observer blind to the treatment group. Overall pain intensity was rated in 120 sec intervals, according to the following numerical scale (adopted from (Dubuisson and Dennis, 1977):

**0** Both hind paws are firmly placed on the floor, and weight is evenly distributed. Injected paw is not significantly favored during locomotion.

**1** The injected paw rests on the other part of the body or is lightly touching the floor surface, with little or no weight placed upon it. Moderate to significant limp is

present during locomotion. Normal grooming with both paws being elevated and attempts to sleep with both paws off the floor are also given this rating.

**2** The injected paw is elevated and is not touching the floor or any other surface, while the uninjected paw is placed on the floor. The injected paw is held of the floor or tuckered under the body while animal is curled up or sleeping.

**3** The injected paw is shaken, licked, or bitten, while the uninjected paw exhibits none of these actions. This behavior should be differentiated from the normal grooming patterns.

Afterwards, the same animals were also tested for thermal sensitivity during the first 24 h after noxious stimulation, using the previously described protocol.

***Statistical Analyses***

Data from all the experiments were analyzed using analysis of variance (ANOVA) with Student-Newman-Keuls' statistical tests used for post-hoc comparisons, except for the mechanical sensitivity experiment, where nonparametric Kruskal-Wallis analysis with Dunn's post-test was used (InStat, GraphPad Software, Inc., San Diego, CA). Significance was considered to be $p \leq 0.05$.

## RESULTS

### *Analgesic and anti-inflammatory effects of indomethacin and imipramine*

The analgesic and anti-inflammatory properties of NSAID and antidepressant drugs were evaluated using paw sensitivity thresholds and levels of edema. In animals that did not receive a drug treatment (CFA alone group), 50 μL injection of CFA produced significant thermal (Figure 2A) and mechanical (Figure 2B) hyperalgesia of the ipsilateral paws at 24 h post-injection. Administration of ethanol (indomethacin vehicle) had no considerable effect on the lowered sensitivity. However, pretreatments with either 10 or 20 mg/kg of indomethacin (INDO) fully inhibited both forms of the hyperalgesia, as the thresholds recorded in these animals were similar to baseline latencies. The highest dose of indomethacin (30 mg/kg) attenuated only the mechanical hypersensitivity, while the mechanical thresholds were only partially affected. When given at 20 mg/kg, indomethacin seemed to exhibit optimal anti-nociceptive properties, so this dose was used throughout subsequent experiments. Pretreatment with 15 mg/kg/day imipramine (IMI) for 21 consecutive days showed no significant effects on mechanical hyperalgesia, while thermal sensitivity was only slightly decreased. Similarly to ethanol, administration of saline (imipramine vehicle) did not affect CFA-induced hyperalgesia.

CFA produced robust local edema of the ipsilateral hind paw at 24 h post injection, as shown by the significant increase in weight compared to the contralateral paw (Figure 3). The anti-inflammatory effect of indomethacin was confirmed, as edema of the ipsilateral paw was completely blocked at this time and the ipsilateral paw weight was almost identical to that of the contralateral side (Figure 3A). In contrast, imipramine

showed no anti-inflammatory properties; ipsilateral paws in these animals were similar in weight and redness to the group that received CFA alone (Figure 3B). Additionally, acute immobilization stress had no effect on the appearance or weight of either paw.

***Effects of indomethacin and imipramine on gene expression in the CNS***

The effects of analgesic and antidepressant drugs on the nociception- or stress-induced regulation of NK-1 receptor and BDNF gene expression in "sensory" (spinal cord) and the "affective" (hippocampus) regions of the CNS were assessed using solution hybridization-nuclease protection assays. As indicated in Figures 4 and 5, 24 h after CFA injection into the hind paw, NK-1 receptor (Figure 4A) and BDNF (Figure 4B) mRNA levels were robustly increased in the ipsilateral spinal cord dorsal horn (by 45% and 67%, respectively) compared to sham-treated controls. Administration of indomethacin (20 mg/kg) one hour prior to CFA injection completely inhibited the nociception-evoked up-regulation of both genes in the spinal cord (Figure 4). Not surprisingly, a single 45 min bout of immobilization stress had no effect on either NK-1 receptor or BDNF mRNA levels in the dorsal horn (Figures 4 and 5). Administration of imipramine (15 mg/kg/day) for 21 days did not alter spinal gene expression by itself (Fig. 5). Interestingly, imipramine pretreatment did diminish CFA-induced increases in NK-1 receptor and BDNF mRNA levels (Figure 5) to an extent comparable to that produced by indomethacin (Figure 4).

In the hippocampal formation, both inflammatory nociception and immobilization stress robustly decreased NK-1 receptor and BDNF gene expression, as shown in previous reports (Duric and McCarson, 2005). Neither pain nor stress produced

significantly sided differences; thus, hippocampal mRNA levels are shown as bilateral averages. Surprisingly, indomethacin pretreatment was unable to block the damaging modulatory effects of pain or stress on the hippocampus (Figure 6). As shown in Figure 6, hippocampal NK-1 receptor and BDNF mRNA levels are similar in vehicle- or indomethacin-treated rats receiving pain or stress model treatments. This contrasts with the ability of indomethacin to reverse the pain-evoked changes in gene expression in the spinal cord (Figure 4). Administration of the antidepressant drug imipramine clearly prevented the immobilization stress-induced decreases in hippocampal NK-1 receptor and BDNF mRNA levels (Figure 7), as predicted by previous reports (Nibuya et al., 1995). Furthermore, the effects of CFA-induced changes in NK-1 receptor and BDNF gene expression were also fully inhibited by imipramine pretreatment (Figure 7).

Levels of β-actin mRNA were determined to serve as gel loading controls and to further ensure that the detected changes in NK-1 receptor and BDNF mRNA levels are not due to a pain- or stress-related global modulation of gene expression within the CNS. β-actin mRNA levels were unaffected by peripheral inflammatory nociception, immobilization, or drug treatments alone in either the spinal cord or hippocampus.

***Effects of indomethacin on pain-related behaviors and thermal sensitivity during the first 24 hours after CFA***

Since the results of the first two experiments demonstrated that indomethacin inhibited hyperalgesia, peripheral inflammation and gene expression up-regulation in the spinal cord after CFA administration without prevention of its effects on the hippocampus, an additional experiment assessed the onset of indomethacin-produced

analgesia. The quantitation of pain-related behaviors during the first 26 min after CFA is shown in Figure 8A. Baseline behaviors were measured prior to the nociceptive stimulation. The animals received an average rating of 0.1, mainly due to routine grooming and rearing behaviors. In rats that did not receive indomethacin, CFA injection evoked robust withdrawal, elevation, and frequent licking and biting of the ipsilateral hind paw that peaked at 6 min post inoculation (Figure 8A) as well as redness and edema (Figure 2). In animals pretreated with indomethacin (20 mg/kg), initial redness and swelling was also observed, but the pain-related behaviors were considerably attenuated (Figure 8A). Pain-related behaviors were quantified by measuring the area under the curve in Figure 8A; the CFA-only group had an AUC value of 17.0 ± 1.5, while CFA + indomethacin produced AUC value of 6.5 ± 1.6 (mean ± S.E.M.). Nevertheless, the rats still appeared agitated; the CFA-inflamed paws generally bore little to no weight or were slightly elevated with occasional licking and biting despite the presence of indomethacin (i.e., the AUC of pain-related behaviors of CFA + indomethacin rats was greater than zero). Thermal sensitivity of the CFA-inflamed paws is shown in Figure 8B. At 30 min post injection, animals injected with CFA alone displayed a robust hyperalgesia that persisted for at least 24 h (Figure 8B). Notably, the indomethacin-treated group also showed significant hypersensitivity during the initial 60 min; however, by 90 min after CFA the drug's analgesic effect was clearly present as the withdrawal latencies returned to baseline levels (Figure 8B).

## DISCUSSION

The use of NSAIDs and antidepressants in the management of various chronic pain conditions has been well documented in clinical settings (Walker, 1995; Richeimer et al., 1997; Korzeniewska-Rybicka and Plaznik, 1998; Minotti et al., 1998; Fishbain, 2000; Fishbain et al., 2000). Their analgesic properties have been associated with alterations of pain signaling in the sensory pathways within the CNS, primarily the spinal cord and brainstem. However, pain may also have an impact on the emotion- and cognition-processing components of the CNS. The possibility of stress-like modulation in higher brain centers has been previously supported by numerous clinical observations that people suffering from chronic pain are often also depressed (Bair et al., 2003). The effects of NSAIDs and antidepressants on mood-regulating brain regions such as the hippocampal formation are poorly characterized in animal models of persistent pain. The experiments in this study were designed to assess the effects of either indomethacin or imipramine pretreatment on gene expression within sensory and affective pain processing regions of the CNS during peripheral inflammatory nociception or immobilization stress.

Initially, the effects of analgesic and antidepressant drugs on inflammation-evoked hyperalgesia were determined. As reported by other studies, animals treated with CFA developed robust swelling and inflammation of the injected limb, as well as significant thermal and mechanical hyperalgesia (Figure 2) measured at the plantar surface of the paw (Ma and Woolf, 1996). Three different doses (10, 20 and 30 mg/kg) of indomethacin were used in this experiment that were comparable to previously suggested doses (Nagakura et al., 2003). The results indicated that at 24 h post CFA administration, pretreatment with indomethacin induced a complete reversal of both

thermal and mechanical hyperalgesia in the injected paw (Figure 2). The observed analgesia was fully attributable to the effects of the drug alone, since the vehicle (ethanol) alone did not produce a similar outcome. Interestingly, the highest dose of the NSAID indomethacin (30 mg/kg) evoked only partial inhibition of mechanical hypersensitivity, likely due to the gastrointestinal side-effects of indomethacin and its overall toxicity at high doses (Bauerova et al., 2004). Indomethacin given at a dose of 20 mg/kg emerged as the most efficacious analgesic treatment, so this dose was used in subsequent experiments addressing peripheral inflammation and gene expression within the CNS. Moreover, the CFA-evoked redness and edema observed in the injected paws at 24 h were completely inhibited by indomethacin (20 mg/kg) pretreatment (Fig. 2A), clearly confirming the anti-inflammatory properties of this drug.

Other reports have suggested that tricyclic and various other classes of antidepressant drugs produce both anti-nociceptive and anti-inflammatory effects in rats exposed to painful stimuli (Abdel-Salam et al., 2003). However, the administration of imipramine for 21 days at 15 mg/kg/day produced no considerable thermal or mechanical analgesia (Figure 2) and had no effect on the CFA-evoked edema (Figure 3B). This treatment paradigm was chosen for this study since it has been shown to induce antidepressant effects in the hippocampus, as manifested by increased neurogenesis and expression of growth factors needed for neuronal survival (Nibuya et al., 1995; Malberg et al., 2000). In these experiments, the imipramine administration was long-term, with the analgesic and anti-inflammatory properties measured 24 h after inflammatory nociception challenge. In previous studies, a single dose of antidepressants was usually applied 30 – 90 min prior to painful stimulation and the effects were observed soon after

(Yokogawa et al., 2002; Zarrindast and Sahebgharani, 2002; Abdel-Salam et al., 2003; Nagakura et al., 2003). Thus, the differences between the antidepressant and analgesic actions of antidepressant drugs may be a function of both the time of initiation and the duration of the drug treatment. Furthermore, the relevant sites of action within the CNS may be different with regard to antidepressant versus analgesic activity. The antidepressants effects are thought to be related to activity in the hippocampus and other mood-regulating regions of the limbic system, while the analgesic properties are thought to involve modulation of endogenous pain pathways projecting from the brainstem to the spinal cord. This is supported by clinical studies showing that analgesic effects of antidepressant drugs may occur without the antidepressant effects, occurring with a much more rapid onset and at lower doses (McQuay et al., 1996; Lynch, 2001; Raja et al., 2002).

The main goal of this study was to investigate the effects of analgesic and antidepressant drugs on changes in NK-1 receptor and BDNF gene expression evoked by pain or stress. Results confirm that CFA produced significant increases in NK-1 receptor and BDNF mRNA levels in the ipsilateral dorsal horn, consistent with previous results (McCarson and Krause, 1994) and with the role of these neuromodulators in the generation of hyperalgesia and amplification of nociceptive signaling during persistent pain (Woolf and Salter, 2000). Treatment with the NSAID analgesic drug indomethacin (Figure 4) or the antidepressant drug imipramine (Figure 5) did not alter spinal gene expression when the drugs were administered alone. However, at the level of the spinal cord, both indomethacin and imipramine provided full protection against CFA-induced increases in NK-1 receptor and BDNF gene expression. Indomethacin-evoked inhibition

of spinal gene activation may mechanistically support the analgesic effects initially observed in pain-related behaviors and hyperalgesia. Even though imipramine did not attenuate thermal or mechanical hyperalgesia (Figure 2), the gene expression results suggest that tricyclic antidepressants (Figure 5), like NSAIDs (Figure 4), may exhibit analgesic-like properties at the level of gene expression within nociceptive sensory neuronal pathways of the CNS. Of course, nociception induces a myriad of cellular and molecular changes within the spinal cord, and these data do not imply that nociception-induced changes in expression of NK-1 receptor and BDNF genes are the sole factors important in spinal modulation of inflammation and hyperalgesia.

Activation of peripheral and central nociceptive pathways may have an impact on the limbic system through initiation of cellular transduction mechanisms similar to those characterized as important in stress and depression. Contrary to the pain-evoked *increases* in gene expression previously characterized in the spinal cord (see above), significant *decreases* in hippocampal NK-1 receptor and BDNF mRNA levels were observed after either peripheral inflammation or immobilization stress. These data further support our previous findings (Duric and McCarson, 2005; Duric and McCarson, 2006), and provide additional evidence that both pain and stress activate the same "non-sensory" brain regions and induce potentially detrimental modulatory effects. Despite its anti-inflammatory (Figure 3) and analgesic (Figure 2) effects in the periphery and inhibitory effects in the spinal cord (Figure 4), indomethacin had no significant effect on nociceptive activation of the brain, as shown by a lack of inhibition of pain-evoked decreases in hippocampal NK-1 receptor or BDNF gene expression. Thus, under these conditions, analgesic therapy that effectively blocked the development of inflammation

and hyperalgesia was insufficient to prevent the initiation of stress-like effects in the limbic system.

Therefore an additional experiment was performed to address CFA-induced behaviors and thermal hypersensitivity at early time points to clarify the interpretation of findings from the gene expression experiments. Since all measurements of behavior and gene expression to this point were conducted only at the 24 h time point, the final experiment showed the onset of indomethacin's analgesic activity during the first 24 h after CFA. As indicated in Figure 8, pretreatment with indomethacin only partially inhibited pain-related behaviors during the first 30 min after CFA injection, and analgesic effects on thermal hyperalgesia were not apparent until 90 min after CFA injection. This behavioral result clearly shows that there is an initial window of approx. 90 min immediately after CFA administration when pain-related behaviors are apparent before the analgesic effects of indomethacin begin. Therefore, even brief, temporary nociceptive stimulation may be sufficient to induce delayed and long-lasting hippocampal plasticity. This finding is similar to recent observations that early life exposures to short-term stress can lead to delayed onset of cellular mechanisms causing profound, progressive hippocampal impairment (Brunson et al., 2005). Taken together, the current results suggest that analgesic drugs such as indomethacin are very effective in diminishing nociception in the periphery and spinal cord, but may have little effect on the negative emotional and cognitive impact of pain.

Repetitive administration of antidepressants have been shown to enhance synaptic levels of norepinephrine and serotonin in the hippocampus, with concomitant increases in BDNF and trkB mRNAs and protein levels (Duman et al., 1997). Regulation of hippocampal BDNF has been linked to increased expression and activation of cAMP response element binding protein (CREB), and is thought to be associated with the reversal of stress-related limbic pathophysiology (Nibuya et al., 1995). In the current study, prolonged antidepressant treatments alone did not affect NK-1 receptor and BDNF mRNA levels in the spinal cord or hippocampus (Figures 5 and. 7), which is consistent with previous observations (Coppell et al., 2003; Sartori et al., 2004). However, in rats that were exposed to pain or stress, imipramine completely inhibited the pain- or stress-evoked down-regulation of hippocampal NK-1 receptor and BDNF gene expression (Figure. 7). Thus, antidepressants apparently block the changes in expression of genes that contribute to long-term nociceptive sensory plasticity not only in the spinal cord, but also in limbic regions involved in the modulation of affect. Furthermore, these findings suggest that early antidepressant drug administration as part of persistent pain therapy may prevent predisposition toward development of pain-related depression.

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**FOOTNOTES**

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## LEGENDS FOR FIGURES

**Figure 1.** Schematic diagram showing drug treatment paradigms. Rats were subjected to intraperitoneal (i.p.) pretreatments with either a single dose of **(A)** indomethacin (10, 20 or 30 mg/kg for 1hr) or repetitive **(B)** imipramine doses (15 mg/kg for 21 days), and then challenged with either an inflammatory stimulus (subcutaneous injection of 50 μL of CFA) into the plantar aspect of the right hind paw or an acute (45 min) immobilization stress. Sham control animals were not exposed to pain or stress paradigms, but were administered (i.p.) vehicle (ethanol or saline) of the same volume as the drug injections. Otherwise, controls were handled identically to the treatment animals.

**Figure 2.** Histograms showing **(A)** thermal nociceptive withdrawal latencies and **(B)** mechanical withdrawal thresholds to stimulation of the plantar surface of the ipsilateral (right) rat hind paw following CFA-induced inflammation. Rats were pretreated with either ethanol (vehicle) or indomethacin (INDO; 10, 20 or 30 mg/kg) for 1 h, while animals that received saline (vehicle) or imipramine (IMI; 15 mg/kg/day) were pretreated for 21 days prior to CFA administration. Baseline groups represent all animals (combined from all treatment groups) prior to injection of CFA. Note that CFA produced both thermal and mechanical hypersensitivity that was reversed by indomethacin but not long-term imipramine treatment. Thermal withdrawal latencies are expressed in seconds, while mechanical withdrawal thresholds are reported as grams of force (mean ± S.E.M.; n = 4). *$p < 0.05$, ***$p < 0.001$ compared to baseline; ††$p < 0.01$ compared to vehicle (ethanol) group (Thermal data: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[7,48] = 14.0$; Mechanical data: Kruskal-Wallis and Dunn's *post-hoc* test).

**Figure 3.** Histograms showing the effects of **(A)** indomethacin (INDO) or **(B)** imipramine (IMI) on peripheral inflammation (edema) 24 h after CFA injection. Edema was evaluated by weighing the contralateral vs. ipsilateral hind paws. CFA injection produced significant inflammation of the ipsilateral paw that was completely attenuated by indomethacin (20 mg/kg) pretreatment but not by 21 day imipramine (15 mg/kg/day) pretreatment. Data are reported as weight of the paw in grams (mean ± S.E.M.; n = 4). ***$p < 0.001$ compared to sham control groups that were administered vehicle (**A**: ethanol, ANOVA and Student-Newman-Keuls' *post-hoc* test, Ipsilateral paw $F[5,18] = 92.7$; Contralateral paw $F[5,18] = 3.3$; **B**: saline, ANOVA and Student-Newman-Keuls' *post-hoc* test; Ipsilateral paw $F[5,18] = 73.3$; Contralateral paw $F[5,18] = 4.8$).

**Figure 4.** Histograms showing the effects of indomethacin (INDO; 20 mg/kg) treatment on **(A)** NK-1 receptor and **(B)** BDNF mRNA levels in the ipsilateral spinal cord dorsal horn following either an injection of CFA into the right hind paw or a single exposure to immobilization stress for 45 min. Note that CFA-induced increases in both NK-1 receptor and BDNF gene expression in the spinal cord were blocked by indomethacin. Immobilization stress had no effect on gene expression in the spinal cord dorsal horn. Data are expressed as pg specific mRNA/ ng β-actin mRNA (Mean ± S.E.M.; n = 4). **$p < 0.01$, ***$p < 0.001$ compared to the sham control (ethanol) group (**A**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 5.8$; **B**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 12.1$).

**Figure 5.** Histograms showing the effects of imipramine (IMI; 15 mg/kg/day for 21 days) treatment on **(A)** NK-1 receptor and **(B)** BDNF mRNA levels in the ipsilateral spinal cord dorsal horn of following either an injection of CFA into the right hind paw or a single exposure to immobilization stress for 45 min. Similar to indomethacin treatment, imipramine reversed CFA-evoked increases in NK-1 receptor and BDNF gene expression. Data are expressed in pg specific mRNA/ ng β-actin mRNA (Mean ± S.E.M.; n = 4). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the sham control (saline) group (**A**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 9.7$; **B**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 5.0$).

**Figure 6.** Histograms showing the effects of indomethacin (INDO; 20 mg/kg) treatment on **(A)** NK-1 receptor and **(B)** BDNF mRNA levels in the bilateral hippocampal formation of rats following an injection of CFA into the right hind paw or a single exposure to immobilization stress for 45 min. Both peripheral inflammation and immobilization stress evoked significant *decreases* in NK-1 receptor and BDNF mRNA levels in the hippocampus. Unlike its effects in the spinal cord, indomethacin had no effect on pain- or stress-evoked changes in hippocampal gene expression. Data are expressed as pg specific mRNA/ ng β-actin mRNA (Mean ± S.E.M.; n = 4). $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the sham control (ethanol) group (**A**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 6.0$; **B**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 8.6$).

**Figure 7.** Histograms showing effects of imipramine (IMI; 15 mg/kg/day) treatments on **(A)** NK-1 receptor and **(B)** BDNF mRNA levels in the bilateral hippocampal formation of rats following an injection of CFA into the right hind paw or a single exposure to immobilization stress for 45 min. Administration of imipramine alone did not alter gene expression in animals that were not challenged with pain or stress. However, note that imipramine blocked inflammation- or immobilization stress-evoked down-regulation of hippocampal NK-1 receptor and BDNF gene expression. Data are expressed as pg specific mRNA/ ng β-actin mRNA (Mean ± S.E.M.; n = 4). $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the sham control (saline) group (**A**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 8.8$; **B**: ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[5,18] = 14.7$).

**Figure 8.** Graphs showing the effects of indomethacin (20 mg/kg) treatment on **(A)** spontaneous CFA-evoked pain-related behaviors and **(B)** thermal paw withdrawal latencies of rats immediately following CFA injection. Baseline levels (average rating = 0.1) were measured immediately prior to administration of CFA. Note that indomethacin significantly reduced, but did not completely eliminate, pain-related behaviors within first 26 min following CFA injection (**A**; CFA only group AUC = 17.0 ± 1.5; CFA + indomethacin group AUC = 6.5 ± 1.6; two-tailed Student's p value = 0.0027). Similarly, the analgesic effects of indomethacin were not apparent until 90 min after CFA administration, but persisted until 24 hours after CFA injection. Behavioral assessment was numerically quantified (score range: 0-3), while thermal withdrawal latencies are expressed in seconds, (mean ± S.E.M.; n = 4). $^{a}p < 0.001$ compared to baseline

(ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[8,27] = 17.0$); $^{b}p < 0.01$ compared to baseline (ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[8,27] = 6.25$); $^{c}p < 0.001$ compared to CFA only group (ANOVA and Student-Newman-Keuls' *post-hoc* test, $F[15,48] = 16.0$).

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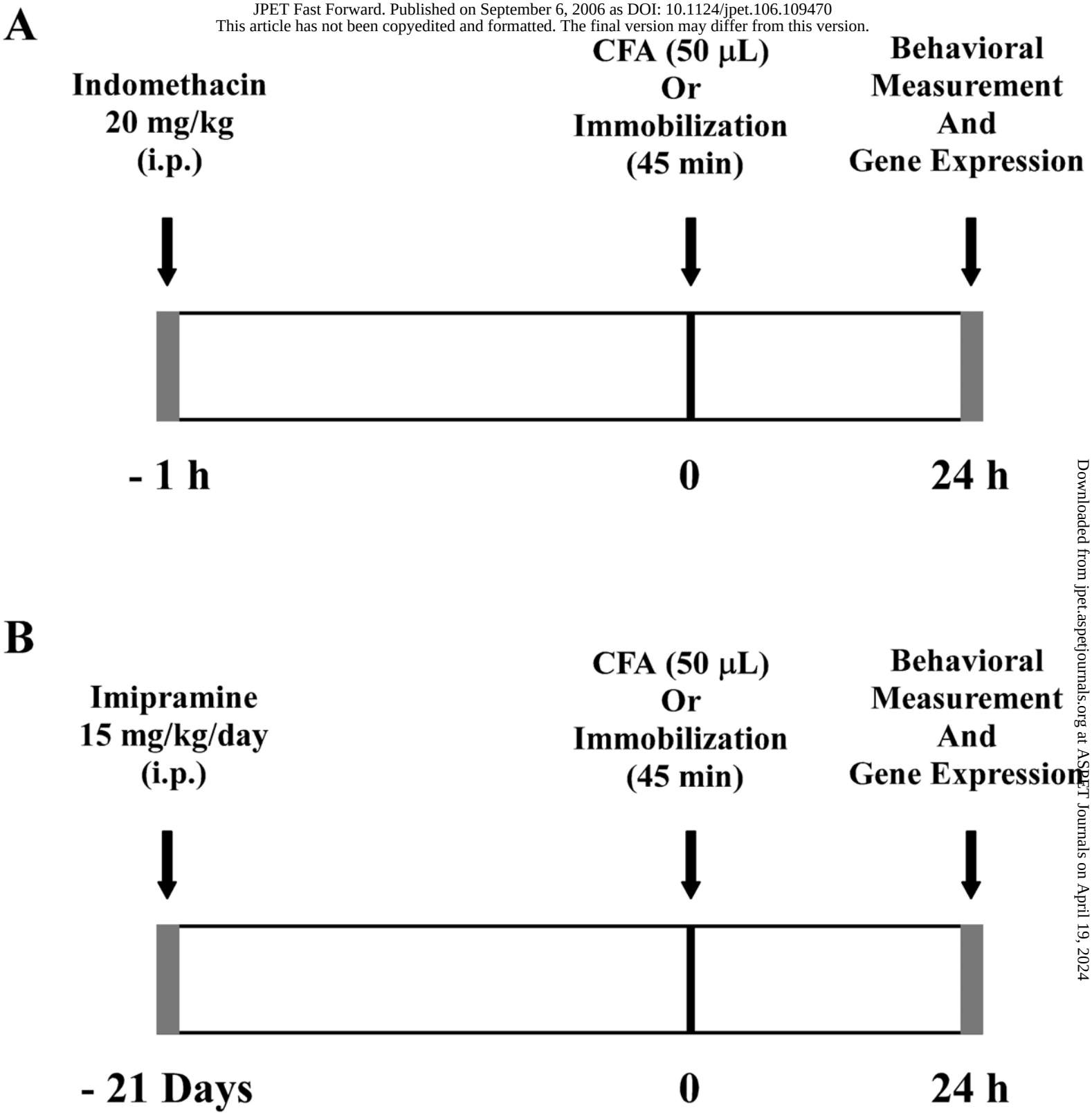




**Figure 1**

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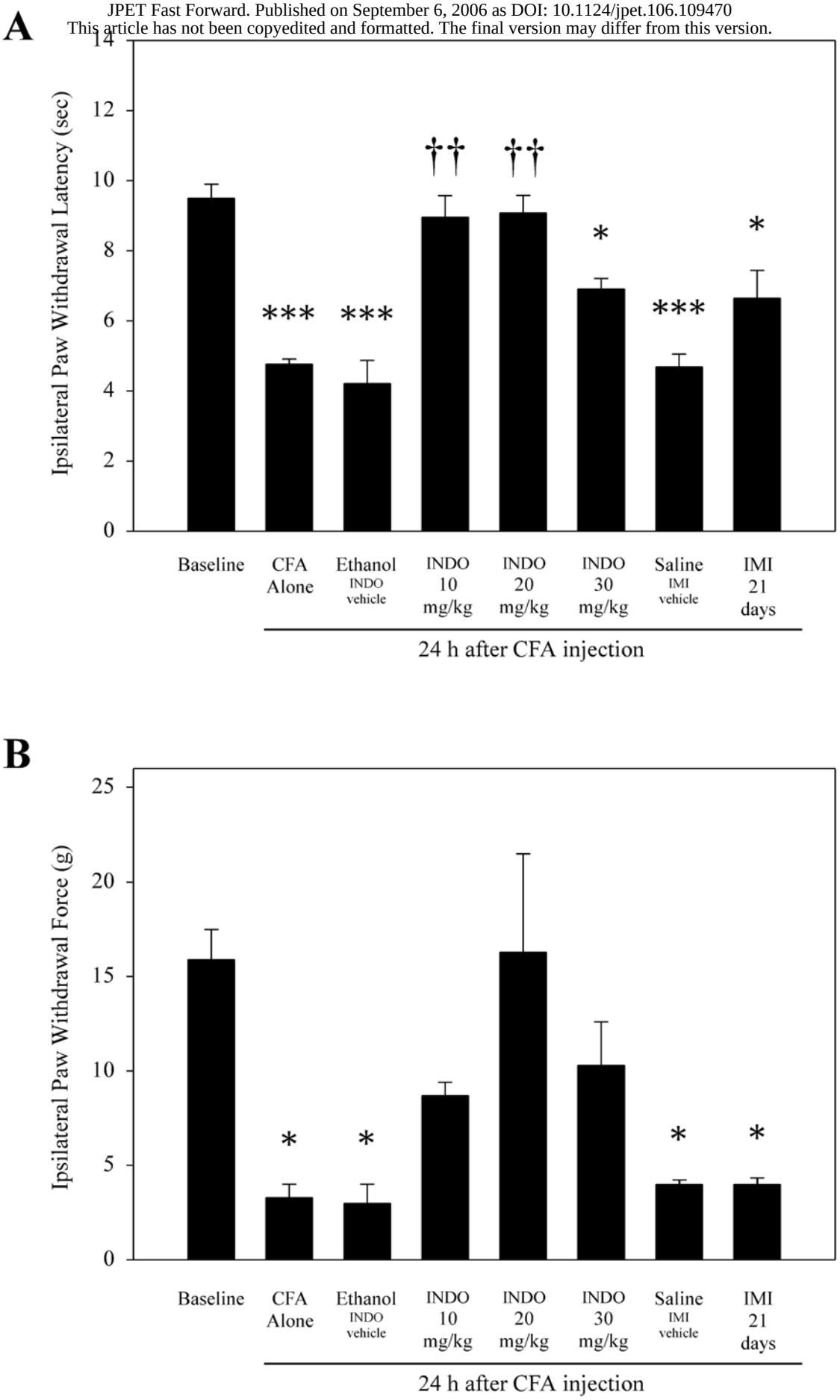


**Figure 2**

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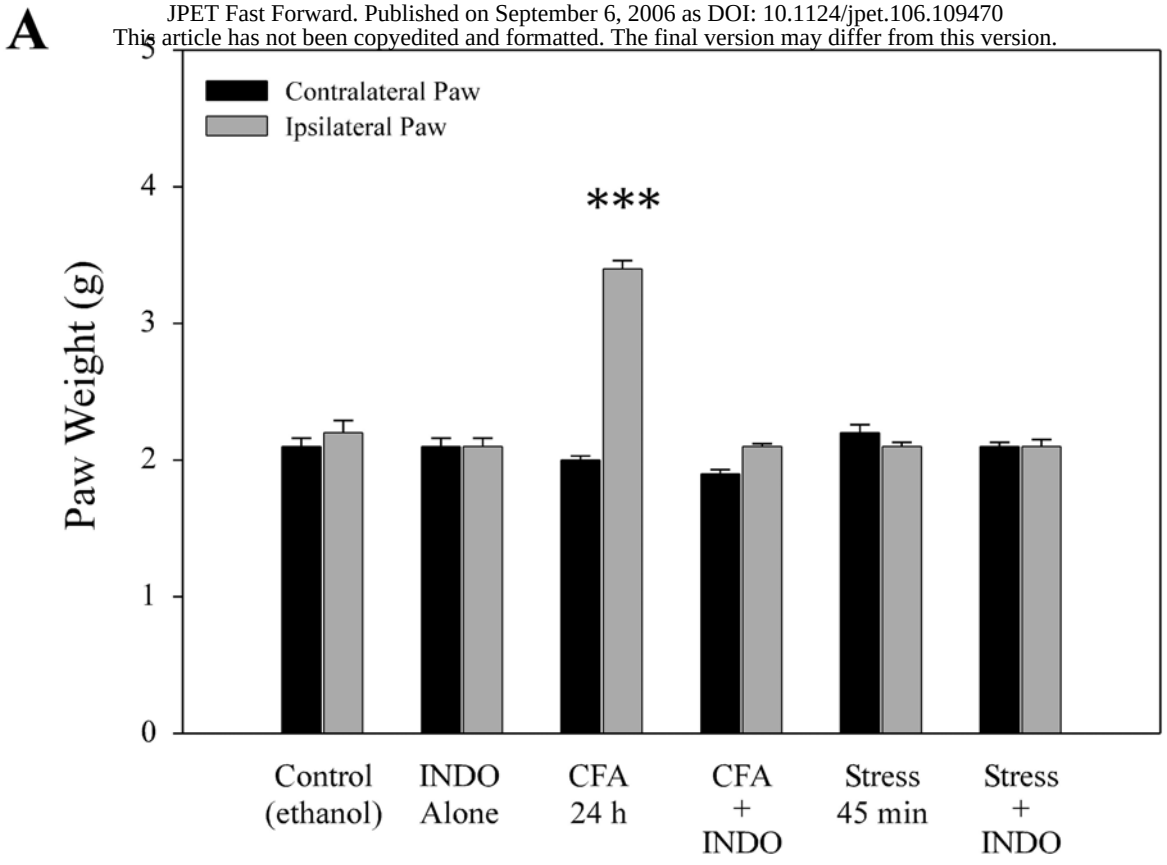


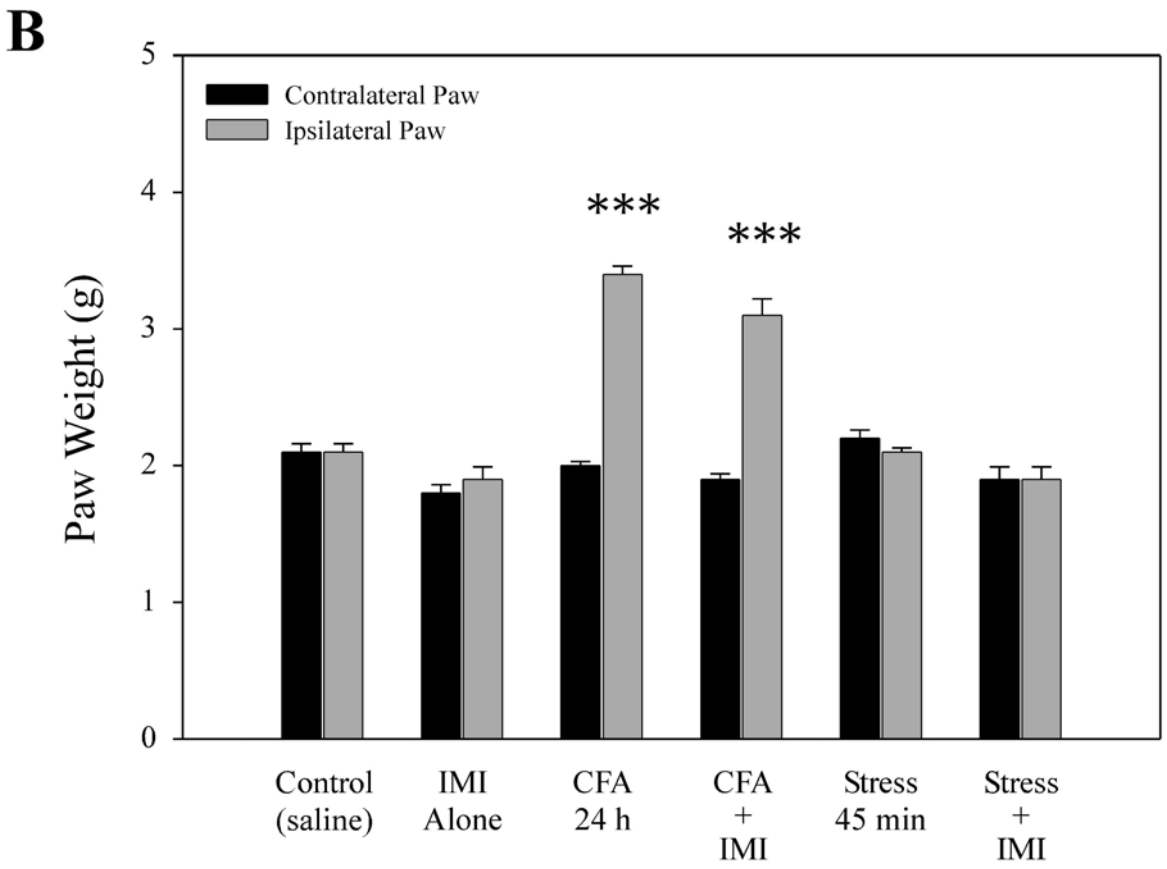


**Figure 3**

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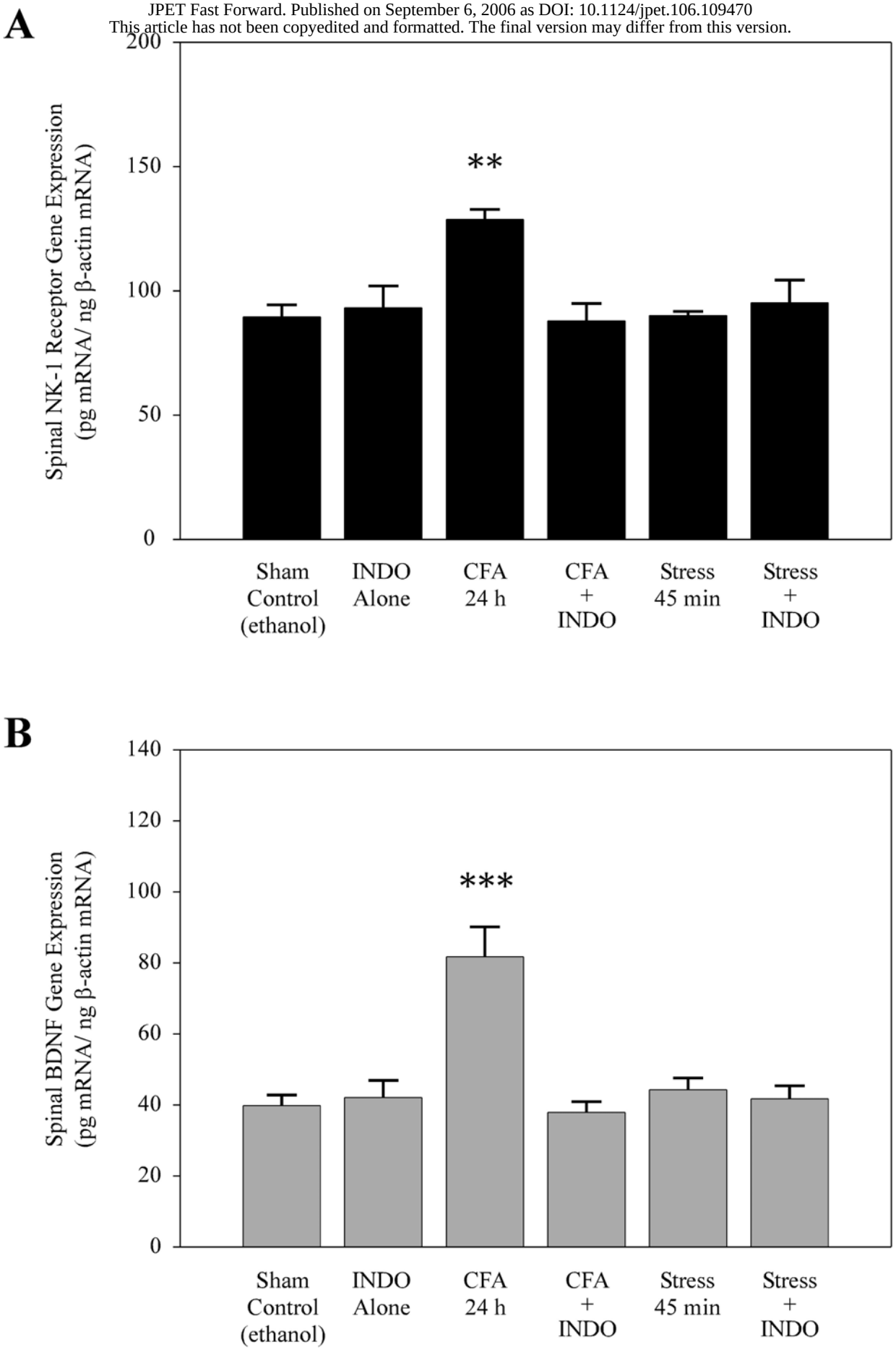


**Figure 4**

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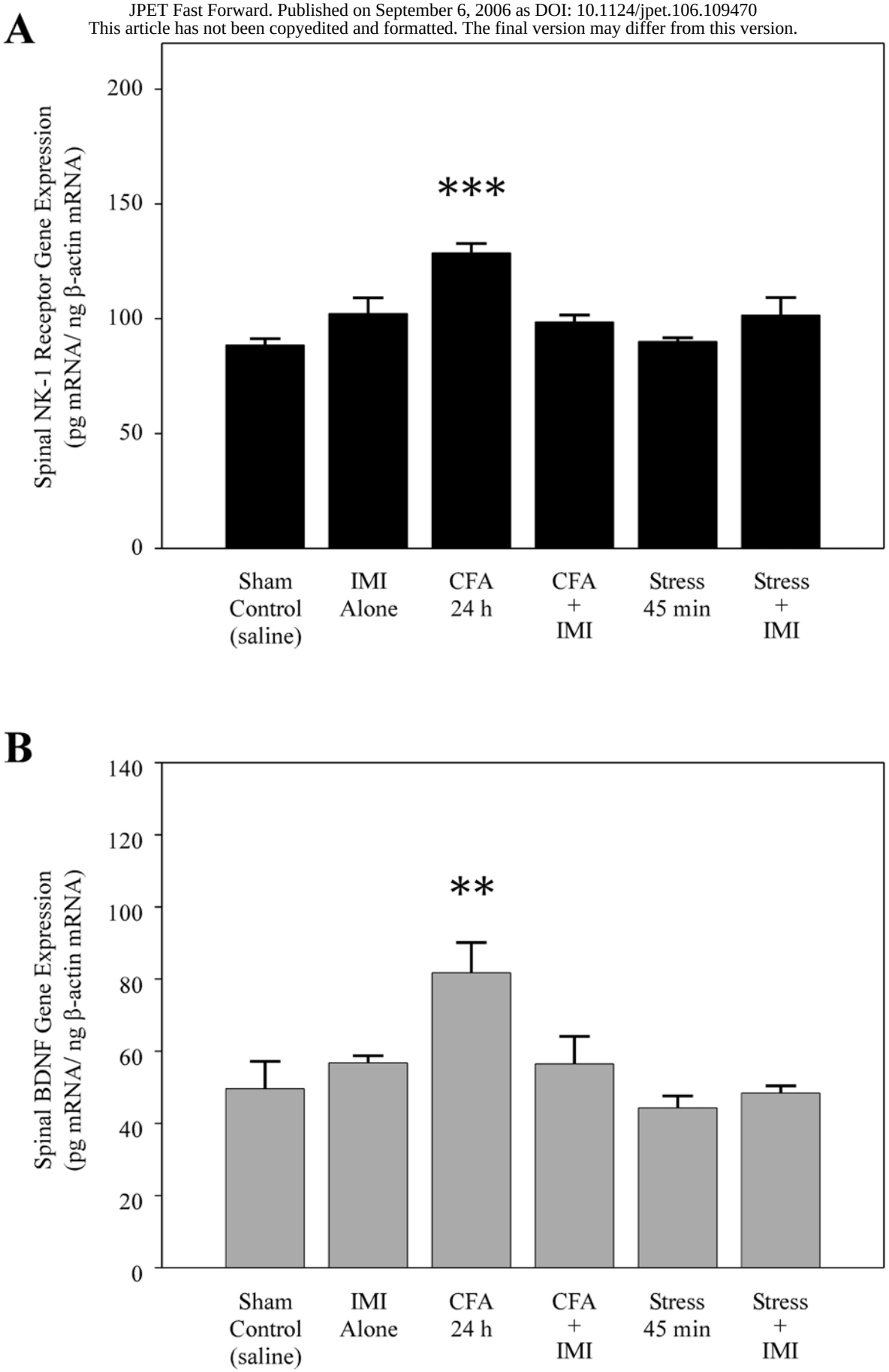


**Figure 5**

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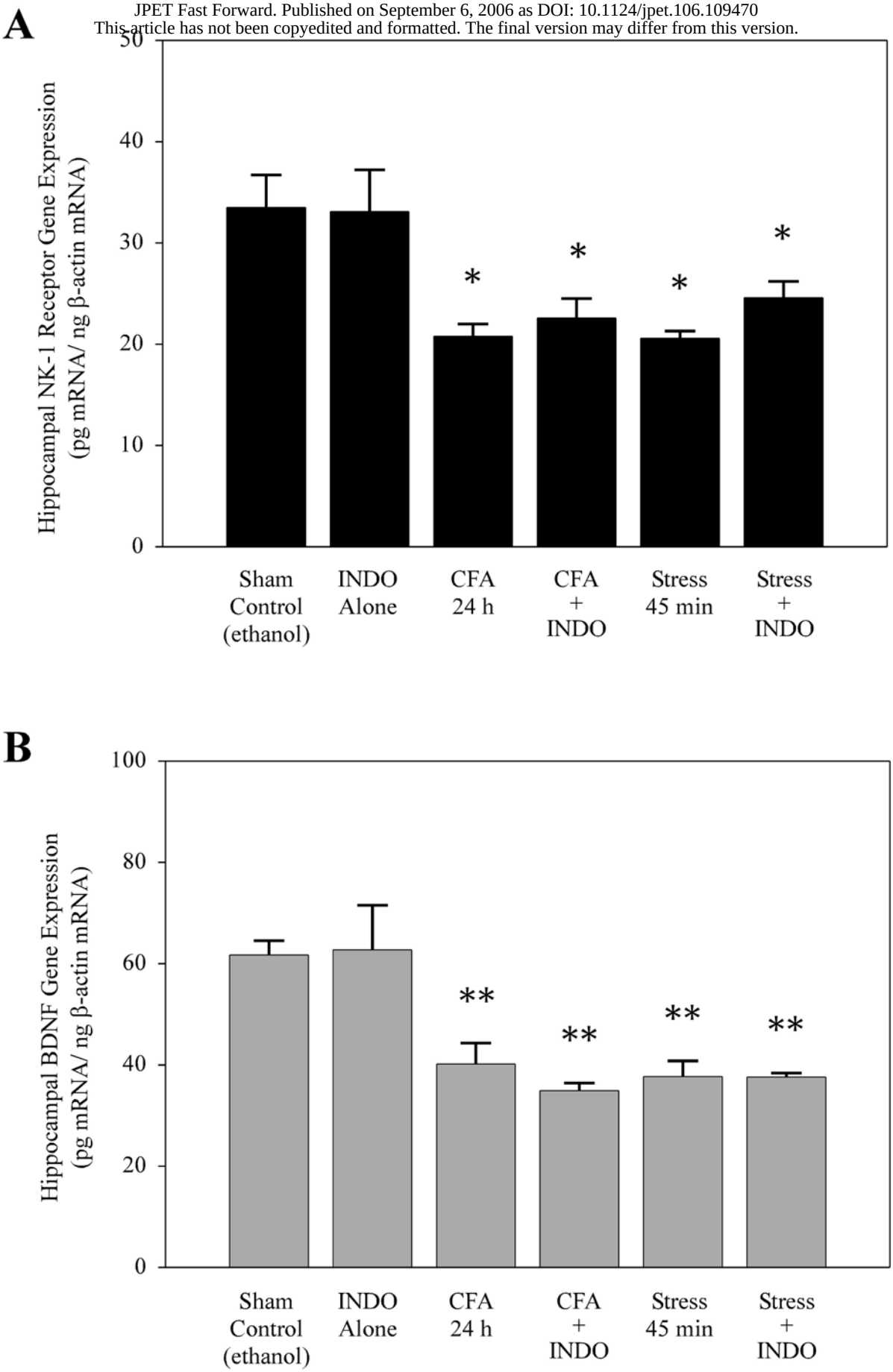


**Figure 6**

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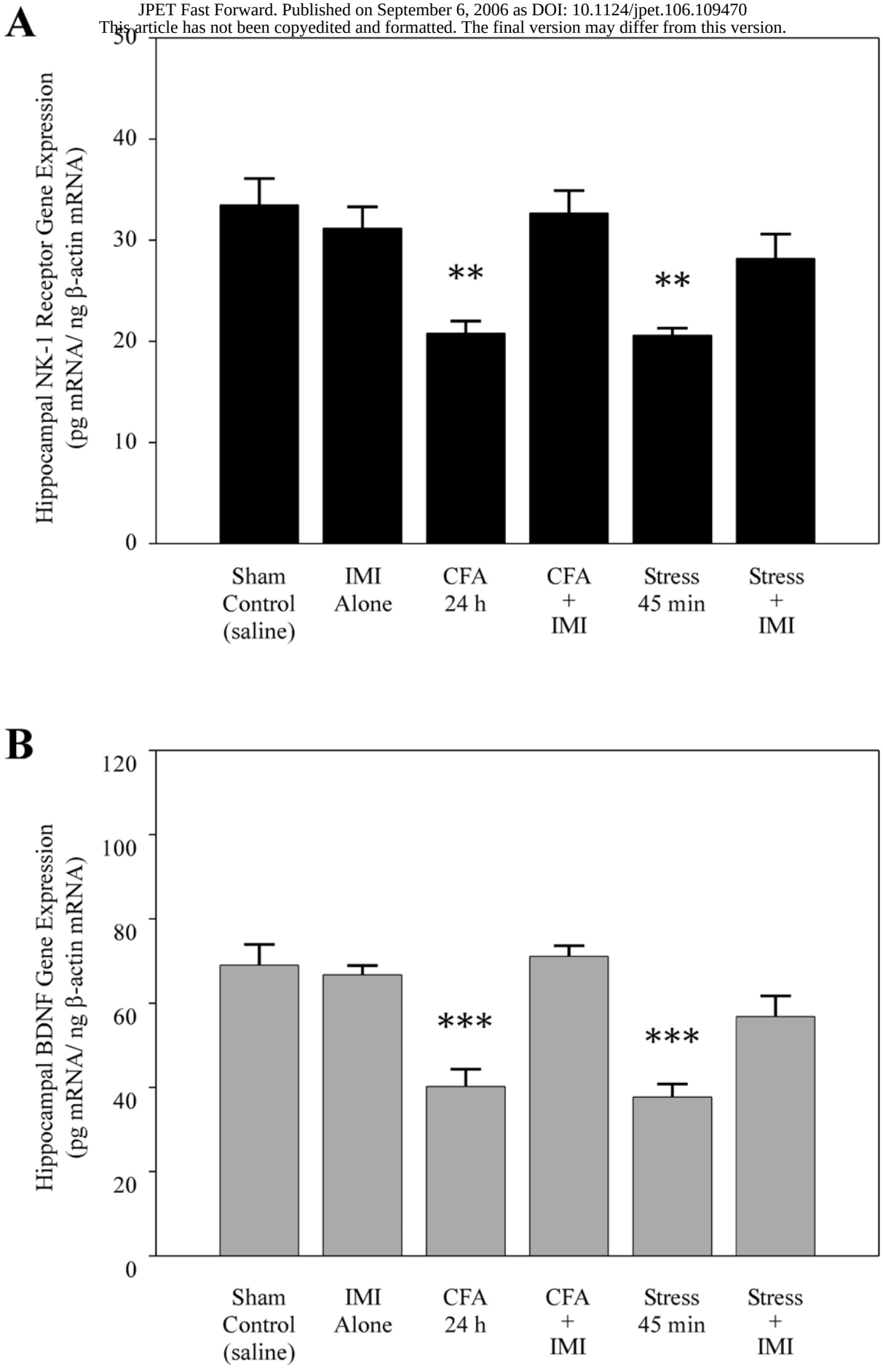


**Figure 7**

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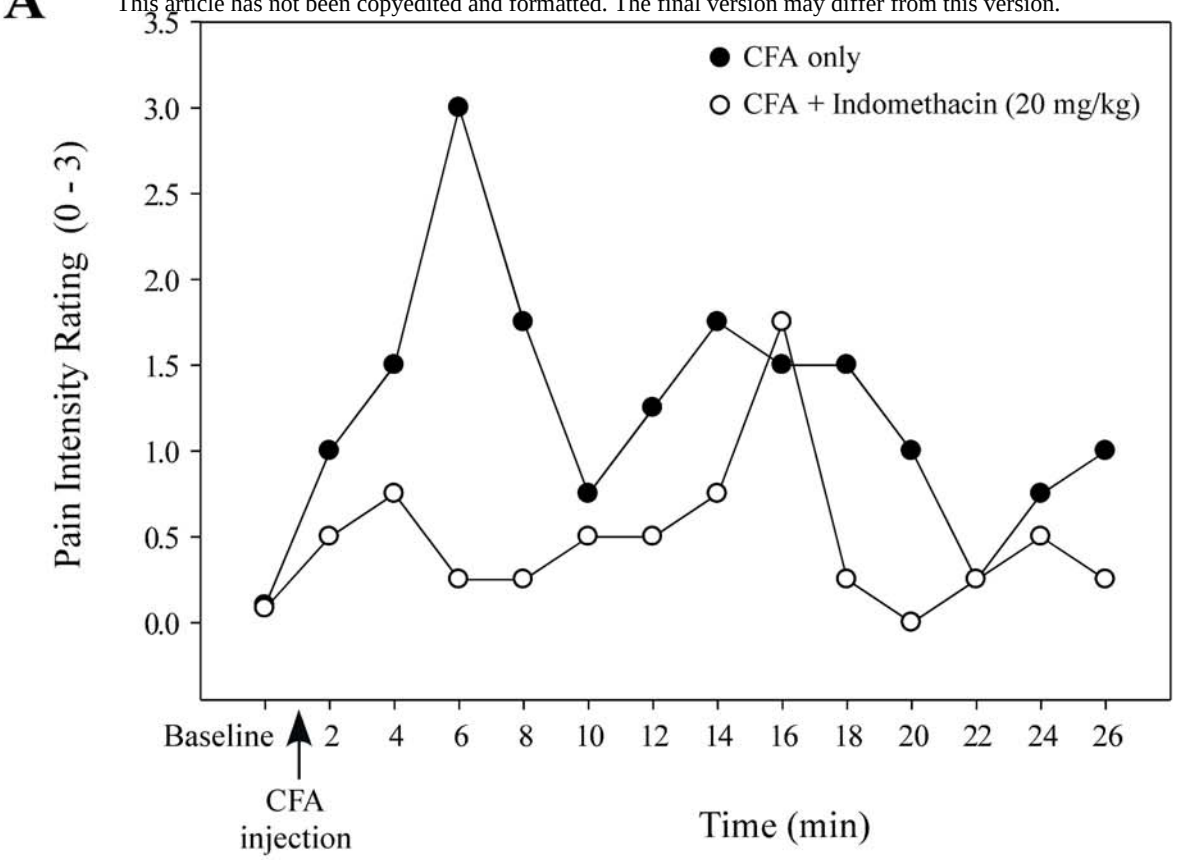


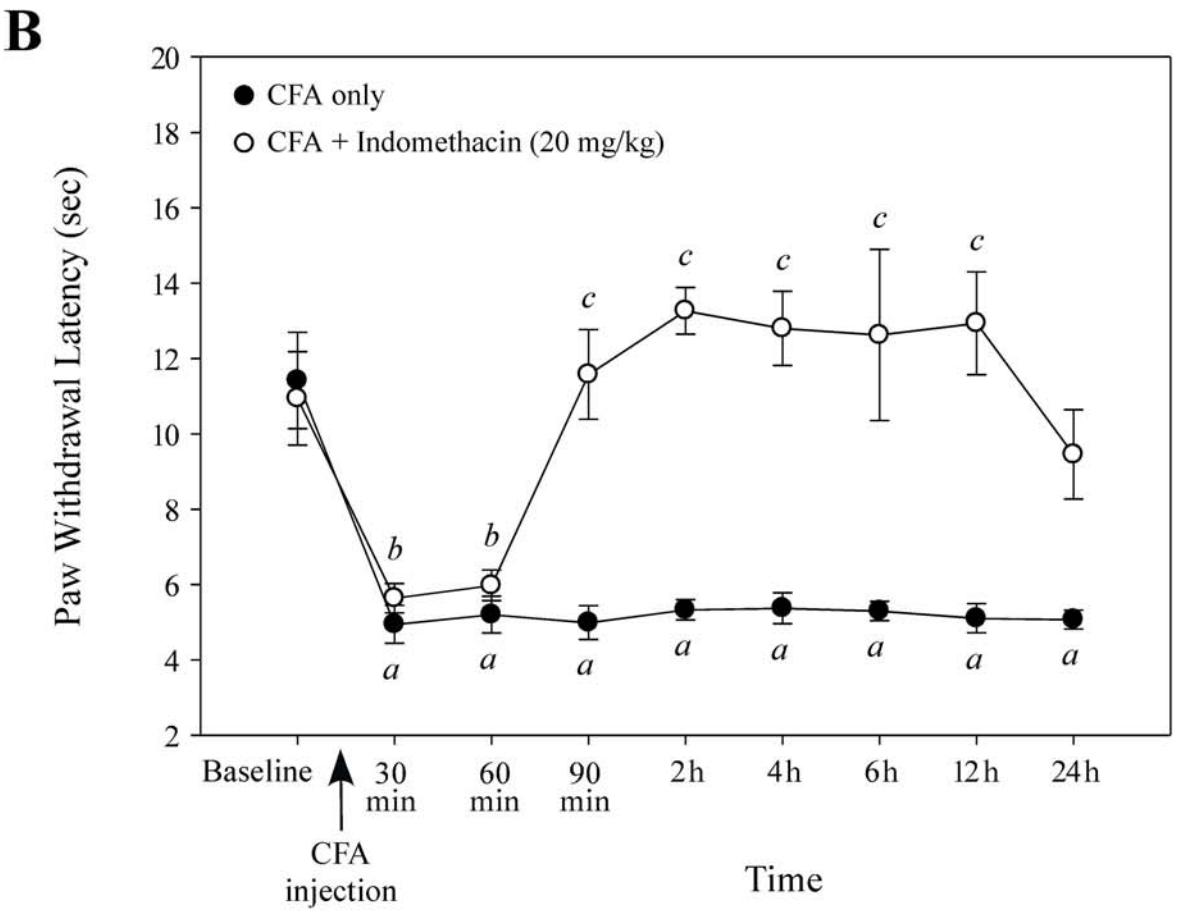


**Figure 8**