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Opiate Withdrawal-Induced Fos Immunoreactivity in the Rat Extended Amygdala Parallels the Development of Conditioned Place Aversion

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Low doses of naloxone have been shown to affect the motivational aspects of opiate withdrawal in morphine-dependent rats. Conditioned place aversion to opiate withdrawal is one of the most sensitive of motivational indices of opiate withdrawal and is thought to be mediated by the basal forebrain. Expression of the transcription factor Fos is known to increase during opiate withdrawal, but its presence during low-dose antagonist-precipitated withdrawal has not previously been established. In order to determine if there is a relationship between withdrawal-induced neuronal activity and conditioned place aversion, immunocytochemical localization of Fos was examined in the basal forebrain of opiate-dependent animals receiving one of several doses of naloxone (0, 3.25, 7.5, 15, 30, or 1000 μg/kg). In separate groups of opiate-dependent animals, naloxone doses of 3.25 - 30 μg/kg were paired with a specific chamber in a single-pairing conditioned place aversion paradigm. Significant increases in both immunocytochemical detection of Fos and conditioned place aversion were seen at doses ≥ 7.5 μg/kg. The shell of the nucleus accumbens and central nucleus of the amygdala were most sensitive to low doses, thus supporting the hypothesis that the extended amygdala plays a role in opiate-induced condition place aversion. ***[Neuropsychopharmacology 24:152–160, 2001]***

KEY WORDS: *Conditioned place aversion; Extended amygdala; Fos; Immunocytochemistry; Withdrawal; Opiate*

Chronic opiate use is partially characterized by the emergence of withdrawal symptoms upon cessation of drug administration. These symptoms include a well-characterized group of both physical and affective changes. In humans, affective symptoms often involve feelings of anxiety, restlessness, tension, irritability, and dysphoria (Haertzen and Hooks 1969; Henningfield et al. 1987; Jaffe 1990). Animal models of withdrawal include a number of characteristic somatic signs (Blasig et al. 1973; Gellert and Sparber 1977; Martin et al. 1963; Wei et al. 1975), as well as behavioral changes which are thought to reflect the affective symptoms associated with opiate withdrawal (Koob et al. 1993; Koob et al. 1989).

In opiate-dependent rats, affective or motivational signs of withdrawal are apparent at low doses of opiate antagonists at which physical signs of withdrawal are not seen (Higgins and Sellers 1994). Animals experiencing low-dose antagonist-precipitated opiate withdrawal show suppressed locomotor activity (Brady and Holtzman 1981) and operant responding for food (Gellert and Sparber 1977; Higgins and Sellers 1994; Koob et al. 1989), increased intracranial self-stimulation (ICSS) thresholds (Schaefer and Michael 1986), and aversion to

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the environment associated with withdrawal (Hand et al. 1988; Mucha 1987; Schulteis et al. 1994; Stinus et al. 1990). Conditioned place aversion is one of the most sensitive of these indices and can be shown in dependent animals administered doses of opiate antagonists well below those able to induce physical signs of withdrawal (Schulteis et al. 1994).

Many of the behaviors associated with the motivational aspects of opiate withdrawal have been shown to be modulated by certain structures of the basal forebrain known to be a part of the extended amygdala (Heinrichs et al. 1995; Koob et al. 1989; Stinus et al. 1990), notably the nucleus accumbens (Acb) and the central nucleus (ACe) of the amygdala (Alheid and Heimer 1988). Studies by Stinus et al. (1990) showed that both regions are involved in opiate withdrawal-induced conditioned place aversion.

The transcription factor c-fos is commonly used as a marker of neuronal activity. Several studies have shown that opiate withdrawal increases the expression of the c-fos protein (Fos) both in brain regions associated with the physical (Beckmann et al. 1995; Chieng et al. 1995; Hayward et al. 1990; Rohde et al. 1996; Stornetta et al. 1993) and motivational aspects of opiate dependence (Rasmussen et al. 1995; Stornetta et al. 1993). While the Acb and amygdala have been shown to have increased Fos levels during opiate withdrawal (Hayward et al. 1990; Rasmussen et al. 1995; Stornetta et al. 1993), these studies used doses of opiate antagonists that produce dramatic physical withdrawal signs. The induction of Fos in response to naloxone doses associated only with motivational signs of withdrawal has not previously been assessed.

The purpose of this study was to use both immunocytochemical and behavioral techniques to assess the effects of naloxone at doses that produce motivational but not physical signs of opiate withdrawal in opiate-dependent rats. The immunocytochemical localization of Fos was examined in regions of the extended amygdala of opiate-dependent animals over a range of naloxone doses. Fos immunoreactivity was seen throughout the extended amygdala at 15, 30, and 1000 μg/kg naloxone, but at 7.5 μg/kg, Fos immunoreactivity was seen primarily in the ACe. Conditioned place aversion was produced with naloxone doses of 7.5, 15, and 30 μg/kg. Neither conditioned place aversion nor Fos activation was seen with the 3.25 μg/kg dose. The results suggest that certain neuronal indices and behavioral measures of place aversion have similar sensitivities to naloxone during opiate withdrawal in opiate-dependent rats.

METHODS

Animals

Seventy-seven male Wistar rats (250-300 g at the start of the experiment) were housed three per cage in a 12 hour light/dark cycle vivarium. Lights went on at 10:00 AM, and all experiments were conducted during the rats' active (dark) cycle. The procedures conformed to the procedures established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Wistar rats were bred at the Beckman Laboratories of The Scripps Research Institute from a stock originally derived from Charles River (Kingston, NY). The animals were bred using a circular pair random system of breeding in order to maintain genetic heterogeneity, and new breeders were obtained from Charles River as determined by the internal Genetics Advisory Board.

Experiment 1: Fos Immunocytochemistry

Naloxone pretreatment. Thirty-four animals were implanted with either two subcutaneous morphine base pellets (75 mg [0.13 mmol] each) wrapped in nylon mesh (n = 23) or with two similarly prepared placebo pellets (n = 11). Subcutaneous saline injections (1 ml/kg) were given daily to all groups for two days. On the third day morphine-implanted animals were injected subcutaneously with saline or one of five doses of naloxone (3.25, 7.5, 15, 30, or 1000 μg/kg; Table 1). Placebo-implanted animals were injected subcutaneously with one of the three highest doses (15, 30, 1000 μg/kg) of naloxone. One hour after the injection, the animals were sacrificed by perfusion.

Tissue Preparation. Animals were anesthetized with 200 mg/kg sodium pentobarbitol (Nembutol® Abbott Laboratories, Chicago, IL) and then perfused transcardially through the ascending aorta with 10–20 mls saline followed by 200 mls of 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brains were removed and postfixed in the same fixative for 30 minutes. Forty μm thick sections were cut rostrocaudally through the extended amygdala using a vibrating microtome. The Paxinos and Watson atlas (1997) was used to identify and section the following regions: Acb (plates 11-15), BST (plates 17-20), and ACe (plates 26-31) (Paxinos and Watson 1997).

Immunocytochemical Labeling. Vibratome sections were processed for immunocytochemistry as described in Hsu et al. (1981). Briefly, the sections were rinsed in 0.1 M Tris-buffered saline (TBS), placed in 0.5% bovine serum albumin in 0.1 M TBS for 30 minutes, and then incubated in sheep anti-Fos antibody (Chemicon, Temecula, CA) diluted in 0.1 M TBS with 0.25% Triton-X for 24 hours at room temperature. The primary antibody was made against a synthetic 14 amino acid peptide conjugated to bovine thyroglobulin. Sections were rinsed three times in 0.1 M TBS and then incubated in biotinylated donkey anti-sheep secondary antibody (Jackson, Westgrove, PA; 1:200) for 30 minutes. After the tissue was rinsed again in 0.1 M TBS, it was placed

Table 1. Naloxone Pretreatment

		Fos Immunoreactivity		
Group	Pellet	Naloxone Treatment (μg/kg)	Rats/group	Tissue Sections Examined/Group
1	m	1000	4	4
2	m	30	4	4
3	m	15	4	4
4	m	7.5	4	4
5	m	3.75	4	4
6	m	0 (1ml/kg saline)	3	0
7	p	1000	3	4
8	p	30	4	0
9	p	15	4	0
Conditioned place aversion				
10	m	30	10	
11	m	15	10	
12	m	7.5	10	
13	m	3.75	10	

Abbreviations: *m = morphine pellet; p = placebo pellet.

for 30 minutes in avidin-biotin complex (Vector Laboratories Inc. Burlingame, CA). The sections then were rinsed in 0.1 M TBS several times. The peroxidase reaction product was visualized with a 6 minute incubation in 22 mg of 3-3′diaminobenzidine (Aldrich, St. Louis, MO) with 10 μl of 30% hydrogen peroxide in 100 mls of 0.1 M TBS. After successive washes in 0.1 M TBS and 0.1 M PB, the sections were transferred to 0.05 M PB and mounted on charged microscope slides (Fischer, Pittsburgh, PA). The slides were dehydrated through an ascending alcohol series, placed in Hemo-De (Fischer, Pittsburgh, PA) and coverslipped using Permount (Aldrich, St. Louis, MO). They then were examined and photographed on a Zeiss Axiophot microscope.

Control sections, in which the primary antibody was omitted, were processed as described above. In these sections, there was virtually no reaction product, suggesting that nonspecific labeling was minimal. Tissue within each treatment group was pooled. It was then processed in tandem with tissue from placebo-pelleted animals treated with the same naloxone dose and with at least two other treatment groups in order to minimize variations in immunocytochemical labeling.

Fos-immunoreactive cell nuclei were counted in the Acb shell, BST, and ACe of 4-6 randomly-chosen vibratome sections from the brains of morphine-dependent animals in each naloxone dose using visual reference to anatomical markers. The results were compared to the number of Fos-immunoreactive nuclei seen in 5-6 randomly chosen tissue sections from the extended amygdala of placebo-pelleted animals receiving the highest dose of naloxone (1000 μg/kg). Changes in Fos immunoreactivity induced by different doses of naloxone in the Acb, BST, and ACe were compared using a one-way analysis of variance (ANOVA), with dose as the repeated measure for each region. *Post hoc* comparisons using a Dunnet's test were made where main effects were observed.

Experiment 2: Conditioned Place Aversion

The place aversion conditioning apparatus is described in detail in Stinus et al. (1990) and consisted of three rectangular boxes (40 × 33 × 34 cm), each with distinctive visual (white, black and white striped, or dotted walls), tactile (rough, smooth, or semi-rough floors), and olfactory (acetic acid, anise, or none) cues. Boxes were spaced at 120° angles and accessible from a triangular central compartment. The compartments were equipped with photocells which recorded the animals' position at all times. A 11W red bulb located 144 cm above the apparatus provided illumination, and a white noise (70 dB) masked external noise.

Preconditioning. Sixty-eight rats were anesthetized with halothane and implanted subcutaneously with two morphine base pellets (75 mg [0.13 mmol] each) wrapped in nylon mesh. On the third day after pellet implantation, animals were placed in the central triangular compartment of the apparatus and allowed to explore all three compartments for 20 minutes. Animals that showed a strong unconditioned preference (more than 44% of the session time) or aversion (less than 17%) were eliminated from the study. This criterion eliminated 25 rats. For each remaining rat, the two compartments in which it spent the most similar amount of time were randomly assigned as the naloxone or the saline compartment. The pairing assignments were made so there was an equal number of animals experiencing naloxone-induced withdrawal in each of the three com-

partments. Animals were then randomly assigned to one of four dose groups (Table 1).

Conditioning. On day 4, half the animals received a subcutaneous saline injection (1 ml/kg) before they were confined in the compartment previously paired with saline for 20 minutes. The other half of the rats received a subcutaneous naloxone injection (3.25, 7.5, 15, or 30 μg/kg in 1 ml/kg saline) before they were confined in the compartment previously paired with naloxone for 20 minutes. On day 5, the animals received the opposite injection and pairing. The highest dose (1000 μg/kg) used in the Fos immunocytochemistry study was not tested in the conditioned place aversion study because of the strong withdrawal symptoms it induces (Schulteis et al. 1994).

Postconditioning Test. On the testing day (day 7), animals were allowed to freely explore all three arms of the apparatus for 20 minutes. The difference in the time spent in the naloxone-paired compartment during the testing phase versus the preconditioning phase served as an index of place aversion. The preconditioning and postconditioning test scores were compared using the non-parametric Wilcoxon matched-pairs signed-ranks test. Because of the need to perform multiple tests, the significance value was set at the $p < .02$ level.

RESULTS

Fos Immunoreactivity in the Extended Amygdala Is Induced by Low Doses of Naloxone in Morphine-Dependent Animals

Fos immunoreactivity in the basal forebrain of morphine-dependent rats appeared dose-dependent (Figure 1). In the Acb shell, ANOVA showed a main effect of dose for naloxone: $F(5,26) = 12$ ($p < .01$). A *post hoc* Dunnett's test showed significant increases in Fos-immunolabeled nuclei at doses 7.5, 30, and 1000 μg/kg naloxone compared to placebo-pelleted animals treated with the highest dose of naloxone ($p < .05$, Figures 1 and 2). In morphine-dependent animals, immunoreac-

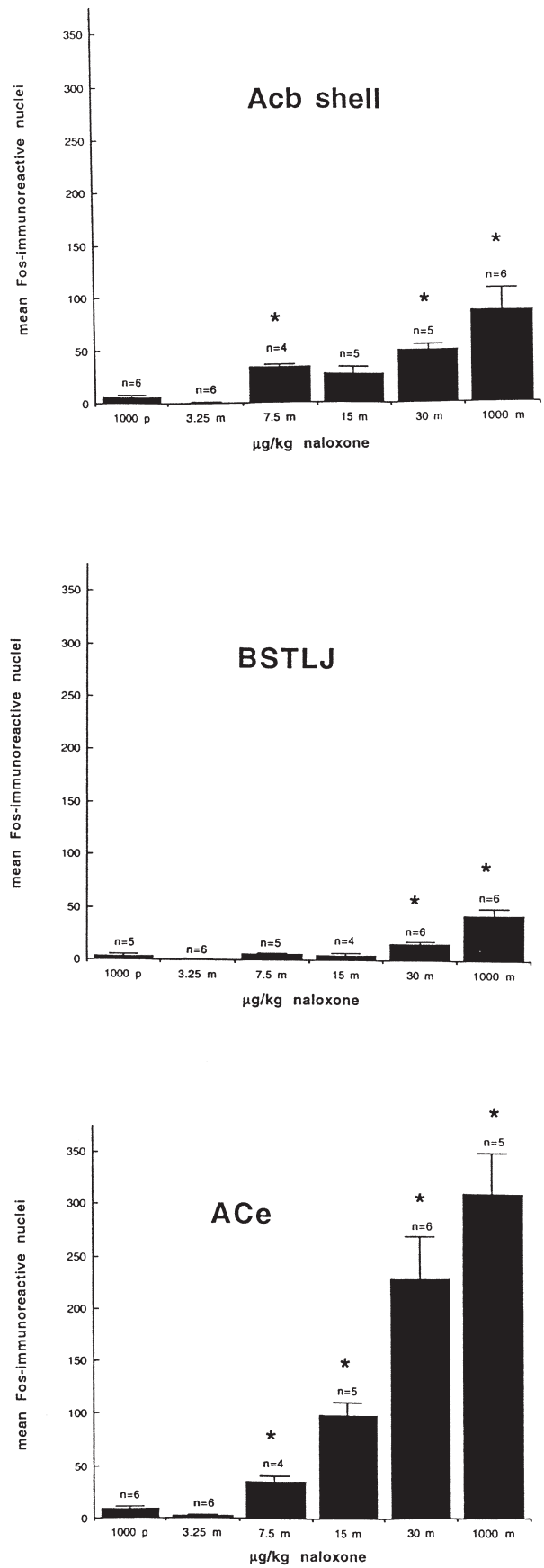

Figure 1. Bar graph showing the mean number of Fos-immunoreactive nuclei (±SEM) produced in the Acb shell, BSTLJ and ACe. ANOVA showed a main effect of dose in the Acb shell [$F(5,26) = 12$; $p < .01$], BSTLJ [$F(5,26) = 25$; $p < .01$], and ACe [$F(5,26) = 33$; $p < .01$]. Significant increases (*) in Fos-immunoreactive nuclei in tissue from morphine-pelleted animals (m) compared to that seen in tisse from placebo-pelleted animals (*p*) treated with the highest dose of naloxone were observed at naloxone doses 7.5, 30, and 1000 μg/kg in the Acb shell, =30 μg/kg in the BSTLJ and =7.5 μg/kg in the ACe ($p < .05$; Dunnett's).

tive nuclei were seen throughout the shell region of the Acb, while the core region contained very few Fos-labeled nuclei (Figure 2). The nearby septum also showed labeling at the three highest doses.

The BST appeared to be the region least sensitive to low doses of naloxone. There was a main effect of dose [$F(5,26) = 25$ ($p < .01$)], but only doses ≥ 30 μg/kg naloxone produce significant ($p < .05$, Dunnett's test) increase in Fos-labeled nuclei in morphine-pelleted animals compared to the 1000 μg/kg naloxone-treated, placebo-pelleted group (Figures 1 and 3). The immunoreactivity was seen specifically in the lateral region of the BST termed the lateral juxtacap (BSTLJ; Figure 3). While the highest dose of naloxone produced faint labeling of the ventral lateral BST, no other regions of the BST contained labeling at any other doses in tissue from morphine-dependent animals.

The most robust Fos immunoreactivity was seen in the ACe of morphine-pelleted animals (Figure 1). ANOVA revealed a main effect of naloxone dose: $F(5,26) = 33$ ($p < .01$). A *post hoc* Dunnett's test showed that doses of naloxone ≥7.5 μg/kg significantly ($p < .05$) increased Fos levels in morphine-pelleted animals compared to placebo-pelleted animals treated with 1000 μg/kg naloxone (Figures 1 and 4). No dose of naloxone produced substantial labeling in any other region of the amygdala complex. In the rest of the forebrain, only the paraventricular nucleus of the hypothalamus showed faint labeling in morphine-dependent animals treated with the highest dose of naloxone (1000 μg/kg).

There was virtually no Fos labeling seen in tissue from placebo-pelleted animals treated with 1000 (Figures 1, 2, 3, and 4), 30, or 15 μg/kg naloxone (data not shown). Lower doses of naloxone in placebo-pelleted animals were therefore not tested. Morphine-pelleted animals treated with saline vehicle rather than naloxone on day three also showed no Fos immunoreactivity in the basal forebrain (data not shown).

Conditioned Place Aversion Is Established by One Pairing with Low Doses of Naloxone in Morphine-Dependent Animals

A separate group of rats was tested in a conditioned place aversion protocol. After one pairing with 7.5, 15, or 30 μg/kg naloxone, animals spent significantly less time in the chamber where they had previously experienced naloxone-induced withdrawal ($p < .02$; Figure 5). The lowest dose of naloxone (3.25 μg/kg) did not induce significant place aversion after one pairing. At the highest dose (30μg/kg) some animals experienced diarrhea, but no other somatic signs of withdrawal were seen at this or lower doses.

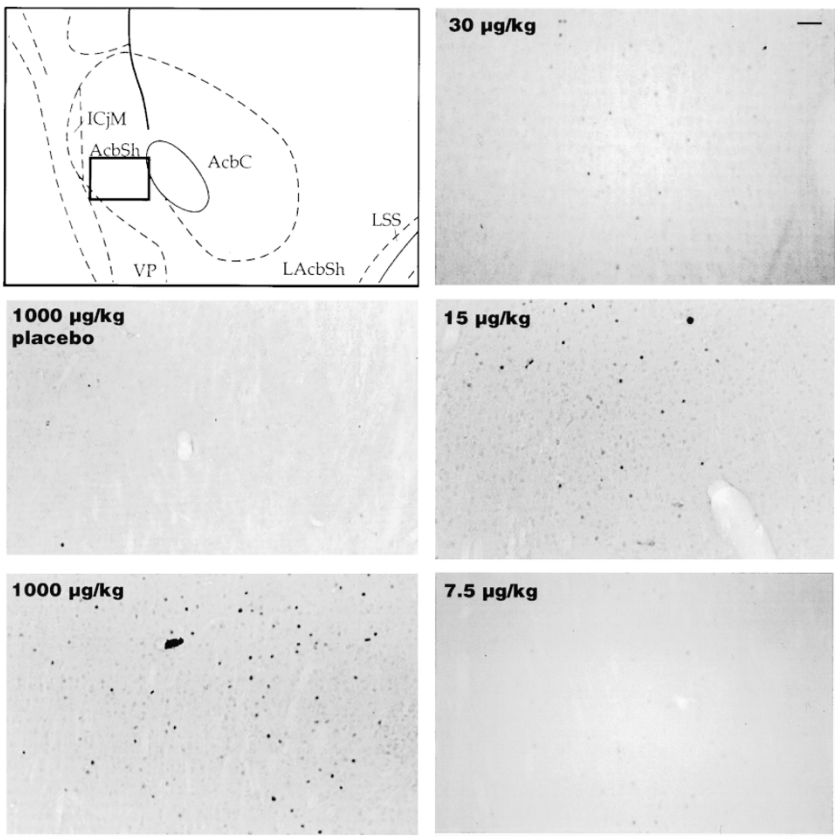

Figure 2. Light microscope photographs showing the distribution of Fos immunoreactivity in the Acb shell. The square shown in the atlas drawing represents the approximate area shown in the photographs. The panel labeled "1000 μg/kg placebo" shows tissue from placebo-pelleted animals treated with 1000 μg/kg of naloxone. All other panels show tissue from morphine-pelleted animals treated with the naloxone dose indicated. AcbSh = accumbens shell, AcbC = accumbens core. Bar =25 μm

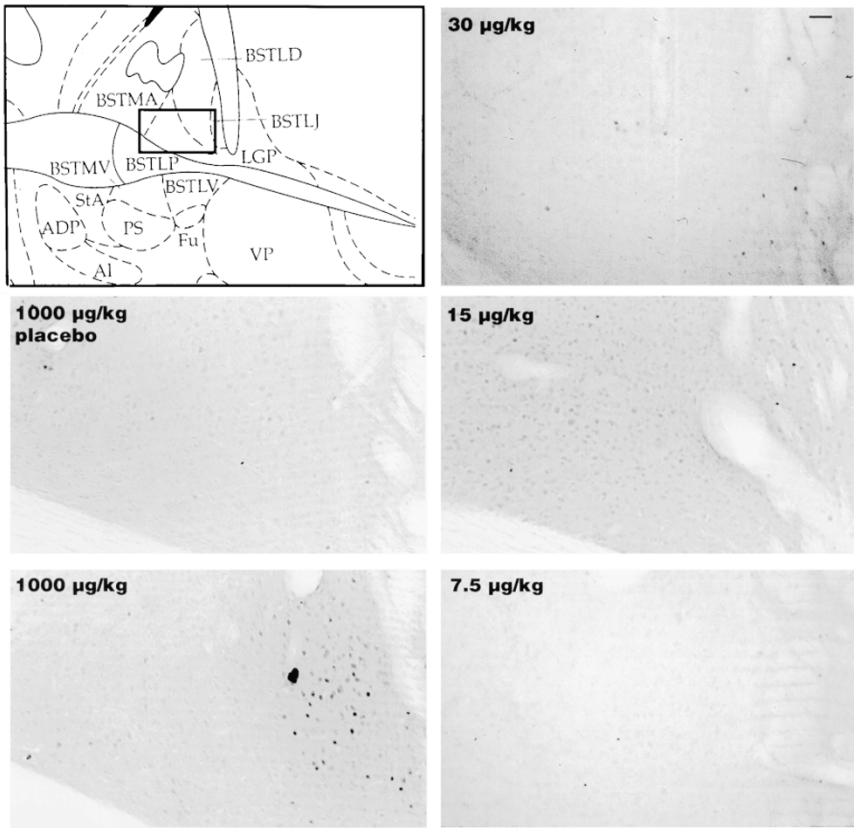

Figure 3. Light microscope photographs showing the distribution of Fos immunoreactivity in the BST. The square shown in the atlas drawing represents the approximate area shown in the photographs. The panel labeled "1000 μg/kg placebo" shows tissue from placebo-pelleted animals treated with 1000 μg/kg of naloxone. All other panels show tissue from morphine-pelleted animals treated with the naloxone dose indicated. Tissue from morphine-pelleted animals treated with 1000μg/kg naloxone showed Fos-labeled nuclei only in the bed nucleus of the stria terminalis, lateral juxtacap division (BSTLJ). Bar = 25 μm

DISCUSSION

Doses of naloxone that produce motivational but not somatic signs of withdrawal induced Fos in the basal forebrain of morphine-pelleted animals. Significant increases in both immunocytochemical detection of Fos and conditioned place aversion were seen at doses ⩾ 7.5 μg/kg. The fact that the Acb shell, BSTLJ and ACe appear robustly affected at these low doses supports the hypothesis that the extended amygdala plays a role in opiate-induced condition place aversion. These studies further establish Fos immunoreactivity as a sensitive indicator of neuronal activity in brain regions thought to be involved in motivational withdrawal.

Opiate withdrawal is known to cause induction of c-fos mRNA in the Acb and amygdala (Hayward et al. 1990; Rasmussen et al. 1995). Immunohistochemical localization of Fos protein in tissue from opiate withdrawn animals has been previously described in the amygdala (Stornetta et al. 1993) and the BNST (Aston-Jones et al. 1999; Delfs et al. 2000). The doses of opiate antagonist (1-100 mg/kg naltrexone) used in these studies, however, produced physical signs of withdrawal.

While the extended amygdala is rich in opiate receptors (George et al. 1994; Hiller et al. 1994), it is not thought to be highly involved in the expression of physical withdrawal from opiates (Maldonado et al. 1992). Instead, it appears to be sensitive to low doses of opiate antagonists which selectively produce motivational withdrawal signs (Stinus et al. 1990). The studies presented here establish the ability of naloxone to induce Fos in specific brain structures at doses producing motivational but not physical signs of withdrawal.

The reciprocal interconnections between the structures of the extended amygdala and its association with regions involved in emotion, aversion, stress and autonomic responses support the idea that the region is important in the modulation of the motivational component of opiate withdrawal. The Acb shell is considered distinct from the core region (Heimer et al. 1997a) and is thought to act as an interface with motor systems and emotional reactions (Heimer et al. 1997b). The BST projects to the Acb shell as well as to regions associated with autonomic processing such as the periaqueductal gray, parabrachial area, dorsal vagal complex and paraventricular nucleus of the hypothalamus (de Olmos et al. 1985). The BST is part of the continuum connecting the ACe to the Acb shell and the neurons there closely resemble those in the lateral region of the ACe (Alheid et al. 1995). Like the Acb shell and BST, the ACe connects not only to other structures within the extended amygdala but also sends axons to regions, such as the lateral hypothalamus and caudal brain stem (Alheid et al. 1995).

The extended amygdala was seen to be uniquely sensitive to low doses of opiate antagonists. Naloxone

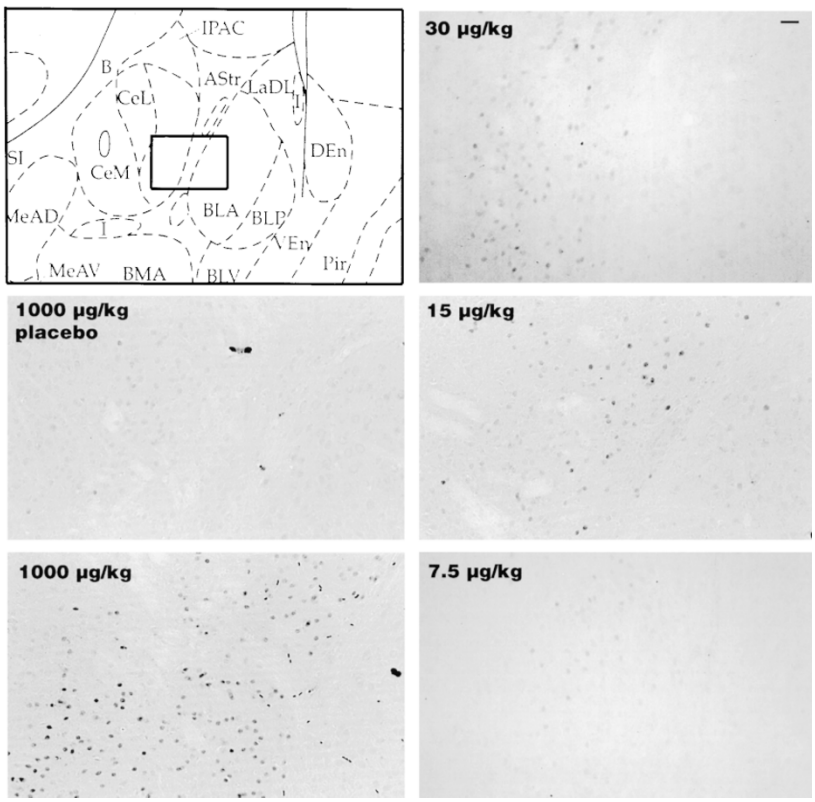

Figure 4. Light microscope photographs showing the distribution of Fos immunoreactivity in the ACe. The square shown in the atlas drawing represents the approximate area shown in the photographs. The panel labeled "1000 μg/kg placebo" shows tissue from placebo-pelleted animals treated with 1000 μg/kg of naloxone. All other panels show tissue from morphine pelleted animals treated with the naloxone dose indicated. BLA = basolateral amygdala, CeM = medial central nucleus, CeL = lateral central nucleus Bar = 25 μm

doses as low as 7.5μg/kg were able to significantly increase Fos-immunoreactive nuclei in the region. At the lowest dose, Fos was seen primarily in the Acb shell and ACe. The behavioral significance of these findings are supported by studies that used microinjection of opiate antagonists to establish the Acb and ACe as the brain regions most sensitive to conditioned place aversion (Stinus et al. 1990).

The fact that Fos was not seen in the Acb core, in medial regions of the BST or in amygdaloid nuclei other than the ACe suggests that opiate withdrawal induces neuronal activity in a specific pathway within the extended amygdala. Outside the extended amygdala, opiate withdrawal-induced Fos immunoreactivity was observed in the septum, suggesting that this structure may also be involved in motivational responses to opiate withdrawal. In contrast, none of the regions of the forebrain known to show increased Fos levels in response to high-dose opiate withdrawal (Stornetta et al. 1993) contained Fos labeling at these low doses. Only the paraventricular nucleus of the hypothalamus showed faint labeling in this study and only at the highest dose of naloxone (1000 μg/kg). This selective effect of Fos induction in the extended amygdala alone supports a causal role for this region in opiate-withdrawal induced conditioned place aversion.

Place aversion is one of the most sensitive behavioral indices of naloxone-precipitated withdrawal (Schulteis et al. 1994), and doses of naloxone as low as 4.0 μg/kg are capable of producing place aversion after three pairings (Schulteis et al. 1994). One pairing with an opiate antagonist, however, also has been shown to successfully produce conditioned place aversion (Heinrichs et al. 1995). In the present study, one naloxone pairing was used in order to replicate as closely as possible the immunocytochemical study, in which animals received one dose of naloxone before they were sacrificed. Place aversion was established with one pairing using subcutaneous doses of naloxone as low as 7.5, but not 3.25 μg/kg. As the study by Schulteis et al. (1994) showed that 4.0 μg/kg of naloxone but not 2.0 μg/kg could produce place aversion after three pairings, there appears to be little difference between the efficacy of one versus three pairings with naloxone.

The fact that both Fos immunoreactivity and conditioned place aversion are produced by naloxone doses ⩾ 7.5μg/kg in morphine-dependent rats, suggests a strong relationship between neuronal activation in regions of the extended amygdala and motivational signs of opiate withdrawal. Since the lowest naloxone dose able to produce conditioned place aversion produced Fos induction primarily in the Acb shell and ACe, these findings support other studies implicating the importance of these regions in the motivational aspects of opiate withdrawal.

Several different mechanisms may affect the component of opiate withdrawal mediated by the extended amygdala. Opiate withdrawal induces downregulation

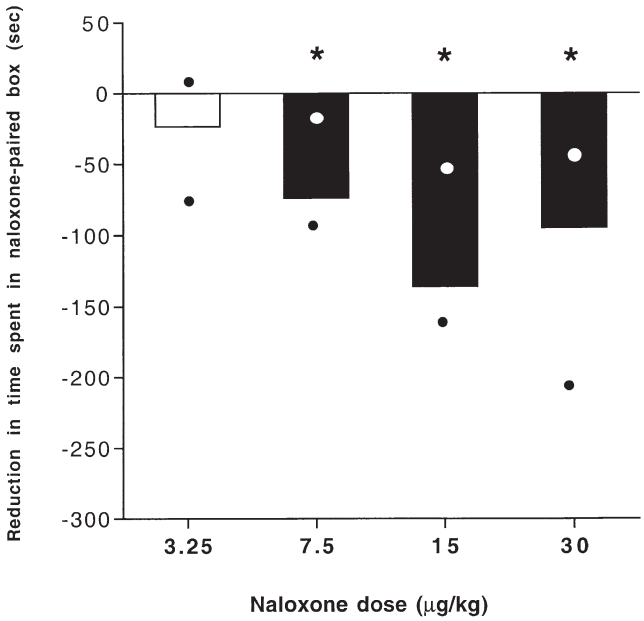

Figure 5. Bar graph showing place aversion produced by one pairing with naloxone in morphine-dependent rats. The values show the median difference between the time spent in the naloxone-paired chamber during postconditioning versus preconditioning. The dots represent the interquartile range of this distribution. The preconditioning and postconditioning test scores were compared using the non-parametric Wilcoxon matched-pairs signed-ranks test. Animals spent significantly (*) less time in the naloxone-paired chamber after one pairing with naloxone doses ⩾7.5 μg/kg ($p < .02$).

of corticotropin-releasing factor in both the nucleus Acb and amygdala (Iredale et al. 2000) and upregulation of prodynorphin in the Acb (Przewlocka et al. 1996). Noradrenergic agonists and glutamate receptor antagonists attenuate withdrawal symptoms when microinjected into the ACe (Taylor et al. 1998). Despite indications that the Acb and ACe may be most sensitive to low doses of opiate antagonists, the potential role of the BST should not be ignored. Recent studies have shown that systemic administration of noradrenergic antagonists decreases withdrawal-induced Fos in the BST. Furthermore, microinjections of noradrenergic antagonist into the lateral BST block withdrawal-induced place aversion (Aston-Jones et al. 1999; Delfs et al. 2000). Continued studies in the extended amygdala will further elucidate the mechanism of Fos activation and its role in the motivational aspects of opiate withdrawal.

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REFERENCES

Alheid G, de Olmos JS, Beltramino CA (1995): Amygdala and Extended Amygdala. In: Paxinos G (ed), The Rat Nervous System, 2nd edition. San Diego, Academic Press, pp 495–579

Alheid GF, Heimer L (1988): New perspectives in basal forebrain organization of special relevance for neurospychiatric disorders: The striatopallidal, amygdaloid and corticopetal components of substantia innominata. Neurosci 27:1–39

Aston-Jones G, Delfs JM, Druhan J, Zhu Y (1999): The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann NY Acad Sci 29:486–498

Beckmann AM, Matsumoto I, Wilce PA (1995): Immediate early gene expression during morphine withdrawal. Neuropharmacol 34:1183–1189

Blasig J, Herz A, Reinhold K, Zieglgansberger S (1973): Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia 33:19–38

Brady LS, Holtzman SG (1981): Locomotor activity in morphine-dependent and post-dependent rats. Pharmacol Biochem Behav 14:361–370

Chieng B, Keay KA, Christie MJ (1995): Increased fos-like immunoreactivity in the periawueductal gray of anaesthetised rats during opiate withdrawal. Neurosci Lett 183:79–82

de Olmos JS, Alheid GF, Beltramino CA (1985): Amygdala. In: Paxinos G (ed), The Rat Nervous System. New York, Academic Press, pp 223–334

Delfs JM, Zhu Y, Druhan JP, Aston-Jones G (2000): Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature 403:430–434

Gellert VF, Sparber SB (1977): A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. J Pharmacol Exp Ther 201:44–54

George SR, Zastawny RL, Briones-Urbina R, Cheng R, Nguyen T, Heiber M, Kouvelas A, Chan AS, O'Dowd BF (1994): Distinct distribution of mu, delta, and kappa opioid receptor mRNA in rat brain. Biochem Biophy Res Com 205:1438–1444

Haertzen CA, Hooks NT (1969): Changes in personality and subjective experience associated with the chronic administration and withdrawal of opiates. J Nerv Ment Dis 148:606–614

Hand TH, Koob GF, Stinus L, LeMoal M (1988): Aversive properties of opiate receptor blockade: Evidence for exclusively central mediation in naive and morphine-dependent rats. Brain Res 474:364–368

Hayward MD, Duman RS, Nestler EJ (1990): Induction of the c-fos proto-oncogene during opiate withdrawal in the locus coeruleus and other regions of the rat brain. Brain Res 525:256–266

Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS (1997a): The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci 9:354–381

Heimer L, Harlan RE, Alheid GF, Garcia MM, de Olmos JS (1997b): Substantia innominata: A notion which impedes clinical-anatomical correlations in neuropsychoatric disorders. Neurosci 76:957–1006

Heinrichs SC, Menzaghi F, Schulteis G, Koob GF, Stinus L (1995): Suppression of corticotropin releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. Behav Pharmacol 6:74–80

Henningfield JE, Johnson RE, Jasinski DR (1987): Clinical procedures for the assessment of abuse potential. In: Bozarth MA (ed), Methods of Assessing the Reinforcing Properties of Abused Drugs. New York, Springer-Verlag, pp 573–590

Higgins GA, Sellers EM (1994): Antagonist-precipitated opioid withdrawal in rats: Evidence for dissociations between physical and motivational signs. Pharmacol Biochem Behav 48:1–8

Hiller JM, Zhang Y, Bing G, Gioannini TL, Stone EA, Simon EJ (1994): Immunohistochemical localization of mu-opioid receptors in rat brain using antibodies generated against a peptide sequence present in a purified mu-opioid binding protein. Neurosci 62:829–841

Hsu S, Raine L, Fanger H (1981): Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comperison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Iredale PA, Alvaro JD, Lee Y, Terwilliger R, Chen YL, Duman RS (2000): Role of corticotropin-releasing factor receptor-1 in opiate withdrawal. J Neurohem 74

Jaffe JH (1990): Drug addiction and abuse. In: Gilman AG, Rall TW, Nies AS, Taylor P (eds), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 8th edition. New York, Pergamon Press, pp 522–573

Koob GF, Markou A, Weiss F, Schulteis G (1993): Opponent process and drug dependence: neurobiological mechanisms. Seminars Neurosci 5:351–358

Koob GF, Wall TL, Bloom FE (1989): Nucleus accumbens as a substrate for the aversive stimulus effects of opiate withdrawal. Psychopharm 98:530–534

Maldonado R, Stinus L, Gold LH, Koob GF (1992): Role of different brain structures in the expression of the physical morphine withdrawal syndrome. J Pharmacol Exp Ther 261:669–677

Martin WR, Wikler A, Eades CG, Pescor FT (1963): Tolerance to and physical dependence on morphine in rats. Psychopharmacologia 4:247–260

Mucha RF (1987): Is the motivational effect of opiate withdrawal reflected by common somatic indices of precipitated withdrawal? A place conditioning study in the rat. Brain Res 418:214–220

Paxinos G, Watson C (1997): The rat brain in stereotaxic coordinates, third edition. San Diego, Pubisher

Przewlocka B, Turchan J, Lason W, Przewlocki R (1996): The effect of single and repeated morphine administration on the prodynorphin system activity in the nucleus accumbens and striatum of the rat. Neurosci 70:749–754

Rasmussen K, Brodsky M, Inturrisi CE (1995): NMDA antagonists and clonidine block c-Fos expression during morphine withdrawal. Synapse 20:68–74

Rohde DS, Detweiler DJ, Basbaum AI (1996): Spinal cord mechanisms of opioid tolerance and dependence: Fos-like immunoreactivity increases in subpopulations of spinal cord neurons during withdrawal. Neurosci 72:233–242

Schaefer GJ, Michael RP (1986): Changes in response rates and reinforcement thresholds for intracranial self-stimulation during morphine withdrawal. Pharmacol Biochem Behav 25:1263–1269

Schulteis G, Markou A, Gold LH, Stinus L, Koob GF (1994): Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. J Pharmacol and Exp Ther 271:1391–1398

Stinus L, LeMoal M, Koob GF (1990): Nucleus accumbens and amygdala are possible substrates for the aversive stimulus effects of opiate withdrawal. Neurosci 37:767–773

Stornetta RL, Norton FE, Guyenet PG (1993): Autonomic areas of rat brain exhibit increased Fos-like immunoreactivity during opiate withdrawal in rats. Brain Res 624:19–28

Taylor JR, Punch LJ, Elsworth JD (1998): A comparison of the effects of clonidine and CNQX infusion into the locus coeruleus and the amygdala on naloxone-precipitated opiate withdrawal in the rat. Psychopharm 138: 133–142

Wei E, Loh HH, Way EL (1975): Application of the pellet implantation technique for the assessment of tolerance and physical dependence in the rodent. In: Ehrenpreis S and Neidle A (eds), Methods in Narcotics Research. New York, Marcel Dekker, pp 243–289