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Enhanced Morphine Preference Following Prolonged Abstinence: Association with Increased Fos Expression in the Extended Amygdala

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We previously found that chronically morphine-pretreated, abstinent rats show stronger preferences for morphine-associated environments than placebo-pretreated rats. Here we show that this increased preference persisted for at least 5 weeks after withdrawal of chronic morphine. To determine brain regions involved in this behavior, we examined neural activation (as indexed by Fos-like proteins) induced by a morphine-conditioned place preference test. Placebo-pretreated (P) morphine-conditioned rats showed significantly elevated Fos in the anterior cingulate cortex (Cg), nucleus accumbens core (Ac-C) and shell (Ac-S), ventral lateral and dorsal lateral bed nucleus of the stria terminalis (BNST-VL and -DL), and central and basolateral amygdala nuclei (ACE, ABL) when compared to nonconditioned P rats. Chronically morphine-pretreated (M) rats that exhibited enhanced morphine preference 5 weeks after morphine withdrawal showed significantly greater Fos in all the same areas except the BNST-DL relative to conditioned P or nonconditioned M rats. Place preference measures and Fos expression were positively correlated in the Cg and ABL, for conditioned P animals, and in the Cg, ABL and BNST-VL for conditioned M animals. These results indicate a relationship between place preference behavior and neural indices of activation in the forebrain in response to morphine-conditioned cues that may be chronically modulated by prior morphine exposure.

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INTRODUCTION

Drug addiction is a chronic disorder characterized by compulsive drug taking at the expense of other behaviors (Jaffe, 1990). Intense craving for the drug is a fundamental feature of addiction and is thought to trigger drug use and relapse. Environmental cues associated with drug taking behavior are potent stimulators of opiate craving in humans (Childress *et al*, 1986; Sell *et al*, 1999). A better understanding of brain mechanisms involved in producing drug craving would lead to better treatments for drug addiction.

Human brain imaging studies have shown that drug-associated cues strongly activate the anterior cingulate cortex (Cg), the amygdala and other basal forebrain regions (Childress *et al*, 1999; Sell *et al*, 1999). Environments associated with drug taking can strongly motivate drug-seeking behavior in human addicts (Childress *et al*, 1986; Wallace, 1989) and the conditioned place preference (CPP) paradigm is an animal model of this type of cue-elicited conditioning (Bardo *et al*, 1995; Tzschentke, 1998). In the CPP paradigm, drug preference is measured, in a drug-free state, by the amount of time an animal spends in an environment that has been previously associated with drug administration. A recent study by Schroeder *et al* (2000) reported that exposure to a morphine-paired environment significantly increased Fos expression in the prefrontal and cingulate cortices, nucleus accumbens (NAc) and the preoptic area of nondependent rats, findings that are consistent with the human imaging results.

Recently, we reported that chronically morphine-pretreated animals abstinent for 2 weeks before CPP testing exhibited significantly greater preference for morphine-paired environments than placebo-pretreated animals (Harris and Aston-Jones, 2001). The present study sought to determine whether this increased preference after chronic morphine exposure is long-lasting, and which brain regions are involved in this enhanced preference. For the latter, we used Fos protein expression as a marker of neuronal stimulation (Sharp *et al*, 1993; Herrera and Robertson, 1996).

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We focused our analysis of Fos induction on brain areas previously associated with conditioned or affective properties of opiates. The brain areas analyzed for this study were the anterior Cg, nucleus accumbens, bed nucleus of the stria terminalis (BNST), and amygdala. The Cg is an area consistently activated by drug cues in human cocaine and heroin addicts (Childress *et al*, 1999; Sell *et al*, 1999), and has been shown to be important for learning to associate environmental stimuli with cocaine administration in animals (Weissenborn *et al*, 1997). Although the basolateral amygdala is not necessary for the acquisition of cue-induced heroin seeking (Alderson *et al*, 2000), this area is important for cue-induced reinstatement of extinguished heroin-seeking behavior (Fuchs and See, 2002). Other studies found that the BNST is important for the reinforcing effects of opiates (Walker *et al*, 2000), while both the BNST and central nucleus of the amygdala (ACE) play roles in opiate withdrawal aversion (Delfs *et al*, 2000; Gracy *et al*, 2001). Neurons in the NAc appear to be critically involved in both the reinforcing properties of opiates (Dworkin *et al*, 1988; Zito *et al*, 1985) and opiate withdrawal responses (Harris and Aston-Jones, 1994). We also counted Fos-positive neurons in the periventricular nucleus of the thalamus (PVT) as a control region because it is interconnected with the NAc, ACE, ABL and BNST (Moga *et al*, 1995; Otake *et al*, 1995), but has not previously been linked to opiate abuse.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (200–250 g) from Harlan (Indianapolis, IN) were used in both experiments. Rats were group housed in accordance with NIH guidelines on a 12-h light/dark cycle with food and water available *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committees of the Philadelphia Veterans Administration Medical Center and the University of Pennsylvania. A total of 26 animals were used for the experiment reported here, with individual group numbers of six or seven animals.

Drugs

Morphine pellets and morphine sulfate powder were provided by the National Institute on Drug Abuse. Morphine sulfate was dissolved in sterile saline, and was administered via intraperitonal injection. All vehicle injections consisted of sterile saline.

Chronic Drug Treatment

Morphine pretreatment was carried out in 13 rats by subcutaneously implanting two 75-mg morphine tablets under halothane anesthesia. Placebo-pretreated rats ($n=13$) were implanted at the same time with placebo pellets. In this report, chronically morphine-pretreated animals are denoted by the abbreviation 'M', and all placebo-pretreated animals are denoted by the abbreviation 'P'. Previous studies have shown that morphine pellets are a reliable way to induce physical dependence (Gold *et al*, 1994; Yoburn *et al*, 1985). The signs of physical dependence wane by day 14 as the morphine pellets dissolve (Gold *et al*, 1994). Here, deprivation withdrawal, a model of abstinence, was induced by removing the pellets after 14 days.

Conditioned Place Preference Procedure

Training and testing occurred in a Plexiglas apparatus consisting of two distinct compartments ($37 \times 39 \times 30\,cm^3$) separated by a tunnel ($31 \times 11 \times 19\,cm^3$). One compartment had a grid floor with black walls, and the second compartment had a mesh floor with black and white stripes on the walls. Each compartment was equipped with photocells to automatically record time in each compartment (MED Associates, East Fairfield, VT). Rats were conditioned with acute injections of morphine in one of these environments beginning 4 weeks after pellet removal, with testing completed 5 weeks after the withdrawal from morphine. We choose to examine behavior and Fos expression after this period of abstinence, because by this time all morphine has been cleared from the body and all apparent withdrawal signs have dissipated. Also, it is a time period in which addicts trying to maintain abstinence are vulnerable to relapse. It has been estimated that 80% of addicted people will relapse by 1 month after treatment (Hunt *et al*, 1971; Vaillant, 1992). On the first day of the procedure (the preconditioning day), rats were allowed to freely explore all of the apparatus for 15 min, and the amount of time spent in each compartment was recorded. None of the animals had an initial bias for either compartment and rats were randomly assigned to one compartment for morphine conditioning in a balanced design. Conditioning began 5 days later. On each of the next 3 days, all to-be conditioned rats were injected with either saline or morphine (10 mg/kg i.p.) in the morning and afternoon. Rats were confined to one side of the box after each injection by means of an opaque Plexiglas divider for 30 min. The morphine and saline treatments were alternated in morning and afternoon sessions for every conditioned animal, so that rats given morphine in the morning were given saline in the opposite compartment in the afternoon, and on subsequent days received saline in the morning, and morphine in the afternoon. The morning and afternoon injections were at least 4 h apart. Five days after conditioning, a preference test was conducted, in which animals were given free access to the apparatus for 15 min. The amount of time spent in each compartment and the amount of activity were recorded. As a conditioning control, additional groups of P ($n=7$) and M animals ($n=7$) were given injections of saline in both compartments and morphine injections (10 mg/kg) in their home cages (1600 h).

Fos Protein Immunoreactivity

Two hours after the preference test animals were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with ice-cold 0.9% saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4. The brains were removed and stored overnight in 4% paraformaldehyde. They were

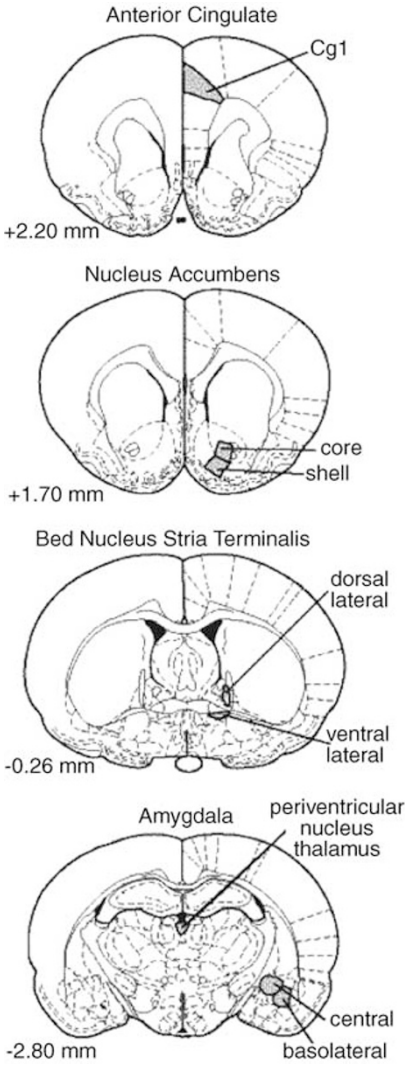

Figure 1 Schematic representations of regions analyzed for Fos-positive protein expression. Gray shaded regions indicate the areas where cells were counted. Numbers in the lower left-hand corner of each brain section represent the distance from bregma. Cg1 refers to anterior Cg area 1. Drawings were adapted from Paxinos and Watson (1998).

then transferred to a 20% sucrose solution and stored at 4°C for 5 days. Coronal sections (40 μm) were cut using a freezing microtome. Sections from P and M groups were processed together to equalize staining between groups. Sections were placed in a solution of 0.01 M PBS with 0.3% Triton-X added (PBS-Tx, pH 7.4) containing 2% normal donkey serum for 3 h. Sections were incubated overnight at room temperature in this same solution with the addition of primary antibody (rabbit antiserum against Fos-related antigens at 1:20,000, Oncogene Sciences, Cambridge, MA). Sections were rinsed three times in PBS-Tx and then incubated for 2 h with the secondary antibody (biotinylated donkey anti-rabbit 1:1000, Jackson Immunoresearch Laboratories, West Grove, PA). After three rinses in PBS-Tx, sections were transferred to an avidin–biotin complex (1:1000, Jackson Immunoresearch Laboratories) for 1.5 h. Sections were again rinsed two times with PBS-Tx and once with 0.05 M Tris buffer. Fos-related antigen-positive (denoted here as simply Fos) cells were visualized by placing the tissue in 3,3′-diaminobenzidine (DAB, 0.02%, Sigma, St Louis, MO) with 0.0002% H_2O_2 and 0.6% nickel ammonium sulfate in 0.01 M Tris buffer for 3.5 min. This reaction was arrested by immediate transfer into 0.05 M Tris buffer. The sections were mounted on gelatin-coated slides, stained with neutral red to identify specific structures, dehydrated through graded alcohols, cleared in xylene, and cover-slipped with Permount.

Quantification of Fos-positive cells was done using Openlab image processing software (Improvision, Ltd.; Coventry England) on a Macintosh computer that was linked to a microscope and digital camera. Color images of the areas of interest were taken and saved to a disk. The numbers of Fos-positive nuclei in regions of interest were counted with a point counter tool on the saved image. This tool simultaneously marked and counted each cell so that no cells could be counted twice and the total of the number of cells counted was available. One section at each level was randomly selected from each animal. The levels chosen corresponded to the following distances from bregma (Paxinos and Watson, 1998); anterior Cg (Cg, +2.20 mm), accumbens shell and core (Ac-S, Ac-C, +1.70 mm), ventral and dorsal lateral bed nucleus of the stria terminalis (BNST-VL, BNST-DL, −0.26 mm), amygdala central and basolateral nuclei (ACE, ABL −2.80 mm) and the periventricular nucleus of the thalamus (PVT −2.80 mm; Figure 1). Both the right and left sides were counted and averaged into a single score.

Data Analysis

Place conditioning data were analyzed by calculating the time spent in the morphine-paired chamber minus the time spent in the saline-paired chamber to determine chamber preference. The resulting difference score was compared

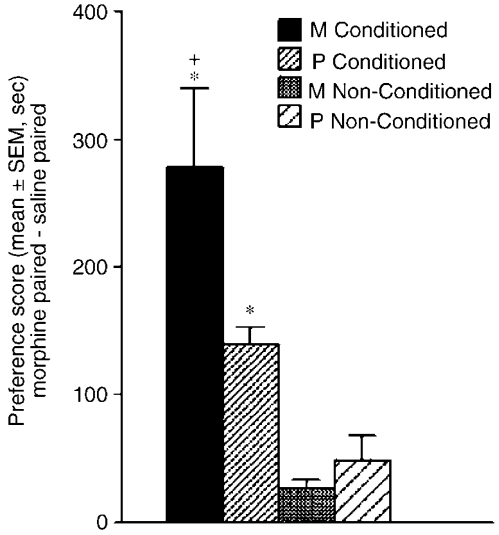

Figure 2 Preference scores for the morphine-paired environment expressed as the mean time in seconds spent in the morphine-paired side minus the mean time in seconds spent in the saline-paired side on the test day. *Significantly different ($p<0.01$) from non-conditioned groups; $^{+}$significantly different ($p<0.01$) from conditioned P groups.

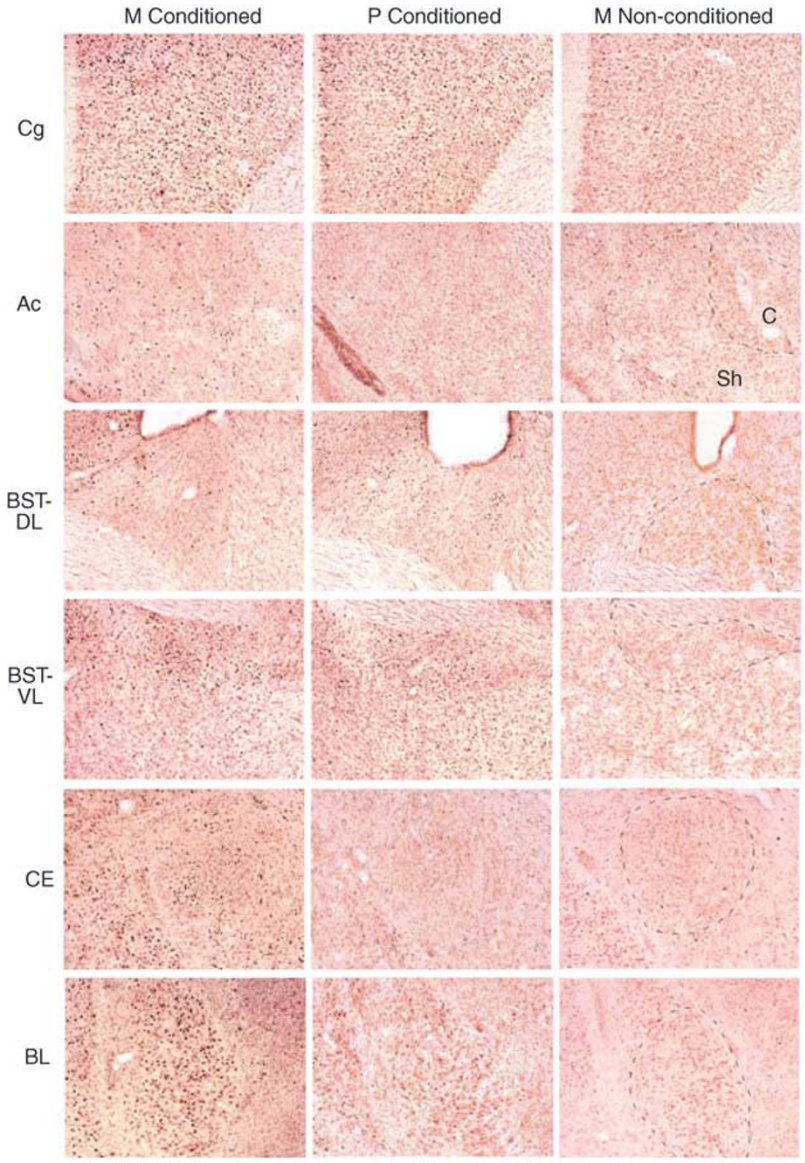

Figure 3 Photomicrographs of frontal sections showing Fos expression from representative sections from conditioned M, conditioned P and nonconditioned M groups. Sections compare the Cg, the nucleus accumbens (Ac) (C = core, Sh = shell), the dorsal lateral (BST-DL) and ventral lateral (BST-VL) BNST, and the central (CE) and basolateral (BL) amygdala. Sections are counter-stained with neutral red. Dashed lines in the M nonconditioned pictures delineate the approximate area of each structure. For all sections dorsal is up, for the Cg, accumbens, and amygdala sections medial is right and for the BNST sections medial is left.

between groups. In addition, a within-group measurement of conditioned place preference was assessed by comparing the difference in time spent in the morphine- and saline-paired side preconditioning *vs* postconditioning. Behavioral and Fos data were analyzed using a one-way or two-way analysis of variance. Where necessary, *post hoc* analysis was performed with a Newman–Keuls test.

RESULTS

Place Conditioning

As expected, our paradigm produced a significant preference for the morphine-paired environment in all conditioned rats ($p<0.01$; $F_{(1,10)}=73.14$ for P rats, and $F_{(1,10)}=101.65$ for M animals). Notably however, the preference for the morphine environment was greatly increased by morphine pretreatment 5 weeks prior to CPP testing. A two-way ANOVA (drug treatment × conditioning) revealed a significant main effect for drug treatment ($F_{(1,22)}=16.63$, $p<0.006$), conditioning ($F_{(1,22)}=184.06$, $p<0.006$), and an interaction between drug treatment and conditioning ($F_{(1,22)}=23.51$, $p<0.006$). Follow-up tests revealed that all conditioned groups were significantly different from non-conditioned groups ($p<0.01$), and that conditioned M rats showed enhanced preference for the morphine-paired environment relative to conditioned P rats ($p<0.01$, Figure 2). The nonconditioned P and M groups given saline injections paired with both compartments, plus morphine in the home cage, did not show significant place preferences ($p=0.12$; $F_{(1,24)}=2.4$ for P and M animals combined).

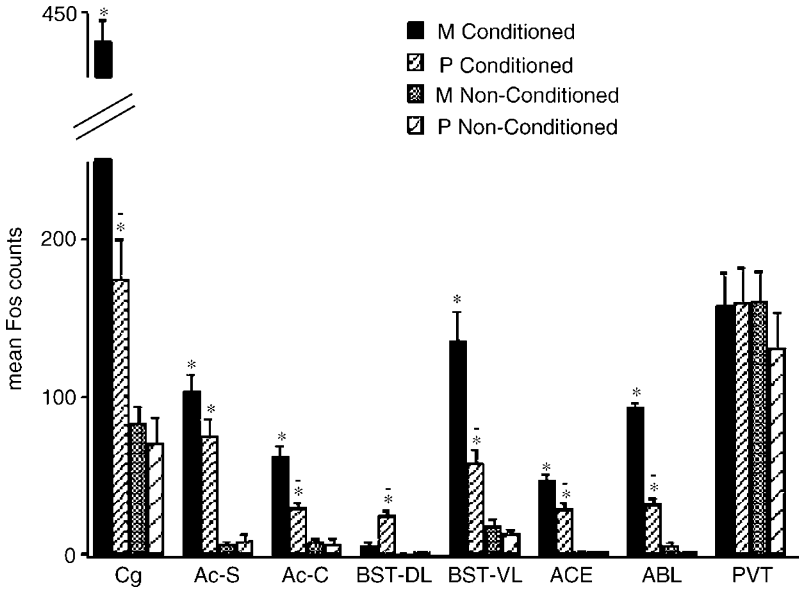

Figure 4 Number of Fos-positive nuclei (± SEM) counted in the Cg, Ac-S, Ac-C, BST-DL, BST-VL, ACE, ABL, PVT. *Significantly different ($p<0.01$) from nonconditioned groups; ¯significantly different ($p<0.05$) from conditioned M groups.

Fos Measurements

Figures 3 and 4 show the results for Fos immunoreactivity in the various brain regions. The results of two-way ANOVAS (similar to those performed for preference data above) on each brain area are presented in Table 1. All brain areas, except the PVT, showed a significant main effect for conditioning. Follow-up tests indicated that the conditioned P and M animals showed significantly more Fos than nonconditioned animals ($p<0.01$). Furthermore, the conditioned M group showed significantly higher Fos levels than either the conditioned or nonconditioned P groups in the Cg, Ac-C, BNST-VL, ACE and ABL ($p<0.01$ for each area, Figures 3 and 4). The conditioned P group exhibited significantly greater Fos than the nonconditioned groups in the CG, Ac-S, Ac-C BNST-VL, ACE, ABL, and significantly greater Fos levels than all groups (including the conditioned M group) in the BNST-DL ($p<0.01$). No significant changes were seen in the PVT of any group.

Correlations Between Preference Scores and Fos Expression

To determine the degree of association between the preference for the morphine environment and Fos induction, we calculated the Pearsons product-moment correlation coefficient (r) between the number of cells expressing Fos and the degree of preference expressed for each brain area in each group. Significant positive correlations (r values >0.72, df $=4$) were found in the Cg and ABL for the conditioned P group ($p<0.05$ each; Table 2). In the conditioned M group, significant positive correlations between preference and Fos staining were found in the Cg, ABL and BNST-VL (r values >0.81, $p<0.02$ each). Nonconditioned animals had no significant correlations.

DISCUSSION

This study found that M animals show significantly greater preferences for morphine-associated environments when tested 5 weeks after withdrawal compared to P animals. Conditioned M rats also showed significantly greater Fos expression in a number of limbic and cortical areas relative to conditioned P rats. Conditioned P rats showed significantly greater Fos expression in most of the same brain areas as the conditioned M group when compared to nonconditioned rats. Significant positive correlations were found between the levels of behavioral preference in conditioned groups and Fos staining in the Cg and ABL. Fos in the BNST-VL was also significantly correlated with preference only in the conditioned M groups.

The increased preference in the conditioned M animals was found at 5 weeks postwithdrawal and replicates our previous findings for animals tested at 2 weeks after withdrawal (Harris and Aston-Jones, 2001). It is noteworthy that the degree of preference was similar in these two

Table 1 Two-way ANOVA Results

Cg	drug tx: F = 14.61, cond: F = 74.54, cond × drug tx F = 11.09
Ac-S	cond: F = 112.19
Ac-C	drug tx: F = 16.57, cond: F = 94.54, cond × drug tx F = 14.86
BST-DL	drug tx: F = 31.66, cond: F = 62.54, cond × drug tx F = 24.31
BST-VL	drug tx: F = 16.23, cond: F = 63.92, cond × drug tx F = 12.58
ACE	drug tx: F = 9.23, cond: F = 145.36, cond × drug tx F = 8.1
ABL	drug tx: F = 126.65, cond: F = 426.28, cond × drug tx F = 99.04
PVT	NS

Results listed are significant at $p<0.006$, df = (1, 22). The probability level of 0.006 was determined using the Bonferroni correction for multiple comparisons. Cg = cingulate cortex, Ac-S = accumbens shell, Ac-C = accumbens core, BST-DL = dorsal lateral BNST, BST-VL = ventral lateral BNST, ACE = amygdala central nucleus, ABL = amygdala basolateral nucleus, PVT = periventricular nucleus of the thalmus. The factors were pretreated with morphine or placebo pellets (drug tx) and conditioning *vs* non conditioning (cond).

Table 2 Correlation results

	Cg	Ac-S	Ac-C	BST-DL	BST-VL	ABL	ACE	PVT
M conditioned	0.88*	0.12	0 0.53	−0.01	0.84*	0.89*	−0.57	−0.06
P conditioned	0.77*	0.65	0 0.24	−0.26	0.01	0.81*	0.54	0.09
M nonconditioned	0.21	0.08	0 0.14	0.10	0.04	0.01	0.17	0.05
P nonconditioned	0.20	0.11	−0.25	0.05	0.07	0.14	0.09	0.01

M and P refer to morphine and placebo pretreated animals, respectively. *$p < 0.05$.

studies, indicating that enhanced preference had not decreased by 5 weeks postwithdrawal and that it is a long-lasting effect. Stress-induced reinstatement of heroin self-administration also follows a similar time course, becoming greater at 6 and 12 days postwithdrawal (Shavlev *et al*, 2001).

Others have also reported that preference scores increase following prolonged exposure to morphine (Lett, 1989; Contarino *et al*, 1997). However, those studies did not examine preference following prolonged drug abstinence. In addition, unlike our study in which a continuous-release morphine pellet was used, the prior reports used a daily injection regimen that is known to produce substantial locomotor sensitization (Vanderschuren *et al*, 1997). It is interesting that both modes of morphine administration produced similar results for enhanced preference following prior morphine administration. This indicates that withdrawal from opiates may be an important factor in generating enhanced preference to opiates, whether it occurs repeatedly as in the case of daily injections or just once following a continuous exposure to opiates.

Our results indicate that prior morphine exposure produces a prolonged increase in preference for morphine-associated stimuli, and furthermore, that prior withdrawal may increase the incentive value of opiates (see Hutcheson *et al*, 2001). Other experiments using the place preference paradigm have shown that morphine-pretreated animals develop a place preference at lower doses than nonmorphine-pretreated animals, suggesting sensitization to the rewarding properties (Shippenberg *et al*, 1996, 1998). The enhanced preference seen in the M groups in our study may be because of sensitization to morphine's rewarding effects which is reflected by changes in neuronal excitability and subsequent Fos production. At 5 weeks postwithdrawal, place preference testing resulted in significantly greater Fos protein induction in the Cg, Ac-C, BNST-VL, ACE and ABL relative to conditioned P rats or nonconditioned M rats. In the Ac-S region, levels of Fos protein in conditioned M rats were significantly elevated relative to nonconditioned animals, but not in relation to conditioned P animals. Thus, conditioning, but not morphine exposure, may be an important factor in stimulation of these neurons.

Conditioned P animals showed significantly greater preference for the morphine-paired environment and significantly greater expression of Fos protein in the Cg, accumbens, BNST, and amygdala areas relative to nonconditioned rats. The conditioned P animals showed an elevation of Fos in the BNST-DL that was not found in the conditioned M animals. This suggests that the chronic morphine treatment may have affected activity in this region. In support of this idea, Walker *et al* (2000) reported that naloxone microinjections into this region of the BNST blocked heroin self-administration in dependent, but not nondependent, animals, indicating that responses in this area may be altered by the chronic morphine pretreatment.

Our results indicate that the increased preference and elevated Fos protein in the conditioned groups were the result of conditioning and not simply because of the morphine treatments. The nonconditioned animals given nonpaired exposures to both morphine and the conditioning chambers did not show a preference for either chamber or increased Fos expression on the test day. Furthermore, the elevations in Fos found in the conditioned groups cannot be explained by differences in locomotor activity because all groups showed similar activity counts on the test day.

Our results are consistent with earlier reports showing that drug-related cues elicit increased Fos expression in the brain (Brown *et al*, 1992; Crawford *et al*, 1995; Franklin and Druhan, 2000; Neisewander *et al*, 2000; Schroeder *et al*, 2000). Those studies found conditioned Fos induction in many of the same cortical and limbic brain areas as in the present study. These data suggest a common neuroanatomical pathway for the ability of cues associated with different addictive drugs to activate the brain. However, no previous study has examined the impact of prior dependence on subsequent conditioned Fos expression.

The changes we found in conditioned Fos protein expression in the anterior cingulate, nucleus accumbens, amygdala, and BNST suggest that these regions may play a role in the expression of morphine-conditioned behavior. Although most of these areas (Cg, accumbens, and amygdala) are not necessary for the acquisition of morphine-rewarded responding (Olmstead and Franklin, 1997a, b; Alderson *et al*, 2000), the finding that Fos expression in these regions was particularly increased in M animals indicates that these areas may be involved in the long-lasting effects of addiction on behavior, especially the expression of preference for drug-associated cues.

Conditioned M animals spent more time (an average of 2.5 min) in the morphine-paired environment than the conditioned P group. This difference may have contributed to the higher Fos levels seen in the M group. However, this explanation for the differences in Fos levels between the M and P groups is unlikely. In a recent study by Schroeder *et al* (2000), animals were confined to a previously morphine-paired environment prior to Fos measurements. Increased Fos expression was found in the prefrontal and anterior cingulate cortices, nucleus accumbens, and preoptic area. Unlike our study, however, they did not find conditioned Fos activation in the amygdala or BNST, and the Fos induction in the anterior cingulate was at a more posterior level than in our study. This indicates that the

activation of these areas by our paradigm may be because of the fact that CPP elicits an operant approach response, and not because of the amount of time spent in the CS+ environment.

The amount of preference strongly correlated with Fos levels in the ABL and Cg. These two areas showed significant correlations in all conditioned groups. The BNST-VL was the one area where a significant positive correlation between Fos and behavior was found only in the M animals, indicating that this area may be particularly involved in the increased preference seen in the M animals.

Recent imaging studies in humans have paralleled the preclinical findings in animal studies. Cocaine- (Childress *et al*, 1999; Maas *et al*, 1998; Grant *et al*, 1996) or morphine-associated cues (Sell *et al*, 1999) that induced high levels of drug craving in drug-experienced subjects also only elicited brain activation in the amygdala and anterior Cg of these subjects. The involvement of the amygdala and Cg in response to conditioned drug cues in our study and others is consistent with the wealth of evidence supporting their role in both Pavlovian conditioning (Hatfield *et al*, 1996; Bussey *et al*, 1997) and cue-elicited drug-seeking behavior (Whitelaw *et al*, 1996; Meil and See, 1997; Weissenborn *et al*, 1997; Everitt *et al*, 2000). Although the amygdala may not be involved in the acquisition of morphine-conditioned responding (Alderson *et al*, 2000), it is involved in the reinstatement of extinguished opiate seeking (Fuchs and See, 2002). This latter finding is particularly relevant to our study.

Chronic morphine alters second messenger systems (Collier, 1980; Nestler *et al*, 1993; Rasmussen *et al*, 1990; Terwilliger *et al*, 1991) and expression of intermediate early genes like *c-fos* (Curran *et al*, 1996; Nye and Nestler, 1996; Erdtmann-Vourliotis *et al*, 1999; Frankel *et al*, 1999). Changes in gene expression could be involved in alterations within the CNS that lead to drug addiction. For example, Fos induction in the accumbens has been found to be necessary for the acquisition but not expression of morphine-induced CPP (Tolliver *et al*, 2000). Thus, Fos itself may not play a large role in the immediate expression of preference, but may instead be involved in associated plasticity, for example maintenance or extinction of the conditioning.

Genes like *c-fos* not only serve as markers of activation but also act as transcription factors that regulate the expression of other genes. Determining brain areas where gene expression is altered following drug dependence could reveal mechanisms for long-term changes in CNS function associated with addiction. Such insights would be valuable for designing new treatments for opiate addiction.

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REFERENCES

Alderson HL, Robbins TW, Everitt BJ (2000). The effects of excitotoxic lesions of the basolateral amygdala on the acquisition of heroin-seeking behavior in rats. *Psychopharmacology* **153**: 111–119.

Bardo MT, Rowlett JK, Harris MJ (1995). Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev* **19**: 39–51.

Brown EE, Robertson GS, Fibiger HC (1992). Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *J Neurosci* **12**: 4112–4121.

Bussey TJ, Everitt BJ, Robbins TW (1997). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavolvian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav Neurosci* **111**: 908–919.

Childress AR, McLellan AT, O'Brien CP (1986). Conditioned responses in a methadone population. A comparison of laboratory, clinic, and natural settings. *J Subst Abuse Treat* **3**: 173–179.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999). Limbic activation during cue-induced cocaine craving. *Am J Psychiatry* **156**: 11–18.

Collier HO (1980). Cellular site of opiate dependence. *Nature* **283**: 625–629.

Contarino A, Zanotti A, Drago F, Natolino F, Lipartiti M, Giusti P (1997). Conditioned place preference: no tolerance to the rewarding properties of morphine. *Naunyn Schmiedebergs Arch Pharmacol* **355**: 589–594.

Crawford AC, McDougall SA, Bolanos CA, Hall S, Berger SP (1995). The effects of the kappa agonist U-50,488 on cocaine-induced conditioned and unconditioned behaviors and fos immunoreactivity. *Psychopharmacology* **120**: 392–399.

Curran EJ, Akil H, Watson SJ (1996). Psychomotor stimulant- and opiate-induced *c-fos* mRNA expression patterns in the rat forebrain: comparisons between acute drug treatment and a drug challenge in sensitized animals. *Neurochem Res* **21**: 1425–1435.

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.

Dworkin S, Guerin GF, Goeders NE, Smith JE (1988). Kainic acid lesions of the nucleus accumbens selectively attenuate morphine self-administration. *Pharmacol, Biochem Behav* **29**: 175–181.

Erdtmann-Vourliotis M, Mayer P, Linke R, Riechert U, Hollt V (1999). Long-lasting sensitization towards morphine in motoric and limbic areas as determined by *c-fos* expression in rat brain. *Brain Res Mol Brain Res* **72**: 1–16.

Everitt BJ, Cardinal RN, Hall J, Parkinson JA, Robbins TW (2000). Differential involvement of amygdala subsystems in appetative conditioning and drug addiction. In: Aggleton JP (ed). *The Amygdala: A Functional Analysis*. Oxford University Press, Oxford, pp 353–390.

Frankel PS, Harlan RE, Garcia MM (1999). Chronic administration of morphine alters immediate–early gene expression in the forebrain of post-dependent rats. *Brain Res* **835**: 204–212.

Franklin TR, Druhan JP (2000). Expression of Fos-related antigens in the nucleus accumbens and associated regions following exposure to a cocaine-paired environment. *Eur J Neurosci* **12**: 2097–2106.

Fuchs RA, See RE (2002). Basolateral amygdala inactivation abolishes conditioned stimulus- and herion-induced reinstatement of extinguished herion-seeking behavior in rats. *Psychopharmacology* **160**: 425–433.

Gold LH, Stinus L, Inturrisi CE, Koob GF (1994). Prolonged tolerance, dependence and abstinence following subcutaneous morphine pellet implantation in the rat. *Eur J Pharmacol* **253**: 45–51.

Gracy KN, Dankiewicz LA, Koob GF (2001). Opiate withdrawal-induced fos immunoreactivity in the rat extended amygdala parallels the development of conditioned place aversion. *Neuropsychopharmacology* **24**: 152–160.

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C *et al* (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proc Natl Acad Sci USA* **93**: 12040–12045.

Harris GC, Aston-Jones G (1994). Involvement of D2 dopamine receptors in the nucleus accumbens in the opiate withdrawal syndrome. *Nature* **371**: 155–157.

Harris GC, Aston-Jones G (2001). Augmented accumbal serotonin levels decrease the preference for a morphine associated environment during withdrawal. *Neuropsychopharmacology* **24**: 75–85.

Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996). Neurotoxic lesions of the basolateral, but not central amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. *J Neurosci* **16**: 5256–5265.

Herrera DG, Robertson HA (1996). Activation of c-Fos in the brain. *Prog Neurobiol* **50**: 83–107.

Hunt WA, Barnett LW, Branch LG (1971). Relapse rates in addiction programs. *J Clin Psychol* **27**: 455–456.

Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A (2001). The role of withdrawal in heroin addiction: enhances reward or promotes avoidance?*Nat Neurosci* **4**: 943–947.

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*. Pergamon, New York, pp 522–573.

Lett BT (1989). Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology* **98**: 357–362.

Maas LC, Lukas SE, Kaugman MJ, Weiss RD, Daniels SL, Rodgers VW *et al* (1998). Functional magnetic resonance imaging of human brain activation during cue-induced cocaine craving. *Am J Psychiatry* **155**: 124–126.

Meil WM, See RE (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behav Brain Res* **87**: 139–148.

Moga MM, Weis RP, Moore RY (1995). Efferent projections of the paraventricular thalamic nucleus in the rat. *J Comp Neurol* **359**: 221–238.

Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LTL, Palmer A, Marshall JF (2000). Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J Neurosci* **20**: 798–805.

Nestler EJ, Hope BT, Widnell KL (1993). Drug addiction: a model for the molecular basis of neural plasticity. *Neuron* **11**: 995–1006.

Nye HE, Nestler EJ (1996). Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. *Mol Pharmacol* **49**: 636–645.

Olmstead MC, Franklin KB (1997a). The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. *Behav Neurosci* **111**: 1324–1334.

Olmstead MC, Franklin KB (1997b). The development of a conditioned place preference to morphine: effects of lesions of various CNS sites. *Behav Neurosci* **111**: 1313–1323.

Otake K, Ruggiero DA, Nakamura Y (1995). Adrenergic innervation of forebrain neurons that project to the paraventricular thalamic nucleus in the rat. *Brain Res* **697**: 17–26.

Paxinos G, Watson C (1998). *The Rat Brain In Stereotaxic Coordinates*. Academic: San Diego.

Rasmussen K, Beitner-Johnson DB, Krystal JH, Aghajanian GK, Nestler EJ (1990). Opiate withdrawal and the rat locus coeruleus: behavioral, electrophysiological, and biochemical correlates. *J Neurosci* **10**: 2308–2317.

Schroeder BE, Holahan MR, Landry CF, Kelley AE (2000). Morphine-associated environmental cues elicit conditioned gene expression. *Synapse* **37**: 146–158.

Sell LA, Morris J, Bearn J, Frackowiak RSJ, Friston KJ, Dolan RJ (1999). Activation of reward circuitry in human opiate addicts. *Eur J Neurosci* **11**: 1042–1048.

Sharp FR, Sagar SM, Swanson RA (1993). Metabolic mapping with cellular resolution: *c-Fos vs* 2-deoxyglucose. *Crit Rev Neurobiol* **7**: 205–228.

Shavlev U, Morales M, Hope B, Yap J, Shaham Y (2001). Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology* **156**: 98–107.

Shippenberg TS, Heidbreder C, LeFevour A (1996). Sensitization to the conditioned rewarding effects of morphine: pharmacology and temporal characteristics. *Eur J Pharmacol* **299**: 33–39.

Shippenberg TS, LeFevour A, Thompson AC (1998). Sensitization to the conditioned rewarding effects of morphine and cocaine: differential effects of the kappa-opioid receptor agonist U69593. *Eur J Pharmacol* **345**: 27–34.

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ (1991). A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res* **548**: 100–110.

Tolliver BK, Sganga MW, Sharp FR (2000). Supression of *c-fos* induction in the nucleus accumbens prevents acquisition but not expression of morphine-conditioned place preference. *Eur J Neurosci* **12**: 3399–3406.

Tzschentke TM (1998). Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol* **56**: 613–672.

Vaillant GE (1992). Is there a natural history of addiction?*Res Publ Assoc Nerv Ment Dis* **70**: 41–57.

Vanderschuren LJ, Tjon GH, Nestby P, Mulder AH, Schoffelmeer AN, De Vries TJ (1997). Morphine-induced long-term sensitization to the locomotor effects of morphine and amphetamine depends on the temporal pattern of pretreatment regimen. *Psychopharmacology* **131**: 115–122.

Walker JR, Ahmed SH, Gracy KN, Koob GF (2000). Microinjections of an opiate receptor antagonist into the bed nucleus of the stria terminalis suppress heroin self-administration in dependent rats. *Brain Res* **854**: 85–92.

Wallace B (1989). Psychological and environmental determinants of relapse in crack cocaine smokers. *J Subst Abuse Treat* **6**: 95–106.

Weissenborn R, Robbins TW, Everitt BJ (1997). Effects of medial prefrontal or anterior cingulate lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. *Psychopharmacology* **134**: 242–257.

Whitelaw RB, Markou A, Robbins TW, Everitt BJ (1996). Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behavior under a second-order schedule of reinforcement. *Psychopharmacology* **127**: 213–224.

Yoburn BC, Chen J, Huang T, Inturrisi CE (1985). Pharmacokinetics and pharmacodynamics of subcutaneous morphine pellets in the rat. *J Pharm Exper Ther* **235**: 282–286.

Zito KA, Vickers G, Roberts DCS (1985). Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. *Pharmacol Biochem Behav* **23**: 1029–1036.