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The Medial Prefrontal Cortex Regulates the Differential Expression of Morphine-Conditioned Place Preference Following a Single Exposure to Controllable or Uncontrollable Stress

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Experiential factors, such as stress, are major determinants of vulnerability to drug addiction and relapse. The behavioral controllability of the stressor is a major determinant of how exposure to a stressor impacts addictive processes. Recent evidence suggests that controllable stressors, such as escapable shock (ES), activate ventral regions of the medial prefrontal cortex (mPFCv), whereas physically identical, but uncontrollable stress (inescapable shock, IS) does not. This activation is critical to the blunting effect that control has on neurochemical and behavioral sequelae of stress. Our laboratory has previously shown that IS, but not ES, potentiates morphine-conditioned place preference (CPP). However, the role of the mPFCv in this phenomenon is unknown. The present experiments investigated the role of the mPFCv during ES and IS in determining the effects of the stressor on subsequent morphine-CPP. Intra-mPFCv microinjection of the $GABA_A$ agonist muscimol 1 h before ES led ES to potentiate morphine-CPP, as does IS. Conversely, the potentiation of morphine-CPP normally observed in IS rats was blocked by intra-mPFCv microinjection of the $GABA_A$ antagonist picrotoxin 1 h before IS. These results suggest that during stress, activation of the mPFCv prevents subsequent potentiation of morphine-CPP, whereas inactivation of the mPFCv during stress does not. Thus, activation of the mPFCv during a stress experience is both necessary and sufficient to block the impact of stress on morphine-CPP, and control over stress blunts stress-induced potentiation of morphine effects by activating the mPFCv.

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INTRODUCTION

Environmental and experiential factors are critical determinants of an individual's vulnerability to drug addiction and relapse (Harrison *et al*, 1997; Koob and Le Moal, 1997; Sinha *et al*, 1999). However, not all individuals exposed to stress develop drug dependence (DuMont *et al*, 2007), and the mechanisms determining vulnerability remain largely unknown. Some of these determinants may relate to the type of stressor to which the individual is exposed.

Acute uncontrollable, but not behaviorally controllable, stress has persistent behavioral effects that occur outside the original stressor environment. These behavioral outcomes, which are dependent upon stressor controllability, have been called 'learned helplessness' (Maier and Seligman, 1976) or 'behavioral depression' (Weiss *et al*, 1981). Uncontrollable stress (inescapable shock, IS), but not controllable stress (escapable shock, ES) intensely activates and sensitizes serotonin (5HT) cells in the dorsal raphe nucleus (DRN) (Amat *et al*, 1998a, b; Grahn *et al*, 1999). Furthermore, these alterations in DRN 5HT activity are both necessary and sufficient to produce 'learned helplessness' (Maier *et al*, 1995a, b, 1994).

Rats exposed to IS, but not ES, exhibit potentiated morphine reward in a long-term, trans-situational manner as measured by conditioned place preference (CPP) (Will *et al*, 1998), a behavioral paradigm used to assess reward (Tzschentke, 2007). As with all consequences of exposure to IS, the potentiation of morphine-CPP by IS is mediated by IS-induced sensitization of DRN serotonergic neurons (Will *et al*, 2004). Consistent with potentiated morphine-CPP following IS, Bland *et al* (2004) reported potentiated dopamine (DA) efflux in the nucleus accumbens (NAc) shell

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following acute morphine in IS, but not ES rats. This morphine-induced potentiation of DA efflux by IS is also dependent on IS sensitization of DRN 5HT neurons (Bland *et al*, 2003).

Although the DRN sends serotonergic projections to the mesolimbic pathway (Van Bockstaele *et al*, 1993) and activation of the DRN during IS is critical in the potentiation of morphine-CPP, the DRN may be a proximate mediator in a more extended neural circuit regulating this phenomenon. Glutamatergic neurons from the ventral regions of the medial prefrontal cortex (mPFCv) project to the DRN (Peyron *et al*, 1998; Vertes, 2004), and synapse preferentially on GABAergic neurons within the DRN (Varga *et al*, 2001). Consistent with this anatomy, stimulation of the mPFCv inhibits DRN 5HT neuronal firing (Celada *et al*, 2001; Hajos *et al*, 1998). Interestingly, the mPFC is implicated in decision making, rule learning, appreciation of event significance, and goal-directed behaviors (Miller and Cohen, 2001). The mPFCv is also associated with affective- (Bremner *et al*, 2007; Killgore and Yurgelun-Todd, 2006; van Reekum *et al*, 2007) and addiction-related (Ballesteros-Yanez *et al*, 2007; Volkow *et al*, 2005) disorders.

Given the anatomy reviewed above and the proximal role of DRN 5HT in IS-induced behaviors, Amat *et al* (2005) investigated the role of the mPFCv in mediating the DRN changes that are produced by ES and IS. ES, but not IS, appeared to activate mPFCv output to the DRN, thereby reducing DRN 5HT activation. Thus, inactivation of mPFCv output by microinjection of the $GABA_A$ receptor agonist muscimol during ES led ES to produce the level of DRN 5HT activation and the behavioral deficits normally produced by IS. That is, having control did not reduce the DRN-activating effects of stress when the mPFCv could not be activated. Indeed, the mPFCv has also been implicated in stressor controllability studies examining fear conditioning (Baratta *et al*, 2007) and behavioral immunization (Amat *et al*, 2006).

Whether the mPFCv is a critical mediator of the effects of stressor controllability on morphine-CPP is unknown. Thus, in the present experiments the mPFCv was inactivated by microinjection of the $GABA_A$ receptor agonist muscimol. This procedure has been used in other experiments that have examined the role of the mPFCv in mediating the impact of stressor controllability on later escape learning and fear conditioning (Amat *et al*, 2005). The present study also employed a new approach. If inactivation of the mPFCv eliminates the protection afforded by behavioral control, then pharmacological *activation* of the mPFCv during *uncontrollable* stressor exposure might be expected to provide such protection. That is, IS should now no longer potentiate later morphine-CPP. The $GABA_A$ receptor antagonist picrotoxin has been used before to activate the mPFCv (Berretta *et al*, 2005), and so picrotoxin was used here.

MATERIALS AND METHODS

Subjects

Adult, male Sprague–Dawley rats (Harlan Inc., Indianapolis, IN) weighing 275–375 g were housed in pairs in Plexiglas cages with food and water available *ad libitum*. Rats were maintained in a climate-controlled colony room at 21°C on a 12 h light–dark cycle (lights on at 0700 hours), and all experiments were conducted during the light phase. Rats were allowed, at minimum, 1 week of acclimation prior to any procedures. All animal care and experimental procedures were in accord with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Surgery and Cannulation

Surgery was performed under halothane (Halocarbon Laboratories, River Edge, NJ) or isoflurane (Webster Veterinary, Sterling, MA) anesthesia. Because of the shortage of halothane, rats in experiments 2 and 3 were anesthetized with isoflurane. All rats were implanted with a 26-gauge dual guide cannula (Plastics One, Roanoke, VA), 1 mm center-to-center distance. For the majority of the rats, the tips of the cannulae were aimed at 1 mm above the infralimbic/prelimbic (IL/PL) regions of the mPFCv: 2.7 mm rostral to bregma, 3.3 mm ventral from the dura mater, and 0.5 mm relative to midline (Paxinos, 1998). Rats used in site-specificity studies were implanted with cannulae aimed at the ventromedial orbital cortex (vmOC): 4.2 mm rostral to bregma, 3.0 mm ventral from the dura mater, and 0.5 mm relative to midline (Paxinos, 1998). Rats were allowed to recover 1–2 weeks before any experimentation.

Cannula Verification

At 24 h following an experiment rats were overdosed with sodium pentobarbital and brains were removed and rapidly frozen in chilled isopentane. A cryostat was used to section 40-μm brain slices, which were mounted on gelatin-treated slides and stained with Cresyl Violet for cannula placement verification. Data were only analyzed from animals whose cannulae were verified to terminate within the IL/PL (mPFCv placements) or vmOC (site-specificity controls). Four rats were eliminated due to cannula misplacement.

Drugs

All drugs were dissolved in 0.9% sterile saline according to required dose. Rats were microinjected with a volume of 0.5 μl of either saline, 100 ng of picrotoxin (Sigma Co., St Louis, MO), or 50 ng of muscimol (Sigma). Morphine sulfate (NIDA) was injected subcutaneously at a dose of 3.0 mg/kg for all CPP experiments at a volume of 1 ml/kg of body weight. This dose was used, as in prior studies (Will *et al*, 1998), to produce only minimal CPP in controls, thereby allowing facilitation to be observed.

Stressor Controllability

In a separate and distinctly different environment than the CPP environment, rats received tailshock in clear Plexiglas boxes measuring 14 cm × 11 cm × 17 cm and containing a wheel mounted on the front wall. Each rat's tail extended from the rear of the box and was taped to a Plexiglas rod. Two copper electrodes coated with electrode paste were affixed to the mid-section of the tail and shocks were delivered to yoked pairs (ES, IS) of rats using a Precision-Regulated Animal Shocker with Graphic State 3.0 software

(Coulbourn Instruments, Allentown, PA). Stress sessions consisted of 80 trials of tailshock with an average intertrial interval of 60 s. This session was divided into 30 trials at 1.0 mA intensity, 30 trials at 1.3 mA, and 20 trials at 1.6 mA. This escalating shock intensity procedure maintains escape behavior in ES subjects. Tailshock was terminated for both ES and IS rats after the ES rat performed the required escape response. The initial response requirement involved a $\frac{1}{4}$ turn of the wheel. The response requirement increased a $\frac{1}{2}$ turn when three consecutive trials were terminated in less than 5 s. Each subsequent response performed in less than 5 s resulted in a 50% increase in the response requirement until the maximum response requirement of four full wheel turns was achieved. If the operant response was not performed within 10 s at any time during the stress session, the response requirement was incrementally decreased. If the correct response was not performed within 30 s, shock was terminated and the response requirement returned to a $\frac{1}{4}$ turn. Prior studies indicate that restraint in the wheel-turn box does not potentiate morphine-CPP (Will *et al*, 1998), for this reason non-stressed homecage control (HC) rats remained undisturbed in the colony.

Microinjections

Drugs for microinjection were delivered via two 10 μl Hamilton microsyringes attached to Kopf Instruments (Tujunga, CA) Model 5000 microinjector. Polyethylene 50 tubing connected the microinjector apparatus to a dual 33-gauge microinjector (Plastics One), which was inserted into the cannula guides, extending 1 mm beyond the tip of the cannula. Injections were made by continuous infusion over a 1-min period. After infusion, the injector remained in place for 2 min. Microinjections were considered successful if upon removal of the microinjector from the guide cannula fluid was dispensable from the microinjector tip.

Conditioned Place Preference

The Plexiglas place preference apparatus measured 72 cm × 30 cm × 30 cm (length, width, and height) and consisted of three distinct environments, two conditioning, and a neutral area. Each conditioning environment measured 30 cm × 30 cm × 30 cm. The environments differed from each other both visually and tactilely. One conditioning environment was striped horizontally with alternating 2 cm black and white electrical tape on the walls, while the other conditioning environment was striped vertically in the same manner. The floor was black sanded Plexiglas with a 2 cm wire grid on the horizontal side and a 3 mm wire mesh on the vertical side. The neutral area measured 12 cm × 30 cm × 30 cm, was painted gray with no texturing. During the conditioning phase, vertically and horizontally striped Plexiglas partitions were inserted on the respective sides of the neutral area to restrict the rats to their conditioning environment. Rat activity was monitored by a Philips TC352A video camera (Lancaster, PA) mounted 1.5 m above the center of the CPP apparatus. The camera relayed information of the rat's location to the Chromotrack Version 4.02 tracking software (Prototype Systems Ltd., Boulder, CO), run on a PC-compatible computer in a separate room. A SA-3 tracker (San Diego Instruments, San Diego, CA) simultaneously measured the time spent by each rat within the three compartments, the distance traveled, and the number of crossings between the environments.

Prior to the experiment, subjects were handled and fitted with a plastic rat collar that fit loosely around the neck (BAS, West Lafayette, IN). A 1 cm × 2 cm piece of reflective tape affixed to the collar was used to track the subjects while they were in the apparatus. On day 1, between 1200 and 1330 hours, all subjects were initially placed in the neutral area and the time spent in each environment of the apparatus was recorded for 20 min. Day 1 served as an assessment of individual subject bias for a given environment, any subject spending less than 20% of total time in either conditioning environment was eliminated from the experiment. Thirty-one rats were eliminated due to bias. On day 2, animals were randomly assigned to receive either ES, IS, or HC control treatment. One hour prior to ES, IS, or HC control, subjects received either an intra-mPFCv or intra-vmOC microinjection of picrotoxin, muscimol, or saline. On day 3, rats were weighed in the morning and, using a counterbalanced conditioning procedure, were randomly assigned to conditioning environments. Conditioning occurred at 1030 and 1430 hours, lasting 45 min per session. All rats received morphine and saline during this day; half were administered s.c. morphine at 1030 hours, while the other half received equivolume of s.c. saline. All rats were injected within 3 min and then placed in the appropriate conditioning environment. At 1430 hours, the injections were alternated such that a rat that had received morphine at 1030 hours, received saline at 1430 hours. Day 4 conditioning was similar to day 3 conditioning, except the order in which the subject was presented morphine and saline on day 3 was reversed. Day 5 was the test of preference. In a drug-free state, subjects were placed in the neutral area and allowed to explore the entire CPP apparatus for 20 min. The length of the pre-exposure and conditioning phases of this CPP protocol were chosen because under these parameters and this dose of morphine, CPP is potentiated in IS, but not ES, rats (Will *et al*, 1998). The dependent variable for measuring preference for each subject was the difference in time spent in the drug-paired environment before drug conditioning sessions (day 1) and after drug conditioning (day 5). Thus, a positive score indicates a shift in preference for the drug-paired compartment. Locomotor and neutral area crossing data are expressed as a difference after and before conditioning; therefore, a positive number reflects the amount by which the measure was reduced.

Statistical Analysis

All data were expressed as mean ± SEM. Data were analyzed by ANOVA to determine differences between groups. All statistically significant main effects and interactions were followed with a Student–Newman–Keuls *post hoc* test ($\alpha = 0.05$).

RESULTS

Figure 1 shows the cannula placements for both mPFCv and site-specificity control microinjections across experiments.

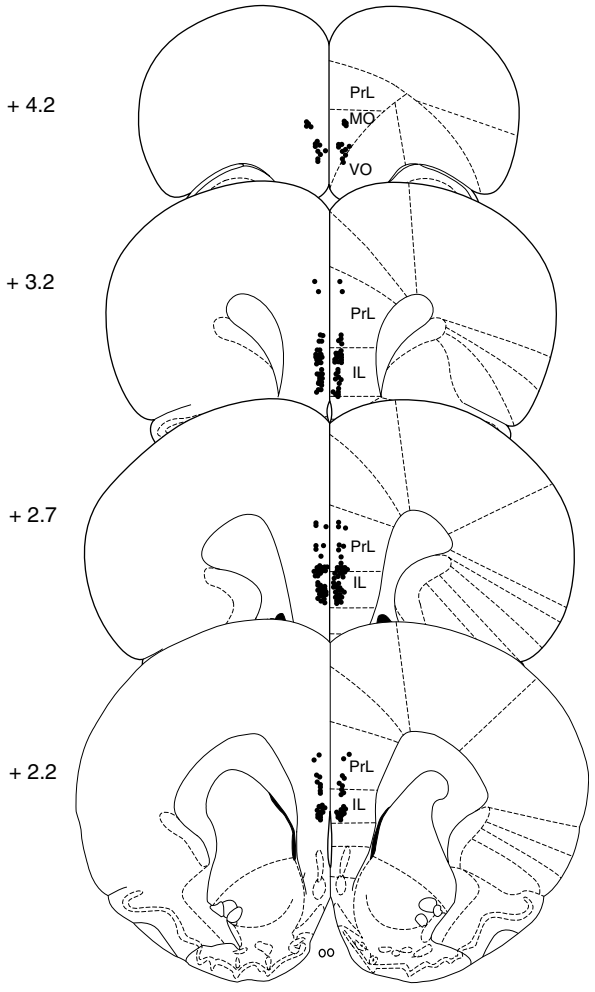

Figure 1 Placements of microinjection cannula. Numerals indicate distance from bregma (mm). Summary of microinjection cannula placements in ventral regions of the medial prefrontal cortex and ventromedial orbital cortex for all experiments. Ventromedial orbital cortex located at +4.2 mm from bregma. Medial prefrontal cortex located from +3.2 to +2.2 mm from bregma. Not all cannulae are shown due to overlapping placements. Medial orbital cortex (MO), ventral orbital cortex (VO), prelimbic cortex (PrL), and infralimbic cortex (IL).

Experiment 1: Intra-mPFCv Muscimol Administered during Stress Selectively Potentiates Morphine-CPP in Rats that Previously Experienced ES

Rats ($n = 7$–8 per group) received an intra-mPFCv microinjection of either muscimol or saline 1 h prior to ES, IS, or HC and subsequently underwent morphine conditioning 24 h later. Importantly, intra-mPFCv microinjection of muscimol at this dose does not affect the learning of the escape response during stress exposure (Amat *et al*, 2005). The CPP data are presented in Figure 2a. As is typical, prior IS potentiated morphine-CPP and ES did not. The important new finding is that intra-mPFCv muscimol microinjection before stressor exposure did not alter the effects of IS, but now ES also potentiated morphine-CPP. A 3×2 ANOVA revealed a significant main effect of stress [$F(2,41) = 7.890$, $p < 0.01$], but no significant effect of microinjection or interaction between stressor treatment and microinjection. This failure to obtain a significant interaction is attributable to the fact that muscimol had no effect in either IS or HC subjects. Student–Newman–Keuls *post hoc* tests revealed that morphine-CPP in saline-microinjected groups did not differ between ES and HC subjects, but morphine-CPP was significantly elevated in rats that received IS. *Post hoc* tests also revealed that intra-mPFCv muscimol significantly potentiated morphine-CPP in ES rats, relative to saline ES and muscimol HC, but not muscimol IS rats. Lastly, *post hoc* tests revealed no effect of intra-mPFCv microinjection in IS and HC groups. The differences in locomotor activity after and before conditioning were calculated and are presented in Figure 3a. All groups showed a reduction in locomotor activity, but a 3×2 ANOVA revealed no significant interaction of stressor treatment and microinjection. The differences in the number of neutral area crossings after and before conditioning were calculated and are presented in Figure 4a. Again, all groups showed a reduction in neutral area crossings and a 3×2 ANOVA revealed no significant interaction.

Experiment 2: Intra-mPFCv Picrotoxin Administered during Stress Selectively Blunts Morphine-CPP in Rats that Previously Experienced IS

Rats ($n = 7$–11 per group) received an intra-mPFCv microinjection of either picrotoxin or saline 1 h prior to ES, IS, or HC and subsequently underwent morphine conditioning 24 h later. The results are shown in Figure 2b. As above, IS but not ES potentiated subsequent morphine-CPP. Picrotoxin did not alter the protective effects of ES, but now IS no longer potentiated morphine-CPP. A 3×2 ANOVA revealed a significant main effect of stress [$F(2,48) = 4.383$, $p < 0.05$], but no significant effect of microinjection or interaction between stressor treatment and microinjection. As in experiment 1, Student–Newman–Keuls *post hoc* tests revealed that within saline-microinjected groups there was no CPP difference between ES and HC subjects, but a significant potentiation of morphine-CPP in IS rats. *Post hoc* tests revealed morphine-CPP in picrotoxin-microinjected IS rats was significantly reduced relative to saline-microinjected IS rats, but there was no effect of microinjection in ES or HC rats. No significant differences of morphine-CPP was found between ES, IS, and HC rats receiving intra-mPFCv picrotoxin. As shown in Figure 3b, locomotor activity after and before conditioning was reduced but a 3×2 ANOVA revealed no significant interaction of stressor treatment and microinjection. The differences in the number of neutral area crossings after and before conditioning were calculated and are shown in Figure 4b. Again, all groups showed a reduction in neutral area crossings and a 3×2 ANOVA revealed no significant interaction.

Experiment 3: Site-Specificity Control Microinjections do not Alter Morphine-CPP

Two site-specificity control studies were conducted. Rats were implanted with bilateral cannulae directed at the vmOC (see Materials and methods) and given the CPP protocol. ES rats received microinjections of muscimol

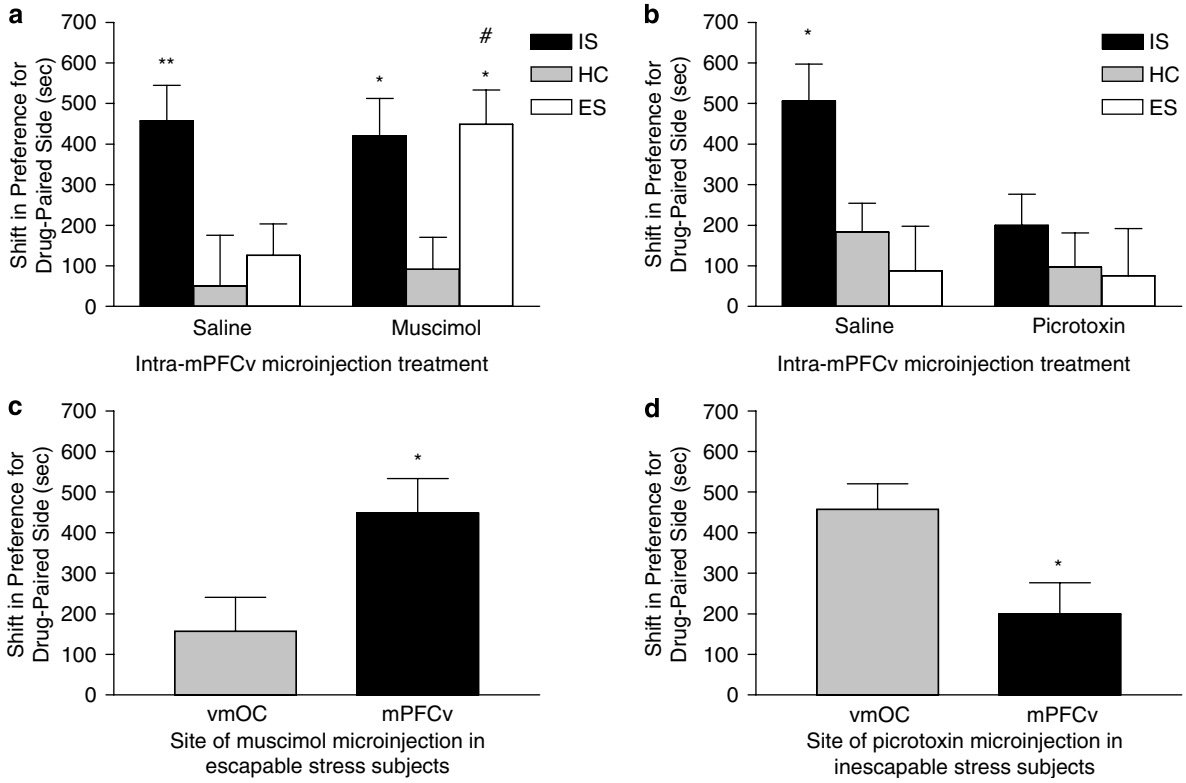

Figure 2 Morphine-conditioned place preference (CPP) following escapable stress (ES), inescapable stress (IS), or homecage control (HC) treatments. Data are expressed as mean ± SEM difference in time (s) spent on the drug-paired side before and after conditioning. Positive scores indicate a shift in preference for the drug-paired compartment. (a) Intra-mPFCv microinjection of the $GABA_A$ receptor agonist muscimol 1 h before stress potentiates morphine-CPP in rats that received ES ($n = 7$–8 rats per group). *Different from muscimol-microinjected HC group ($p < 0.05$). **Different from saline-microinjected ES and HC groups ($p < 0.05$). #Different from saline-microinjected ES group ($p < 0.05$). (b) Intra-mPFCv microinjection of the $GABA_A$ receptor antagonist picrotoxin 1 h before stress attenuates IS-induced potentiation of morphine-CPP ($n = 7$–11 rats per group). *Different from all groups ($p < 0.05$). (c) Intra-vmOC microinjection of the $GABA_A$ receptor agonist muscimol 1 h before stress did not potentiate morphine-CPP in rats that received ES ($n = 6$–8 rats per group). *Different from rats that received intra-mPFCv muscimol 1 h prior to ES ($p < 0.05$). (d) Intra-vmOC microinjection of the $GABA_A$ receptor antagonist picrotoxin 1 h before stress potentiated morphine-CPP in rats that received IS ($n = 7$–9 rats per group). *Different from rats that received intra-mPFCv picrotoxin 1 h prior to IS ($p < 0.05$).

($n = 6$–8 per group) and IS rats were microinjected with picrotoxin ($n = 7$–9 per group) 1 h before stress. Only ES rats were tested with muscimol and IS rats with picrotoxin because mPFCv muscimol only altered the effects of ES and picrotoxin only altered the effects of IS. This strategy saved a large number of subjects. As shown in Figure 2c, rats receiving an intra-vmOC microinjection of muscimol demonstrated significantly less morphine-CPP compared to the intra-mPFCv-microinjected ES rats in experiment 1. That is, muscimol microinjected at the control site did not duplicate the effects of intra-mPFCv muscimol. A one-way ANOVA revealed a significant effect of microinjection location [$F(1,12) = 5.703$, $p < 0.05$). As shown in Figure 3c, locomotor activity was reduced in both groups and a one-way ANOVA revealed no significant difference. The number of neutral area crossings are shown in Figure 4c, both groups showed a reduction and a one-way ANOVA revealed no difference. As shown in Figure 2d, rats receiving an intra-vmOC microinjection of picrotoxin showed potentiated morphine-CPP compared to the intra-mPFCv-microinjected IS rats in Experiment 2. Again, picrotoxin microinjected at the control site did not duplicate the effects of intra-mPFCv picrotoxin. A one-way ANOVA revealed a significant effect of microinjection location [$F(1,14) = 6.139$, $p < 0.05$]. Changes in locomotor behavior and neutral area crossings are shown in Figures 3d and 4d, respectively. Both groups showed a reduction in distance traveled and neutral area crossings, but ANOVA tests revealed no significant differences.

DISCUSSION

The research presented here is the first to investigate the role of the mPFCv in mediating the effects of controllable and uncontrollable stress on subsequent morphine reward. The present study replicates the previous finding that a brief exposure to uncontrollable, but not controllable, tailshock potentiates morphine-CPP (Will *et al*, 1998). The results of these experiments demonstrate that regardless of stressor controllability, activation of the mPFCv during stress is both necessary and sufficient in blocking stress-induced potentiation of morphine-CPP. Indeed, the degree of activation of the mPFCv during stress exposure is a critical determinant of subsequent morphine-CPP. The microinjection of the $GABA_A$ receptor agonist muscimol into the mPFCv during stress led to potentiated morphine-CPP in ES subjects, whereas morphine-CPP in saline-microinjected ES

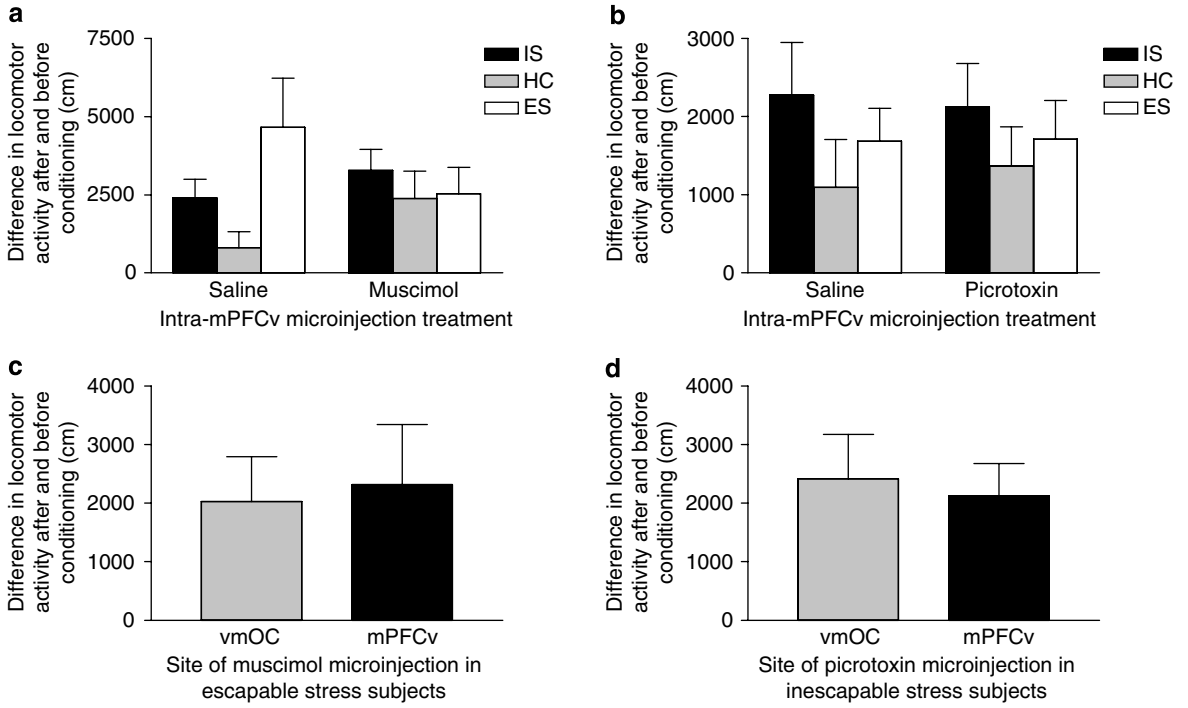

Figure 3 Difference in locomotor behavior following escapable stress (ES), inescapable stress (IS), or homecage control (HC) treatments. Data are expressed as mean ± SEM difference in distance traveled (cm) after and before conditioning. Positive scores represent the decrease in locomotor activity following stressor and conditioning phases. (a) Locomotor behavior is unaltered by intra-mPFCv muscimol 1 h before ES, IS, or HC treatments ($n = 7$–8 rats per group). (b) Locomotor behavior is unaltered by intra-mPFCv picrotoxin 1 h before ES, IS, or HC treatments ($n = 7$–11 rats per group). (c) Locomotor behavior is unaltered by intra-mPFCv or intra-vmOC muscimol 1 h before ES ($n = 6$–8 rats per group). (d) Locomotor behavior is unaltered by intra-mPFCv or intra-vmOC picrotoxin 1 h before IS ($n = 7$–9 rats per group).

subjects remained at the level of non-stressed controls. Interestingly, HC control subjects that received microinjections of either muscimol or saline exhibited similar morphine-CPP. This finding suggests that the stress-induced changes in the expression of morphine-CPP are indeed dependent upon the co-occurrence of stress and the activation state of the mPFCv. Conversely, pharmacological activation of the mPFCv during IS with the $GABA_A$ receptor antagonist picrotoxin significantly blunted potentiation of morphine-CPP, relative to IS subjects microinjected with saline. Additionally, microinjection of muscimol into the vmOC did not potentiate morphine-CPP in ES rats, suggesting that the mediation of the protective effects of ES against facilitated drug reward is specific to the mPFCv. Picrotoxin site-specificity controls also demonstrated that simple neural activation during stress was not sufficient for protection, but that activation had to be within the mPFCv. Furthermore, the stress-induced changes in morphine-CPP were not attributable to reductions in locomotor activity. Lastly, although pharmacological manipulations during IS and ES altered the CPP normally observed, we cannot conclude from these studies that the pharmacological manipulations used exactly mimic mPFCv inactivation/activation during the stress experience.

The present study utilized CPP, a widely used paradigm for measuring reward (Tzschentke, 2007), to assess differences in morphine reward due to stressor controllability. However, a number of interpretations for the IS-induced potentiation of morphine-CPP are possible. One interpretation is that morphine's rewarding properties have increased due to neuronal alterations following IS, but not ES and HC control, treatments. This interpretation is bolstered by neurochemical studies examining NAc DA following morphine injection in ES, IS, and HC rats (Bland *et al*, 2004). However, since CPP is a learning paradigm, another possibility is that IS rats simply condition better than do ES and HC controls, that is, the associative process itself is facilitated. This interpretation would posit that IS rats would also exhibit potentiated CPP to other drugs of abuse; however, IS-induced potentiation of CPP is opioid specific (Der-Avakian *et al*, 2007; Will *et al*, 1998). Lastly, another possible interpretation is that increases in CPP reflect novelty seeking rather than reward. The novelty-seeking confound can be assessed by using a CPP apparatus with three conditioning environments (Bardo and Bevins, 2000). Although the present study used a CPP apparatus with two conditioning environments, morphine-CPP using three conditioning environments has revealed that increased time spent in the drug-paired environment reflects reward rather than novelty seeking (Mucha and Iversen, 1984).

Previously, our laboratory has demonstrated that activation of the mPFCv during ES inhibits the stress-induced serotonergic response of the DRN (Amat *et al*, 2005). That is, aversive experiences activate DRN 5HT release, but when the mPFCv detects the presence of behavioral control over the aversive event, the DRN and other stress-sensitive brain structures are actively inhibited. Indeed, DRN 5HT efflux during IS, but not ES, is elevated almost 500% (Maswood *et al*, 1998). This serotonergic response characteristic of IS

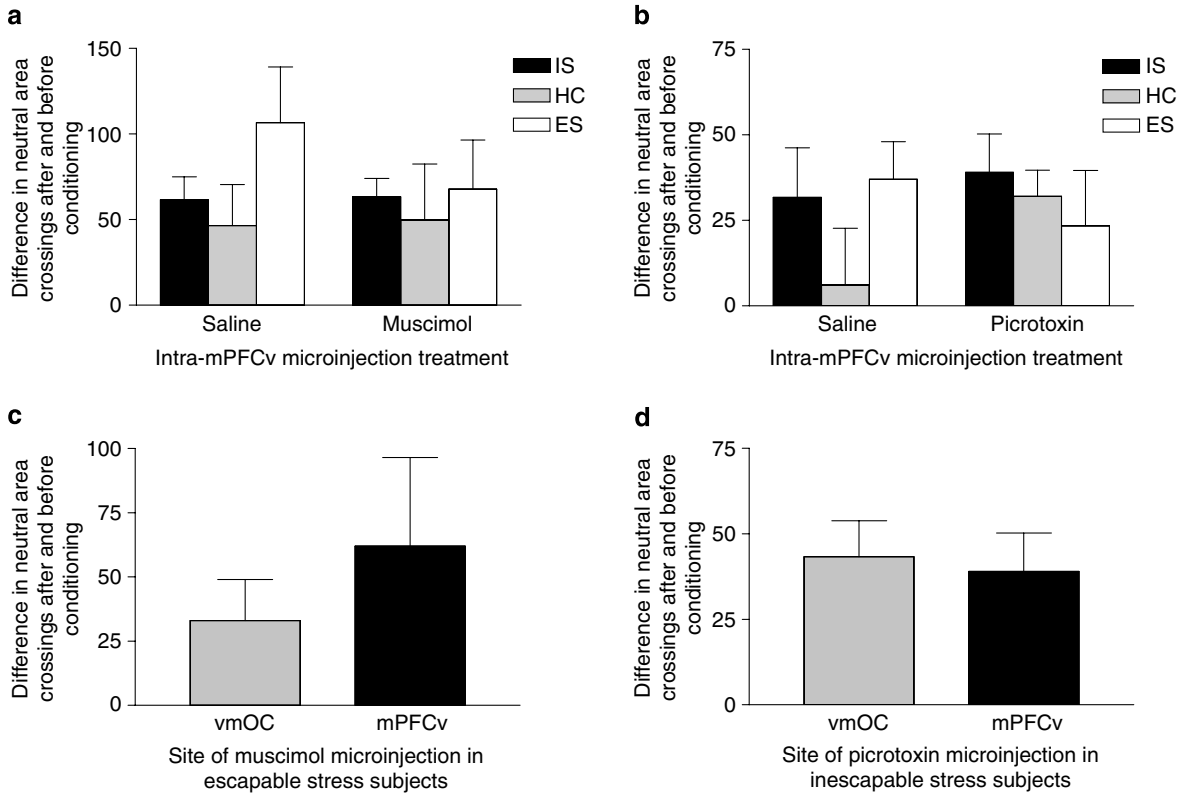

Figure 4 Difference in the number of neutral zone crossings following escapable stress (ES), inescapable stress (IS), or homecage control (HC) treatments. Data are expressed as mean ± SEM difference in neutral zone crossings after and before conditioning. Positive scores represent a decrease in the number of neutral zone crossings following stressor and conditioning phases. (a) The number of neutral zone crossings is unaltered by intra-mPFCv muscimol 1 h before ES, IS, or HC treatments ($n = 7$–8 rats per group). (b) The number of neutral zone crossings is unaltered by intra-mPFCv picrotoxin 1 h before ES, IS, or HC treatments ($n = 7$–11 rats per group). (c) The number of neutral zone crossings is unaltered by intra-mPFCv or intra-vmOC muscimol 1 h before ES ($n = 6$–8 rats per group). (d) The number of neutral zone crossings is unaltered by intra-mPFCv or intra-vmOC picrotoxin 1 h before IS ($n = 7$–9 rats per group).

causes a downregulation of inhibitory $5HT_{1A}$ autoreceptor mRNA in the DRN (Greenwood *et al*, 2003), thereby sensitizing DRN 5HT cells. Thus, activation of the mPFCv during stress either by the presence of behavioral control or by pharmacological manipulation was expected to eliminate sensitization of DRN serotonergic neurons.

Muscimol, a $GABA_A$ receptor agonist known to facilitate neuronal hyperpolarization in the rat brain (Andrews and Johnston, 1979; Edeline *et al*, 2002), has been previously used to inhibit the mPFCv during stress (Amat *et al*, 2005). Using the same dose of muscimol as in the present study, Amat *et al* (2005) demonstrated that intra-mPFCv muscimol potentiates DRN 5HT efflux in ES rats to the levels of that observed in IS rats that received only vehicle microinjections. Muscimol is a potent agonist of the $GABA_A$ receptor and can have long-lasting effects (Martin and Ghez, 1999). Nevertheless, the potentiation of morphine-CPP following intra-mPFCv muscimol in ES rats is unlikely due to a long-lasting presence of muscimol during CPP conditioning because muscimol-microinjected HC controls did not exhibit potentiated morphine-CPP. Picrotoxin, a $GABA_A$ receptor antagonist, has been shown to effectively suppress cortical inhibitory postsynaptic potentials (Connors *et al*, 1988; Yoon *et al*, 1993) and in turn inhibits subcortical structures (Berretta *et al*, 2005). Although picrotoxin can elevate cortical spiking in the rat brain for 12–24 h (Turski *et al*, 1985), this is not problematic because morphine conditioning in the present study occurred 24 h following stress. Additionally, our laboratory has investigated DRN 5HT efflux following intra-mPFCv picrotoxin during ES, IS, and HC control (Amat *et al*, 2008). At the same dose of picrotoxin used in this study, Amat *et al* (2008) found no potentiation of DRN 5HT efflux in rats that received intra-mPFCv picrotoxin during IS compared to vehicle microinjection during ES. However, the possibility of a long-term rebound effect, that is, increased $GABA_B$ receptor activation due to blockade of $GABA_A$ receptors (Bal *et al*, 1995), must be noted and cannot be ruled out in the present study. Lastly, the binding of muscimol and picrotoxin to other substrates besides the $GABA_A$ receptor has been documented (Das *et al*, 2003; Etter *et al*, 1999; Shen and Johnson, 2001); therefore, interpretive cautions regarding $GABA_A$ receptors in the mPFCv and behavioral outcomes of stressor controllability should be exercised.

Anatomical studies indicate that the mPFCv can influence the mesolimbic pathway. Glutamatergic efferents originating in the IL (Hurley *et al*, 1991) and PL (Vertes, 2004) cortices project to the NAc and the ventral tegmental area (VTA). Pyramidal cells from the mPFCv to the NAc appear to synapse on medium spiny GABA neurons (Carr and Sesack, 2000), with these GABAergic neurons then projecting to DA cells in the VTA (Sesack and Pickel, 1992).

Projections from the mPFCv target GABA interneurons within the VTA, which project to both VTA DA cells and DA terminals in the NAc (Carr and Sesack, 2000) and also synapse directly on VTA DA cells (Omelchenko and Sesack, 2007). Additionally, the mPFCv can indirectly influence the VTA via ventral pallidal relays (Groenewegen *et al*, 1993; Heimer *et al*, 1991).

Although the anatomy described above is complex, studies using electrical and pharmacological stimulation provide a clearer account. Initial studies found that stimulation of the mPFC increases NAc DA (Karreman and Moghaddam, 1996; Taber and Fibiger, 1995; You *et al*, 1998). However, later experiments using more physiological levels of stimulation in the mPFC revealed a decrease in release of DA in the NAc (Jackson *et al*, 2001). Moreover, this circuitry appears to be tonically active as blockade of AMPA/kainate receptors in the VTA causes increases in NAc DA efflux (Takahata and Moghaddam, 2000). The implication of the present data is that morphine-induced inhibition of the mPFCv causes disinhibition of NAc DA; however, the pathway by which the mPFCv exerts this effect is unknown.

While the present study emphasizes inhibition of the mPFCv leading to potentiated morphine-CPP, inactivation of the *dorsal* mPFC has been implicated in prevention of both footshock-induced (McFarland *et al*, 2004) as well as cue-induced reinstatement of cocaine self-administration (McLaughlin and See, 2003). These data suggest that the modulatory role of the mPFC in drug-related paradigms is subregion and drug specific, with dorsal and ventral mPFC serving quite different functions. For example, morphine-CPP is attenuated by IL, but not PL or anterior cingulate, lesions and cocaine-CPP is blocked by PL, but not IL or anterior cingulate, lesions (Tzschentke and Schmidt, 1999); moreover, inactivation of the mPFCv, but not the dorsal mPFC, enhances spontaneous recovery of cocaine seeking (Peters *et al*, 2007). Although in the present study inactivation of the mPFCv is hypothesized to potentiate morphine-CPP due to changes induced by IS, the present experiments only address the role of mPFCv during the stress experience.

The results of the present experiments are consistent with studies examining the prefrontal cortex and addiction-related processes. Inhibition/hypoactivity of the mPFC has been linked to other psychiatric disorders (eg PTSD), resulting in reduced inhibitory control over limbic structures (Bremner, 2006). One proposed consequence of reduced mPFCv activity is a disinhibition of structures in the ventral striatum (Ernst *et al*, 2006). Reductions of prefrontal cortical activity have also been correlated with impulsive, perseverative, and deleterious behaviors (Dalley *et al*, 2004; Passetti *et al*, 2002; Soloff *et al*, 2003; Tanabe *et al*, 2007), which can predispose an individual to enter an addictive state (Jentsch and Taylor, 1999; Mitchell, 1999; Perry *et al*, 2005; Poulos *et al*, 1995). Similarly, as in the present experiments, experiential factors that behaviorally (or pharmacologically) inhibit the mPFCv following opioid administration result in a potentiation of approach behavior to previously neutral stimuli paired with morphine.

The present experiments demonstrate that activation of the mPFCv during the stress experience is a critical determinant in whether stress will produce long-term trans-situational changes in morphine reward. Activation of the mPFCv appears to be both necessary and sufficient to attenuate stress-induced potentiation of morphine-CPP. Our findings further suggest a role of the mPFCv in the complex interaction between stress and drugs of abuse. It is possible that an activated mPFCv is responsible for both the detection of stressor controllability and the subsequent dampening of stress-activated neural structures as well as maintaining tonic inhibition of the mesolimbic pathway.

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