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Handling blocks expression of conditioned place aversion but not conditioned place preference produced by ethanol in mice

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Abstract

Previous findings implicate opioid receptors in the expression of the conditioned rewarding and aversive properties of ethanol. We have recently reported that the conditioned rewarding effect of ethanol is mediated by opioid receptors in the ventral tegmental area (VTA). We attempted to determine whether VTA opioid receptors also mediate the expression of the conditioned aversive properties of ethanol. However, the magnitude of conditioned place aversion (CPA) was not consistent with our previous findings and prevented us from making definitive conclusions. We hypothesized that the handling required to make intracranial infusions in mice alters the expression of CPA, but not conditioned place preference (CPP). Therefore, non-operated animals underwent a Pavlovian conditioning procedure for either ethanol CPA or CPP. Just before testing, half of the animals were held by the scruff of the neck to mimic intracranial infusion handling. Animals conditioned for CPA did not express CPA if they were handled. However, animals conditioned for CPP exhibited robust CPP, regardless of handling. These findings provide additional evidence that the conditioned rewarding and aversive effects of ethanol are mediated by different neural mechanisms.

Keywords: Handling; Aversion; Preference; Place conditioning; Reward; Ethanol; Locomotor activity; DBA/2J; Mice

1. Introduction

Systemic non-selective blockade of opioid receptors decreases expression of the conditioned rewarding effect of ethanol in mice (Cunningham et al., 1995, 1998; Kuzmin et al., 2003; Middaugh and Bandy, 2000). We recently demonstrated that the conditioned rewarding effect of ethanol is expressed through a ventral tegmental area (VTA) dependent mechanism that involves both opioid and $GABA_B$ receptors (Bechtholt and Cunningham, in press). Specifically, we showed that the non-selective opioid receptor antagonist, methylnaloxonium, or the $GABA_B$ receptor agonist, baclofen, injected into the VTA decreased the expression of ethanol-induced conditioned place preference (CPP), while intra-nucleus accumbens (NAc) methylnaloxonium was without effect (Bechtholt and Cunningham, in press). Because systemic administration of a non-selective opioid receptor antagonist had also been found to affect expression of ethanol-induced conditioned place aversion (CPA) in mice (Cunningham et al., 1998), we also initiated a series of studies to examine effects of intracranial administration of the antagonist on expression of CPA. However, our ability to draw conclusions from these studies was compromised by the finding of unexpectedly low levels of CPA in vehicle-infused control mice. Thus, we conducted the present studies to test the hypothesis that the prolonged handling involved in administering an intracranial infusion to mice interferes with the expression of CPA, but not with the expression of CPP.

Several studies have previously reported that the temporal relationship between exposure to an abused drug and a paired environmental stimulus (i.e., "conditioned

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stimulus" or CS) plays a critical role in determining whether CPP or CPA is observed. For example, in a series of papers, Fudala and Iwamoto (1986, 1987, 1990) found that pre-CS injections of nicotine produced CPP in rats, whereas post-CS injections produced CPA. Similarly, our lab has reported that ethanol injected just before CS exposure produces CPP in mice, while ethanol injected just after CS exposure produces CPA (Cunningham et al., 1997, 1998; Cunningham and Henderson, 2000; Cunningham et al., 2002a,b, 2003b).

Based on a variety of findings, we have previously suggested that these paradoxically opposite conditioned behaviors reflect independently mediated rewarding and aversive effects of ethanol (e.g., Le et al., 2001; Risinger et al., 2002). One hypothesis is that CPA induced by post-CS injection uniquely reflects the aversiveness of the novel transition from the sober to the intoxicated state. The finding that ethanol pre-exposure decreases expression of CPA, but has no detectable effect on expression of CPP supports this hypothesis (Cunningham et al., 2002b, 2003b). The finding that both CPP and CPA are altered by systemic non-selective opioid antagonism suggests that they may be mediated by similar neural substrates, although in opposite directions (Cunningham et al., 1998). Indeed, while it has been repeatedly suggested that rewarding stimuli activate midbrain dopamine neurons (Schultz, 1998), it has been recently suggested that these neurons might also be responsive to aversive stimuli (Ungless et al., 2004).

Unfortunately, the unexpected finding of weak CPA in our preliminary intracranial studies thwarted efforts to determine the role of VTA neurons in ethanol-induced aversive conditioning. Furthermore, our attempts to assess the effects of intra-NAc treatments on ethanol CPA yielded no significant CPA in vehicle-treated animals, again preventing us from testing our hypothesis. We hypothesized that the nature of the handling technique required to make intracranial infusions in mice was disruptive to the expression of the conditioned aversive effect of ethanol in the CPA procedure. However, because we had successfully conducted similar experiments examining the conditioned rewarding effect of ethanol, we hypothesized that ethanol-induced CPP would not be altered by handling. The present studies were designed to test these hypotheses in mice that were not exposed to intracranial infusions before testing.

2. Method

2.1. Animals

One-hundred and sixty-six male DBA/2J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age. Animals were housed four to a cage with continuous access to food and water and allowed to acclimate to the colony for 4–8 days. In our previous intracranial infusion experiments, animals were housed two per cage after surgery to minimize damage to implanted cannulae. Therefore, to match housing conditions, animals in the present experiments were also housed two per cage after the initial period of acclimation to the colony. Experiments were conducted 5 days/week during the light phase of a 12-h light–dark cycle (lights on at 7:00 a.m.). The NIH "Principles of Laboratory Animal Care" (National Research Council, 1996) were followed in conducting these studies and the protocol was approved by the OHSU IACUC.

2.2. Drugs

A 95% ethanol stock solution was diluted to 20% vol/vol with saline. A dose of 2 g/kg was administered intraperitoneally in an injection volume of 12.5 ml/kg. This dose was chosen because it has been shown to induce robust CPP and CPA (Cunningham et al., 1997).

2.3. Apparatus

The place conditioning chambers consisted of 12 identical acrylic and aluminum boxes (30×15×15 cm) enclosed in separate light and sound attenuating chambers. Locomotor activity and the location of the mouse within the box were determined by six infrared beams located 2.2 cm above the floor of the chamber at 5-cm intervals and recorded with a 10-ms resolution by a computer. The floor consisted of interchangeable halves, which were made of two distinct textures. Specifically, hole floors were made from perforated 16-gauge stainless steel with 6.4-mm round holes on 9.5-mm staggered centers. Grid floors were made of 2.3-mm stainless steel rods mounted 6.4 mm apart on acrylic rails. These floor textures were chosen based on previous studies demonstrating that drug naive mice spend equal time on both floors thereby allowing use of an unbiased CPP procedure (e.g., Cunningham et al., 2003a). The use of interchangeable floor halves allowed presentation of matching floor halves during conditioning trials and different floor halves during the preference test. In this way, floor texture and not the spatial location of the floor was paired with ethanol or saline.

2.4. Place conditioning procedure

Each experiment consisted of three phases: habituation (one session), conditioning (eight sessions) and preference testing (one session). The habituation session was intended to reduce the novelty of the experimental apparatus and injection procedure. On the first day of the experiment, animals were injected intraperitoneally (i.p.) with saline (12.5 ml/kg) either just after removal from (CPA, Experiment 1) or just before placement into the conditioning chamber (CPP, Experiment 2) for 5 min where the floor

Table 1
Group mean±S.E.M. activity counts during conditioning trials

	Conditioning trial activity (counts/min)	
	Saline trials (CS−)	Ethanol trials (CS+)
Experiment 2: handling effects on CPA		
Standard	35.1±2.1	37.4±1.8
Handled	36.6±1.7	37.4±1.7
Experiment 3: handling effects on CPP		
Standard	61.5±2.9	179.6±5.4
Handled	55.7±2.0	177.7±5.0

was made of smooth paper. During the conditioning phase, animals were injected with saline or ethanol on alternating days just after removal from (CPA, Experiment 1) or just before placement into (CPP, Experiment 2) the conditioning chamber for 5 min where both sides of the floor were the same. Animals in the grid+ condition were placed on the grid floor on ethanol injection days and the hole floor on saline injection days. Conversely, animals in the grid− condition were placed on the hole floor on ethanol injection days and the grid floor on saline injection days. The order of ethanol and saline exposure was counterbalanced within groups. After four conditioning trials (4 CS+, 4 CS−; days 2–9), a 30-min preference test was conducted (day 10).

Immediately before the preference test session, animals were either held by the scruff (Handled) or placed into the conditioning chamber without prolonged handing (Standard). Specifically, in order to mimic the handling procedure used to make intracranial infusions, animals were gently, but firmly held by the scruff of the neck with all four paws touching a flat surface for 2.25 min. Animals appeared calm during this procedure and struggled very little. The primary dependent variable during preference testing was the amount of time spent on the grid floor. Seconds spent on the grid floor was divided by total test duration in minutes (i.e., 30) to create a response measure indexed in seconds per minute. This simple transformation readily allows one to evaluate results relative to the full range of possible outcomes (e.g., 0 s/min=complete aversion to grid; 60 s/min=complete preference for grid). Control mice exposed to vehicle injections typically spend about 30 s/min on the grid floor, reflecting equal preference for both floor textures (Cunningham et al., 2003a).

2.5. Data analysis

Conditioning activity data were analyzed using two-way ANOVAs with the factors trial type (ethanol or saline) and handling group (standard or handled). Because the general conclusions were unchanged, conditioning activity data were collapsed across the individual CS+ and CS− trials to simplify presentation. Grid time test data were analyzed with two-way ANOVAs and the factors conditioning group (grid+ or grid−) and handling group. Because we had a specific, directional hypothesis about the outcome of Experiment 1, pair-wise planned comparisons were conducted for the two conditioning subgroups within each handling group to determine whether place conditioning had occurred. To determine whether interpretation of preference data was complicated by group differences in activity, test activity data were analyzed with one-way ANOVAs using the factor handling group.

3. Results

3.1. Experiment 1: handling effects on CPA

Data from six animals were removed from analyses due to an experimental error; one animal was removed due to an injection injury.

3.1.1. Conditioning activity

Activity during conditioning trials was similar on ethanol and saline days across the handling groups (Table 1). This conclusion was confirmed by a two-way ANOVA (trial type×handling group) that yielded no significant effects.

3.1.2. Preference test

In the counterbalanced, unbiased design used here, strength of place conditioning is indexed by the difference between the grid+ and grid− conditioning subgroups (Cunningham et al., 2003a,b). As can be seen in Fig. 1, animals in the grid+ condition spent less time on the grid floor during the test than the animals in the grid− condition, indicating an overall development of CPA. This conclusion was confirmed by a two-way ANOVA (conditioning group×handling group) that revealed a significant main effect of conditioning group

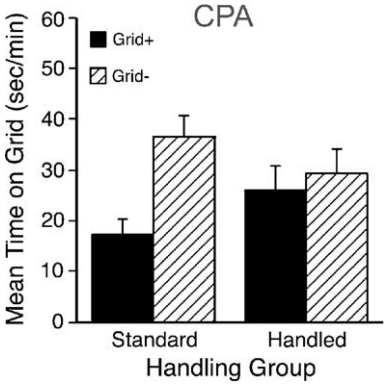

Fig. 1. Mean time s/min (+S.E.M.) spent on the grid floor during the 30-min post-conditioning test for each handling group. Animals in the grid+ condition were given ethanol paired with the grid floor during conditioning trials (standard, n=19; handled, n=19). Animals in the grid−condition were given ethanol paired with the hole floor during conditioning trials (standard, n=20; handled, n=19). Significant main effect of conditioning group (p<0.01). Conditioning group×handling group interaction (p=0.071).

$F(1,73)=6.8$, $p<0.01$. This analysis also revealed a trend toward a conditioning group×handling group interaction $F(1,73)=3.4$, $p=0.071$ for time spent on the grid floor during the test. Planned comparisons between the grid+ and grid− conditioning groups within each handling group demonstrated that animals in the standard group exhibited significant CPA $F(1,37)=13.3$, $p=0.001$, while animals in the handled group did not, suggesting that handling disrupted the expression of ethanol CPA.

3.1.3. Test activity

Mean (S.E.M.) activity rates during the test for standard and handled animals were 24.2 (1.3) and 21.7 (1.5) counts/min, respectively. Handling did not significantly alter locomotor activity during the test. A one-way ANOVA yielded no significant effect of handling group, suggesting that interpretation of floor preference data was not confounded by differences in test activity.

3.2. Experiment 2: handling effects on CPP

3.2.1. Conditioning activity

Animals were more active when treated with ethanol than saline as is typically seen in DBA/2J mice (Table 1) (e.g., Cunningham et al., 1995). This observation was confirmed by a two-way ANOVA (trial type×handling group) that yielded a significant main effect of trial type $F(1,80)=1329.8$, $p<0.001$, but no other significant effects.

3.2.2. Preference test

As expected, animals in the grid+ condition spent more time on the grid floor than animals in the grid− condition, indicating development of CPP. Moreover, handling did not alter the development of CPP (Fig. 2). Two-way ANOVA (conditioning group×handling group) demonstrated a significant main effect of conditioning group $F(1,78)=105.8$, $p<0.001$ and no significant conditioning group×handling group interaction for time spent on the grid floor during the test. Thus, consistent with our prediction, handling did not affect expression of CPP.

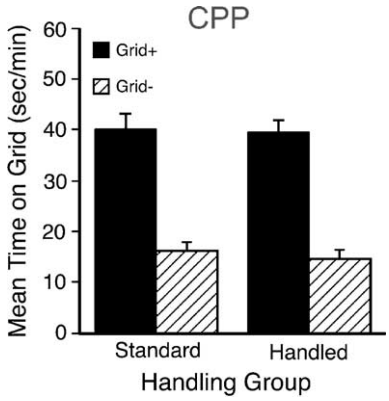

Fig. 2. Mean time s/min (+S.E.M.) spent on the grid floor during the 30-min post-conditioning test for each handling group. Animals in the grid+ condition were given ethanol paired with the grid floor during conditioning trials (standard, $n=21$; handled, $n=20$). Animals in the grid− condition were given ethanol paired with the hole floor during conditioning trials (standard, $n=20$; handled, $n=21$). Significant main effect of conditioning group ($p<.001$).

3.2.3. Test activity

Mean (S.E.M.) activity rates during the test for standard and handled animals were 38.2 (1.4) and 38.4 (1.5) counts/min, respectively. Handling did not significantly alter locomotor activity during the test and therefore interpretation of the preference data was not confounded. A one-way ANOVA supported this observation, yielding no significant effect of handling group.

4. Discussion

These studies show that, in the absence of an intracranial infusion, prolonged handling is sufficient to disrupt ethanol CPA (Experiment 1), but has no discernable effect on ethanol CPP (Experiment 2). The mechanism underlying the handling-induced disruption of CPA remains unknown. Interestingly, previous findings suggest that the endogenous opioid system might be involved in producing handling-related effects such as that reported here. For example, light touch of the dorsal spine resulted in 20–32 kHz ultrasonic vocalizations in rats that decreased with repeated handling (Brudzynski and Ociepa, 1992). These vocalizations (20–32 kHz) are thought to convey an affective state (Miczek et al., 1995) and can be mediated by the endogenous opioid system (Vivian and Miczek, 1998). Similarly handling rats, as for an intraperitoneal injection, induced analgesia and disrupted postshock freezing behavior which was blocked by the non-selective opioid receptor antagonist, naltrexone (Fanselow and Sigmundi, 1986), and was decreased by repeated handling. These findings suggest that routine handling procedures may induce an opioid-mediated response in non-habituated animals. This may be particularly problematic in mice, which do not seem to habituate to handling (Wilson and Mogil, 2001).

The specific effect of handling on CPA, leaving CPP unaffected, might also be explained by endogenous opioid release. As previously mentioned, both of these conditioned behaviors are altered by manipulations of the opioid system. Specifically, systemic administration of naloxone, a non-selective opioid receptor antagonist, enhances the expression of CPA but disrupts the expression of CPP (Cunningham et al., 1998). Based on these findings, one might hypothesize that opioid receptor agonists, including endogenous opioids, could disrupt the expression of CPA and enhance the expression of CPP. While we did not observe effects of handling on CPP, we cannot rule out the possibility that a ceiling effect prevented us from observing enhanced CPP resulting from endogenous opioid release.

Important procedural differences beyond the duration of handling might also explain why handling effects were observed in CPA, but not CPP. That is, it is possible that CPP was preserved, while CPA was disrupted because handling before the test was novel in the CPA experiment. During the conditioning phase in our CPP study (Experiment 2), animals were held by the scruff daily before conditioning trials, with the handling duration being the only difference experienced on the test day. Conversely, animals conditioned for CPA (Experiment 1) were only handled by the scruff for injections as they were taken out of the conditioning chamber on conditioning days. For the first time on the test day, these animals were held by the scruff for a prolonged period before being placed into the conditioning chamber. This procedural difference between CPA and CPP might explain the observed difference in handling effects due to the relatively greater novelty of pre-test handling for CPA mice. Alternatively, it is possible that aversive tasks are simply more sensitive to interference by extraneous stimuli (i.e., due to external inhibition or stimulus generalization decrement). These possibilities can only be addressed by additional experiments investigating possible habituation to prolonged handling during conditioning and by experiments using different aversive stimuli.

Finally, these findings are consistent with the idea that the conditioned aversive and rewarding effects of ethanol are distinct phenomena expressed through different neural mechanisms. This suggestion is intriguing, given that both phenomena are elicited by the same unconditioned stimulus (ethanol) and are determined only on the basis of the temporal relationship of ethanol to the CS. That is, the same dose of ethanol given before CS exposure results in CPP and after CS exposure results in CPA. This dissociation of CPA and CPP is also supported by previous findings demonstrating that these two behaviors are not genetically correlated (Cunningham and Ignatoff, 2000; Hill et al., 2002) and that ethanol pre-exposure decreases CPA but leaves CPP unchanged (Cunningham et al., 2002b).

In summary, our data indicate the handling required for intracranial infusions in mice disrupts CPA, but not CPP. Perhaps, the novelty of the timing and/or duration of handling are important in this selective disruption of CPA. Previous findings suggest that endogenous opioids may be involved in these handling effects. Finally, these findings underscore the need to carefully re-evaluate the potential impact of methodologies developed in rats when they are transferred to studies involving mice.

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