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ROLES OF OPIOID RECEPTOR SUBTYPES IN MEDIATING ALCOHOL SEEKING INDUCED BY DISCRETE CUES AND CONTEXT

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Abstract

The aim of this study was to assess the effects of selective blockade of the delta (DOP) or mu opioid (MOP) receptors on alcohol seeking induced by discrete cues and context. **In** Experiment 1, rats were trained to self-administer alcohol in an environment with distinct sensory properties. After extinction in a different context with separate sensory properties, rats were tested for context-induced renewal in the original context following treatment with the DOP receptor antagonist naltrindole (0 – 15-mg/kg, IP) or the MOP receptor antagonist CTOP (0 – 3-μg/kg ICV). In a separate set of experiments, reinstatement was tested with the presentation of a discrete light+tone cue previously associated with alcohol delivery, following extinction without the cue. In Experiment 2, the effects of naltrindole (0 – 5-mg/kg, IP) or CTOP (0 – 3-μg/kg μg ICV) were assessed. For context-induced renewal, 7.5-mg/kg naltrindole reduced responding without affecting locomotor activity. Both doses of CTOP attenuated responding in the first 15 min of the renewal test session; however, total responses did not differ at the end of the session. For discrete cue-induced reinstatement, 1 and 5-mg/kg naltrindole attenuated responding, but CTOP had no effect. We conclude that while DOP receptors mediate alcohol seeking induced by discrete cues and context, MOP receptors may play a modest role only in context-induced renewal. These findings point to a differential involvement of opioid receptor subtypes in the effects of different kinds of conditioned stimuli on alcohol seeking, and support a more prominent role for DOP receptors.

Keywords

rat; ethanol; relapse; craving

In humans, alcohol-associated cues induce craving in social drinkers and precipitate relapse in problem drinkers (McCusker & Brown, 1990; Monti *et al.*, 1999; Rohsenow *et al.*, 2000). This has been modeled in lab animals. In rats, extinguished alcohol-seeking can be reinstated by alcohol-paired stimuli, such as: 1) discrete response-contingent cues (Liu & Weiss, 2002; Nie & Janak, 2003); 2) discriminative non-contingent cues (Katner *et al.*, 1999; Dayas *et al.*, 2007); and 3) multimodal contextual cue complexes (Burattini *et al.*, 2006; Tsang & Janak, 2006; Zironi *et al.*, 2006). Interestingly, each of these cue types elicit a distinct pattern of drug-seeking (Zhou *et al.*, 2005) that is mediated by separate brain

substrates (Fuchs *et al.*, 2005; Bossert *et al.*, 2007). In addition, context gates alcohol-seeking induced by discrete (Tsiang & Janak, 2006) or discriminative (Burattini *et al.*, 2006) cues.

Naltrexone, a non-selective opioid receptor antagonist, attenuates alcohol-seeking induced by each of these cue types (Katner *et al.*, 1999; Liu & Weiss, 2002; Burattini *et al.*, 2006; Dayas *et al.*, 2007; Marinelli *et al.*, 2007), as well as compounds of these cues (Ciccocioppo *et al.*, 2002; Ciccocioppo *et al.*, 2003). Since it has no effect on stress-induced reinstatement (Lê *et al.*, 1999; Liu & Weiss, 2002) opioids appear to play a more important role in mediating seeking elicited by stimuli conditioned to alcohol. Selective antagonists of both MOP (μ) and DOP (δ) receptor subtypes have been shown to block alcohol self-administration and voluntary intake (Froehlich *et al.*, 1991; Hyytia, 1993; Lê *et al.*, 1993; Krishnan-Sarin *et al.*, 1995a; Krishnan-Sarin *et al.*, 1995b; Honkanen *et al.*, 1996; Krishnan-Sarin *et al.*, 1998; June *et al.*, 1999; Hyytia & Kiianmaa, 2001). One other study demonstrated that DOP antagonists effectively and selectively attenuated reinstatement induced by a discrete-discriminative cue compound in a dose-dependent fashion (Ciccocioppo *et al.*, 2002).

To evaluate the contribution of MOP and DOP receptors in alcohol-seeking induced by discrete cues and context, we used the highly selective peptide D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) (Hawkins *et al.*, 1989) to block activity of MOP receptors and the non-peptide antagonist naltrindole to target DOP receptors (Portoghese *et al.*, 1988). We examined the effect of contextual stimuli on reinstatement of alcohol-seeking using a variation of a *renewal* procedure (Bouton and Bolles 1979) that was adapted by Crombag and Shaham (Crombag & Shaham, 2002; Crombag *et al.*, 2008) to study context-induced drug-seeking. Using this procedure, a context associated with the availability of abused drugs (including alcohol) and natural rewards potently elicits seeking (Crombag *et al.*, 2002; Bossert *et al.*, 2004; Marinelli *et al.*, 2007; Diergaarde *et al.*, 2008; Fletcher *et al.*, 2008).

Methods

Subjects

Male Wistar rats (Charles River, St-Constant, QC, Canada) weighing 200–225 g upon arrival, were individually housed under a 12:12 h light-dark cycle (lights on at 7:00 a.m.). Access to food and water was available in the home cage and the temperature was maintained at 21±1°C. The experimental procedures followed the “Principles of laboratory animal care” (NIH publication no. 85-23, 1996) and were approved by the local animal care and use committee.

Materials and Apparatus

The operant chambers were constructed locally and were equipped with two levers, symmetrically centred on a side panel and a house light near the ceiling that was illuminated at the start of the 1-h session and turned off at the end of the session. The Plexiglas chambers were enclosed in lightproof, sound-attenuating boxes equipped with exhaust fans. During self-administration sessions, responding on one lever (an active lever) activated an infusion pump (Razel Sci., Stamford, CT), while responding on the other lever (an inactive lever) was recorded but had no programmable consequences. Activation of the infusion pump resulted in the delivery of 190 μ l of 12% (w/v) alcohol solution into a drinking receptacle located between the two levers and initiated a 5-sec timeout period. During the timeout, a light over the active lever was illuminated, the house-light was turned off and white noise was emitted from a speaker.

Horizontal locomotor activity was monitored in a room illuminated by a 40W red bulb in stainless-steel chambers with wire mesh floors: 40 cm (L)×25 cm (D)×20 cm (H). Each chamber possessed two infrared photocells mounted 3 cm above the floor that divided it into three equally sized areas. Photo-beam interruptions were recorded and tabulated automatically by computer. Locomotor activity was inferred from the total number of photo-beam interruptions.

Drugs

Alcohol solution was prepared by diluting 95% ethanol (Commercial Alcohols Inc., Tiverton, ON, Canada) in tap water. Opioid receptor antagonist drugs were purchased from Sigma (St-Louis, MO, U.S.A). Naltrindole HCl was dissolved in sterile distilled water and injected intraperitoneally (IP) in a volume of 2-ml/kg. CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂, Sigma, St-Louis, MO, U.S.A) was dissolved in sterile saline and infused intracerebroventricularly (ICV) in a volume of 4-μl/rat.

Intracerebroventricular (ICV) cannulation surgery and microinjection procedures

Surgery was performed under sodium pentobarbital anaesthesia (65-mg/kg IP). Using standard stereotaxic techniques, 23-gauge stainless-steel guide cannulae (Plastics One, Roanoke, VA, USA) were implanted in the right lateral ventricle 1 mm over the target region and affixed to the skull using dental acrylic and jeweller screws. The final coordinates for the injector tip were (from bregma): anterior/posterior: −1.0 mm; lateral/medial: 1.4 mm; dorsal/ventral: −3.7 mm from dura. After surgery, stainless-steel obturators were inserted in the cannulae to maintain patency and rats were allowed 9–14 days in their home cages to recover. ICV infusions were administered with a 10-μl Hamilton microsyringe connected via polyethylene tubing to 30 gauge injectors (Plastics One, Roanoke, VA, USA) that extended 1 mm below the guide cannulae. Infusions occurred over the course of 60 s and injectors remained in place for an additional 60 s. At the end of the experiment, cannula patency was confirmed for all subjects by giving each rat an ICV injection of angiotensin at 50-ng/2 μl and by observing subsequent drinking behaviour. Placements were considered to be accurate if a rat drank within 1 min of the infusion and sustained drinking over 2 min (Sakai *et al.*, 1995).

Procedures

Alcohol Self-Administration Training—Rats were trained to self-administer alcohol using methods described previously (Lê *et al.*, 1998; Lê *et al.*, 1999). Briefly, naïve rats were first given a choice of alcohol solution or tap water in Richter tubes for 30-min/day in drinking cages. The alcohol solution was provided in escalating concentrations: 3% (w/v) for the first 5 days, 6% (w/v) for the next 5 days and 12% (w/v) for the next 10 days. Subsequently, operant self-administration of alcohol (12%) was initiated in 1-h daily sessions on a fixed ratio-1 (FR-1) schedule of reinforcement for 1-2 weeks, followed by a FR-2 schedule for 1 week, and a FR-3 schedule for at least 1 week. In cases where alcohol was left over in the drinking receptacle after a self-administration session, it was measured and taken into account in the calculation of intake.

Experiment 1: Context-Induced Renewal—Rats were trained to self-administer alcohol as described above in one of two counterbalanced contexts distinguishable on a number of sensory properties. One context had 25 μl of peppermint extract (McCormick, London, ON, Canada) applied to gauze hung 5 cm from the top of the cage, a white house light, wallpaper with vertical bars, fans off with the enclosure door slightly ajar, a wire-grid floor and testing that occurred between 8:00 and 11:00 am. The other context was scented

with vanilla extract (McCormick), had a green house light, clear walls, fans on, a bar floor and testing that occurred between 3:00 and 6:00 pm.

Once rats showed stable alcohol self-administration at FR-3 in one context (Context A), they received 1-h/day extinction sessions in the alternate context (Context B). Except for the shift in context and the inactivation of alcohol infusion pumps, the extinction procedure resembled that of self-administration. All rats were matched according to average alcohol intake on the last three days of self-administration and were habituated to the injection procedure on the four days preceding the test day. The extinction criterion was at least two consecutive days with 15 or fewer lever presses. On the test day, rats were either returned to Context A where they previously self-administered alcohol (ABA groups) or remained in Context B where they had undergone extinction sessions (ABB group in Experiment 1a). On the test day, vehicle or drug was administered 15 min prior to the session.

Experiment 1a: Effect of 5- and 15-mg/kg naltrindole—Twenty-nine rats were divided into four groups ($n = 7-8$ per group) and underwent extinction. On the test day, three groups of rats were pre-treated with naltrindole (0, 5 or 15 mg/kg IP) and tested as described above for renewal in context A (ABA). An additional control group received vehicle injection and was tested in context B (ABB).

A separate cohort of rats ($n = 11$) were habituated to locomotor chambers (1h/day), handling and injection procedures (without drug) for at least four days, and then tested for locomotor activity 15 min following injections of vehicle and 15-mg/kg naltrindole IP in a counterbalanced repeated-measures design separated by four habituation days.

Experiment 1b: Effect of 7.5- and 10-mg/kg naltrindole—Based on the results of Experiment 1a, a second experiment was run using intermediate doses of naltrindole. Nine rats underwent extinction in context B. The effect of pre-treatment with vehicle, 7.5 and 10-mg/kg naltrindole on the renewal of alcohol seeking was tested in context A using a Latin square design. Rats were given at least two days in the extinction context (Context B) between tests.

Ten days following the renewal test, rats were habituated to the locomotor chambers as described above and tested 15 min following the administration of vehicle, 7.5 and 10-mg/kg naltrindole IP in a Latin square design. Test days were separated by four habituation days.

Experiment 1c: Effect of CTOP—Rats were trained to self-administer alcohol in Context A. Following recovery from the surgical implantation of ICV cannulae, rats were retrained for five days prior to extinction in context B. On the test day, rats were divided into three groups ($n = 7-8$) and pre-treated with the selective MOP receptor antagonist CTOP (0, 1 and 3- μ g/4 μ l, ICV) before being returned to Context A (self-administration context).

Ten days following the renewal test, rats were habituated to the locomotor chambers as described above, and administered 0, 1 or 3 μ g CTOP ICV 15 min prior to being placed in the activity chamber.

Experiment 2: Discrete cue-induced reinstatement—Rats were trained to self-administer alcohol as described above. After reaching stable alcohol self-administration at FR-3, they received 1-h/day extinction sessions where the discrete light+tone cue previously paired with alcohol delivery was not present and the alcohol infusion pump was inactivated. All rats were matched according to average alcohol intake on the last three days of self-administration and were habituated to the injection procedure on the four days preceding the

test day. The extinction criterion was at least two consecutive days with 15 or fewer lever presses. On the test day, a 5-s light+tone discrete cue (without alcohol delivery) marked the beginning of the test session, and for the remainder of the session the discrete cue was delivered on an FR-3 schedule, as it was during self-administration. Vehicle or drug was administered 15 min prior to the test sessions.

Experiment 2a: Effect of naltrindole—Twenty-six rats that underwent extinction were divided into three groups ($n = 8-9$) and tested as described above following an IP injection of vehicle, 1 or 5-mg/kg naltrindole HCl (Ciccocioppo *et al.*, 2002).

Experiment 2b: Effect of CTOP—Twelve rats demonstrating stable alcohol self-administration underwent surgery for intracranial implantations. Following 11–12 days of recovery, rats were returned to normal FR-3 self-administration conditions for 5 more days in order to re-establish a stable baseline. Rats were then extinguished as described above. Following extinction, rats were tested with vehicle, 1 μ g CTOP and 3 μ g CTOP in a repeated measures Latin square design.

Statistical analysis—All results are expressed as mean $\pm$ SEM. Total active and inactive lever presses were analyzed using two-way mixed ANOVAs, with day (extinction and test) as the repeated variable, and group as the repeated or between variable, depending on the experimental design. In addition, active lever presses during the test session were analyzed in 15-min intervals using two-way mixed ANOVAs, with time-interval as the repeated variable and group as the repeated or between variable. Total photocell counts were analyzed with a repeated measures t-test (2 groups), or a one-way ANOVA (> 2 groups). Newman Keuls was used for post-hoc comparisons on all ANOVAs.

Results

Experiment 1: Context-induced renewal

Experiments 1a and b: Effect of naltrindole—In Experiment 1a, rats drank an average of 0.83 ± 0.05 -g/kg alcohol (last 3 d). The total (1-h session) active and inactive lever presses made on the last day of extinction and on the renewal test day are presented by the treatment groups in Figure 1, panels A and B, respectively. Active lever responses show a dose-dependent effect of naltrindole on the context-induced renewal of alcohol seeking (day $\times$ group interaction: $F_{3,25} = 8.09$, $P < 0.01$). Post-hoc analyses revealed significant renewal (higher levels of bar pressing on the test day relative to the last extinction day) by ABA rats treated with 0 or 5-mg/kg naltrindole, but no renewal with 15-mg/kg naltrindole or vehicle-treated ABB rats. On the test day, ABA rats treated with 0- or 5-, but not 15-mg/kg naltrindole demonstrated greater active lever presses than vehicle-treated ABB rats. There were no differences between groups on the extinction day. For active lever presses, there was a significant main effect of test day ($F_{1,25} = 23.71$, $P < 0.01$) and group ($F_{3,25} = 6.11$, $P < 0.01$). No differences were detected for inactive lever presses.

Figure 1C presents the active lever presses made by all four treatment groups in 15-min intervals during the test session. A significant time $\times$ group interaction ($F_{3,75} = 6.425$, $P < 0.01$) showed that rats exposed to the ABA context sequence and treated with either 0 or 5-mg/kg naltrindole showed significantly higher active lever presses than either the ABB or ABA+5-mg/kg naltrindole groups only in the first 15 min of the session. There was a main effect of time interval ($F_{3,75} = 14.33$, $P < 0.01$) showing greatest responding in the initial 15 min of the session. There was also a main effect of group ($F_{3,75} = 5.37$, $P < 0.01$) showing greatest responding by ABA groups treated with 0- and 5-mg/kg naltrindole compared to the other groups.

A dose of 15-mg/kg naltrindole significantly reduced locomotor activity compared to vehicle (167 ± 26 vs. 308 ± 22 , $t_{10} = 4.90$, $P < 0.01$).

In Experiment 1b, rats drank an average of 1.02 ± 0.26 -g/kg alcohol (last 3 d). Active and inactive lever presses (1h) made on the last day of extinction and on the renewal test day are presented by the treatment conditions in panels D and E, respectively. Naltrindole produced a dose-dependent reduction in responses on the active lever (day $\times$ condition interaction: $F_{2,16} = 8.78$, $P < 0.01$), but had no effect on inactive lever presses. For active lever presses, there was also a significant main effect of test day ($F_{1,16} = 27.81$, $P < 0.01$) and treatment ($F_{2,16} = 7.31$, $P < 0.01$).

Figure 1F presents the active lever presses made in 15-min intervals during the test session. A significant time $\times$ group interaction ($F_{6,48} = 5.77$, $P < 0.01$) showed that treatment with 7.5 and 10-mg/kg naltrindole attenuated active lever presses in the first 15 min of the session relative to treatment with vehicle. There was a main effect of time interval ($F_{3,48} = 9.99$, $P < 0.01$) as rats showed greatest responding in the initial 15 min of the session. There was also a main effect of treatment ($F_{2,48} = 6.58$, $P < 0.01$) showing that 10-mg/kg naltrindole significantly reduced active lever presses relative to treatment with vehicle or 7.5-mg/kg naltrindole.

There was a significant reduction in locomotor activity induced by 10-mg/kg (404 ± 53 , $P < 0.05$), but not 7.5-mg/kg naltrindole (480 ± 50), relative to vehicle (615 ± 90 ; $F_{2,16} = 4.42$, $P < 0.05$).

Experiment 1c: Effect of CTOP—Rats drank an average of 0.65 ± 0.08 -g/kg alcohol on the last 3 d of self-administration. Figure 2 shows the active (A) and inactive (B) lever presses made on the last day of extinction and on the reinstatement test day between the treatment groups used in Experiment 2 in 1-h totals. For active lever presses, there was a significant main effect of test day ($F_{1,20} = 31.26$, $P < 0.01$), but no effect of group and no day $\times$ group interaction. Therefore, renewed lever pressing was not affected by CTOP in 1-h totals. There were no differences for inactive lever presses.

Figure 2C shows the active lever presses during the renewal test session in 15-min block time intervals. A significant time $\times$ group interaction ($F_{6,60} = 4.41$, $P < 0.01$) revealed that in the first 15 min of the test session, saline-infused control rats bar pressed more than rats infused with 1 or 3 μ g CTOP. At subsequent intervals, no differences were apparent between groups. The interaction also showed that saline-infused rats decreased bar presses over subsequent intervals, but that CTOP-treated rats maintained a constant rate of bar pressing throughout the session. A significant effect of time interval ($F_{3,60} = 17.44$, $P < 0.01$) showed the highest activity occurred in the first 15 min of the test session. There was no main effect for treatment group.

Pre-treatment with vehicle (613 ± 52), 1 μ g (617 ± 54) or 3 μ g (807 ± 82) CTOP ICV did not produce a significant difference in locomotor activity.

Experiment 2: Discrete Cue-Induced Reinstatement

Experiment 2a: Effect of Naltrindole—Rats drank an average of 0.54 ± 0.04 -g/kg alcohol on the last 3 d prior to undergoing extinction. Figure 3 shows the active (A) and inactive (B) lever presses made on the last day of extinction (no discrete cues present) and on the reinstatement test day (discrete light+tone cue present for all groups) for the three treatment groups. Reinstatement was observed in the control group but not in either of the naltrindole-treated groups (day $\times$ group interaction: $F_{2,23} = 22.42$, $P < 0.01$). Both 1- and 5-mg/kg doses of naltrindole significantly reduced active lever presses relative to controls. A

main effect of test day was observed ($F_{1,23} = 19.50$, $P < 0.01$), but no main effect for group. There were no significant differences for inactive lever presses.

Figure 3C presents the active lever presses made by the three treatment groups in 15-min intervals during the test session. There was a main effect of time interval ($F_{3,69} = 9.55$, $P < 0.01$) and treatment group ($F_{2,69} = 5.77$, $P < 0.01$), but no time $\times$ group interaction. This indicates that naltrindole reduced active lever presses in a time-independent manner.

Experiment 2b: Effect of CTOP—Rats drank an average of 0.80 ± 0.02 -g/kg alcohol on the last 3 d prior to undergoing extinction. Figure 4 shows the active (A) and inactive (B) lever presses made on the last day of extinction (no discrete cues present) and on the reinstatement test day (discrete light+tone cue present for all groups) of the three treatment groups. For active lever presses, there was a significant main effect of test day ($F_{1,22} = 17.89$, $P < 0.01$); but no effect of CTOP treatment, and no day $\times$ group interaction. CTOP did not significantly affect inactive lever presses.

Figure 4C presents the active lever presses made by all three treatment groups in 15-min intervals during the test session. There was a main effect of time interval ($F_{3,66} = 17.77$, $P < 0.01$) showing that active lever presses were greatest in the first 15 min of the test session relative to all subsequent intervals. There was no effect of CTOP and no time $\times$ group interaction.

Discussion

Consistent with other reports, we show that re-exposure to an alcohol-associated context or a discrete cue associated with alcohol-delivery can induce alcohol-seeking following extinction. Of interest, the first 15-min of the test session appeared to be the critical interval to observe the effect of these cues. In this study, we also show that the blockade of DOP or MOP receptors differentially alters the alcohol seeking induced by these cues. We show that DOP receptor activation is a critical component of alcohol seeking precipitated by discrete and contextual alcohol-associated cues. On the other hand, the results suggest that MOP receptors play a modest role in mediating context-induced renewal of alcohol seeking and are not involved in reinstatement induced by discrete cues. This is the first study that suggests a differential involvement of opioid receptor subtypes in mediating alcohol seeking induced by different types of cues.

Naltrindole, a DOP receptor antagonist, significantly reduced alcohol seeking induced by discrete cues and context. With discrete cues, this effect was observed even at the lowest dose (1-mg/kg). These findings are in line with those of Ciccocioppo and colleagues (2002) who showed that 5-mg/kg naltrindole attenuated reinstatement induced by a discrete (light +tone) plus discriminative (odour) cue compound associated with alcohol. Moreover, they found that the effect was specific to alcohol, as naltrindole had no effect with water-associated cues. In the present study, alcohol seeking induced by a context previously associated with the availability of alcohol appeared to be more resistant to naltrindole than that induced by discrete alcohol-associated cues. In Experiments 1a and 1b, 7.5 to 15-mg/kg naltrindole significantly attenuated context-induced renewal of alcohol seeking. It should be noted, that while at doses of 10 and 15- mg/kg naltrindole produced significant locomotor impairment, this was not the case with 7.5 mg/kg. This indicates that the effects of the higher doses of naltrindole were not specific to alcohol seeking.

Surprisingly, CTOP, the selective MOP receptor antagonist, had no effect on reinstatement induced by discrete alcohol-associated cues. This was unlikely due to an insufficient dose of CTOP since the doses we employed have been shown to interfere with operant alcohol self-

administration (Hyytia & Kiianmaa, 2001) and to alter context-induced alcohol seeking (present study, see below). In the only other study that assessed selective blockade of MOP receptors, 15-mg/kg naloxonazine, an irreversible MOP1 selective antagonist, blocked alcohol-seeking induced by a discrete-discriminative cue compound associated with alcohol (Ciccocioppo *et al.*, 2002). However, the effect of naloxonazine did not appear to be selective for alcohol.

The effect of CTOP on context-induced renewal produced an interesting pattern of responding. CTOP had no effect on alcohol seeking during the total 1-h session; however, when responding on the active lever was analyzed in 15-min intervals over the course of the test session, a different picture emerged. Both 1 and 3 μ g CTOP reduced active lever pressing in the first 15 min (Figure 2c). This initial interval is a critical period to observe alcohol seeking, as control rats demonstrate high rates of responding during this period that then decrease to very low levels for the remainder of the session. Since CTOP affected responding during this interval, it may be argued that CTOP specifically attenuated context-induced renewal.

Interestingly, in the context-induced renewal test session, CTOP-treated rats demonstrated a steady rate of responding that resulted in total active lever presses that did not differ from controls. A similar effect of CTOP was reported for alcohol self-administration (Hyytia & Kiianmaa, 2001). Based on the duration of neurochemical and behavioural effects of CTOP, the effect is not likely due to its rapid elimination (Spanagel *et al.*, 1992; Devine *et al.*, 1993; Badiani *et al.*, 1995). It is also not likely due to a nonspecific motoric effect because CTOP did not affect total inactive lever presses or locomotor activity in the photocell chambers and did not have any effect on discrete-cue induced reinstatement when measured in 15-min intervals. There is no clear explanation for the behavioural effect of CTOP in the renewal paradigm.

In previous studies, we have implicated opioidergic mechanisms in the dorsal hippocampus and lateral and basolateral nuclei of the amygdala in mediating context-induced renewal (Marinelli *et al.*, 2007). It is possible that alcohol seeking induced by discrete cues and contexts each involve distinct brain regions and separate opioidergic circuitries. The present data suggest that opioid peptides acting primarily on DOP receptors play an important role in mediating both discrete cue and context-induced alcohol seeking, but in different capacities given the dose of naltrindole required. It also implicates MOP receptors in the effects of context but not discrete cues. As a next step, the contribution of opioid receptor subtypes in specific brain regions to context and cue-induced reinstatement can be determined.

There are two methodological issues that need to be considered in the present study. First, the levels of alcohol consumption and the magnitude of alcohol seeking were variable between experiments. We and others typically observe a stronger effect of context than discrete cues on alcohol seeking (Tsiang & Janak, 2006). Factors such as cohort, route of drug administration and study design can also affect the variability of behavioural data. Variability in alcohol consumption and seeking is commonly noted between experiments in this type of study, even when using the same design. Importantly, each experiment in the present study was designed to allow the determination of the drug effect relative to a vehicle control group built into the design; therefore, variations in basal activity from experiment to experiment do not seriously compromise interpretation. Notably, all rats in the present study drank pharmacologically relevant concentrations of alcohol, every control condition demonstrated alcohol seeking (expressed as a significant increase in active lever responses relative to extinction levels), and every experiment incorporated appropriate counterbalances to minimize bias.

A second consideration is that context-induced renewal in ABA rats may have resulted from non-specific arousal caused by a switch in context and that opioid receptor blockade modified this effect. However, a number of studies examining context-induced reinstatement have incorporated a novel context control in their experimental design. In these instances, rats exposed to a novel context on the test day did not demonstrate enhanced seeking of alcohol (Burattini *et al.*, 2006; Zironi *et al.*, 2006), nicotine (Diergaarde *et al.*, 2008; Wing & Shoaib, 2008), heroin (Bossert *et al.*, 2004), cocaine (Fuchs *et al.*, 2005) or a heroin-cocaine (speedball) mixture (Crombag & Shaham, 2002). More importantly, findings from our laboratory have demonstrated that naltrexone does not modify the renewal of nicotine seeking (Lê *et al.*, in preparation).

The present study supports previous observations that both response-contingent discrete cues and non-contingent contexts are capable of reinstating or renewing alcohol seeking. The endogenous opioid system is involved in the mediation of alcohol seeking to both types of cues, and DOP receptors, in particular, appear to play an important role. A DOP receptor selective antagonist effectively reduced discrete cue and context-induced alcohol seeking, while the role of MOP receptors in the effects of these conditioned stimuli was less clear. MOP receptor blockade had no effect on discrete cue-induced reinstatement but produced a moderate and short-lived attenuation of context-induced renewal. Based on these results, we propose that future studies investigate therapies targeting DOP receptors in alcohol abuse and relapse.

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Abbreviations

CTOP	D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH ₂
DOP	delta opioid
FR	fixed ratio
ICV	intracerebroventricular
IP	intraperitoneally
KOP	kappa opioid
MOP	mu opioid

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Fig. 1. Effects of naltrindole on context-induced renewal

Active (A & D) and inactive (B & E) lever presses on the last day of extinction (drug-free day) and on the renewal test day. In Experiment 1a (Panels A–C), ABB+NDL0 were tested in the extinction context and administered the vehicle. ABA groups were instead tested in the context where alcohol was previously available following treatment with: vehicle (ABA+NDL0), 5-mg/kg naltrindole (ABA+NDL5) or 15-mg/kg naltrindole (ABA+NDL15), IP. In experiment 1b (Panels D–F), all rats were tested in the context where alcohol was previously available following treatment with: vehicle (ABA+NDL0), 7.5-mg/kg naltrindole (ABA+NDL7.5) or 10-mg/kg naltrindole (ABA+NDL10), IP. Active lever presses are also graphed for each treatment group in 15-min intervals (panels C & F) over the course of the

1-h test session. * denotes a significant difference from the extinction day within the treatment group ($P < 0.05$), † denotes a significant difference from the ABA+NDL0 condition on the test day ($P < 0.05$), § denotes a significant difference from the ABB+NDL0 group on the test day ($P < 0.05$).

Fig. 2. Effects of CTOP on context-induced renewal

Active (A) and inactive (B) lever presses on the last day of extinction (drug-free day) and on the renewal test day. On the renewal test day, all groups were tested in the context where alcohol was previously available following pre-treatment with vehicle (ABA+CTOP0), 1- μ g/4 μ l CTOP (ABA+CTOP1) or 3- μ g/4 μ l CTOP (ABA+CTOP3), ICV. Active lever presses are also graphed for each treatment group in 15-min intervals (C) over the course of the 1-h test session. * denotes a significant difference from the extinction day ($P < 0.05$), and † denotes a significant difference from the ABA+CTOP0 group on the test day ($P < 0.05$).

**A. Discrete Cue-induced Reinstatement:
Active Presses**

**B. Discrete Cue-induced Reinstatement:
Inactive Presses**

**C. Discrete Cue-induced Reinstatement:
Active Presses per 15 min**

Fig. 3. Effects of naltrindole on discrete cue-induced reinstatement

Active (A) and inactive (B) lever presses on the last day of extinction (drug-free day) and on the reinstatement test day. On the reinstatement test day all groups were tested in the presence of the response-contingent light+tone cue following pre-treatment with vehicle (NDL0), 1-mg/kg naltrindole (NDL1) or 5-mg/kg naltrindole (NDL5), IP. Active lever presses are also graphed for each treatment group in 15-min intervals (C) over the course of the 1-h test session. * denotes a significant difference from the extinction day within the treatment group ($P < 0.05$), and † denotes a significant difference from the NDL0 group on the test day ($P < 0.05$).

**A. Discrete Cue-induced Reinstatement:
Active Presses**

**B. Discrete Cue-induced Reinstatement:
Inactive Presses**

**C. Discrete Cue-induced Reinstatement:
Active Presses per 15 min**

Fig. 4. Effects of CTOP on discrete cue-induced reinstatement

Active (A) and inactive (B) lever presses on the last day of extinction (drug-free day) and on the reinstatement test day. On the reinstatement test day all groups were tested in the presence of the response-contingent light+tone cue following pre-treatment with vehicle (CTOP0), 1- μ g/4 μ l CTOP (CTOP1) or 3- μ g/4 μ l CTOP (CTOP3), ICV. Active lever presses are also graphed for each treatment group in 15-min intervals (C) over the course of the 1-h test session. * denotes a significant difference from the extinction day ($P < 0.05$).