Published in final edited form as:

Eur J Neurosci. 2008 December ; 28(11): 2288–2298. doi:10.1111/j.1460-9568.2008.06517.x.

Reinstated ethanol-seeking in rats is modulated by environmental context and requires the nucleus accumbens core

Nadia Chaudhri¹, Lacey L. Sahuque¹, Jackson J. Cone¹, and Patricia H. Janak^{1,2}

¹Ernest Gallo Clinic and Research Center, Department of Neurology, University of California at San Francisco, 5858 Horton Street Suite 200, Emeryville, CA 94608, USA

²Wheeler Center for the Neurobiology of Addiction, University of California at San Francisco, San Francisco, CA, USA

Abstract

The reinstatement of ethanol (EtOH)-seeking induced by an EtOH-predictive light-tone stimulus is enhanced in an environment associated with prior EtOH self-administration (SA) compared with a context associated with EtOH unavailability (Tsiang & Janak, 2006). Here we hypothesized that EtOH-seeking would be elicited by the conditioned sensory stimulus properties of EtOH and that this reinstatement would be similarly modulated by context. We also determined whether pharmacologically inactivating the nucleus accumbens (NAc), a key structure in relapse circuitry, would attenuate reinstated EtOH-seeking. Rats lever-pressed for oral EtOH (10% v/v) in operant conditioning chambers distinguished by specific visual, olfactory and tactile stimuli. Responding was then extinguished by withholding EtOH in a different context. EtOH-seeking, expressed as elevated responding without EtOH delivery, was subsequently tested by presenting an oral EtOH prime (two aliquots of 0.1 mL EtOH) in either the extinction or the prior EtOH-SA context. Rats received a microinfusion (0.3 µL/hemisphere) of saline or GABA agonists (muscimol/baclofen) into the NAc core or shell immediately before the reinstatement test. Robust EtOH-seeking was observed in the prior EtOH-SA but not the extinction context in saline-pretreated rats. This effect was significantly attenuated by inactivating the NAc core but not shell. Conversely, NAc shell inactivation significantly elevated lever-pressing in the extinction context. These data suggest that the sensory stimulus properties of oral EtOH can reinstate EtOH-seeking when experienced in the appropriate context and that functional activity in the NAc core is required for this effect. In contrast, the shell may normally inhibit incorrect behavioral responses.

Keywords

addiction; environment; inactivation; relapse; renewal; ventral striatum

© The Authors (2008). Journal Compilation © Federation of European Neuroscience Societies and Blackwell Publishing Ltd

Correspondence: Dr N. Chaudhri, as above. nchaudhri@gallo.ucsf.edu.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Latency (min) to the third active lever response after saline or muscimol/baclofen (M/B) infusions into the core or shell in the prior EtOH-SA context and the extinction context.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Introduction

Heightened craving and relapse to drinking in alcoholics can be precipitated by discrete stimuli (cues) that acquire conditioned incentive properties via repeated association with the pharmacological effects of ethanol (EtOH) (Niaura *et al.*, 1988; Tiffany & Conklin, 2000; Braus *et al.*, 2001). Cues can include EtOH-related sounds and images (Streeter *et al.*, 2002; Heinze *et al.*, 2007), and the specific sensory properties of EtOH, such as the smell and taste of a preferred alcoholic beverage (Staiger & White, 1991; Davidson *et al.*, 2003). Importantly, the environmental context wherein associations between stimuli and EtOH are learned can exert considerable control over conditioned subjective responses to EtOH cues (Ludwig, 1986; McCusker & Brown, 1990; Collins & Brandon, 2002).

Environmental contexts also influence EtOH-seeking in rats. We have shown that, in addition to directly stimulating EtOH-seeking (Burattini *et al.*, 2006; Zironi *et al.*, 2006), contexts can differentially modulate the reinstatement of EtOH-seeking induced by an EtOH-conditioned light-tone cue (Tsiang & Janak, 2006). In the latter study, mice were trained to lever-press for oral EtOH in operant conditioning chambers distinguished by specific visual, tactile and olfactory stimuli, and EtOH delivery was paired with a 2-s compound light-tone stimulus. Self-administration (SA) was then extinguished in a second, distinct context by withholding EtOH and the discrete cue. Subsequently, response-contingent presentations of the cue reinstated lever-pressing in the prior EtOH-SA context but did not affect behavior in the extinction context. Notably, cue presentations in the prior EtOH-SA context engendered greater reinstatement than that caused by placement into the same context without the cue.

These findings illustrate that the context within which an EtOH-conditioned light-tone cue is encountered can influence consequent behavioral responses. Given that EtOH taste and smell cues stimulate robust cue-reactivity in alcoholics, we hypothesized that EtOH-seeking would be induced by the sensory stimulus properties of EtOH in rats, and that this reinstatement would be modulated by the context in which EtOH was experienced. Thus, we compared reinstatement to a stimulus comprised of the sight, smell and taste of EtOH (two aliquots of 0.1 mL 10% EtOH; oral EtOH prime) presented in a context wherein rats had previously self-administered EtOH and in a context associated with the extinction of responding for EtOH.

We also determined whether functional activity in the nucleus accumbens (NAc) was necessary for EtOH-seeking triggered by the oral EtOH prime. The NAc is a critical component of neural circuitry underlying the reinstatement of drug-seeking (Kalivas & McFarland, 2003; Fuchs *et al.*, 2004; Everitt & Robbins, 2005) and is comprised of two functionally and anatomically distinct subregions, the core and shell, which may serve potentially different functions in addiction (Di Chiara, 2002; Everitt & Robbins, 2005). Given that the core has been implicated in cue-induced reinstatement of cocaine-seeking (Fuchs *et al.*, 2004) and the shell is innervated by hippocampal afferents (Yang & Mogenson, 1984; Thierry *et al.*, 2000) that may convey information about context, we hypothesized that inactivating either subregion would attenuate EtOH-seeking triggered by an EtOH prime in an EtOH-associated context.

Materials and methods

Subjects

Male Long Evans rats (Harlan, Indianapolis, IN, USA), weighing 260–280 g upon arrival, were individually housed in ventilated polycarbonate chambers and acclimated to the temperature- ($21 \pm 1^\circ\text{C}$) and light-regulated (12-h light/dark cycle, lights on at 07:00 hours) colony room. Rats had unrestricted access to standard rat chow and water (except for 4 days; see details below) throughout the study. All procedures were approved in advance by the Gallo Center Institutional Animal Care and Use Committee, and are in agreement with recommendations in

the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, 1996).

Apparatus

Experimental sessions were conducted in operant conditioning chambers (Med Associates Inc., St Albans, VT, USA) located in a different room from where the rats were housed. Chambers were contained within ventilated sound-attenuating cubicles and comprised a clear Plexiglas ceiling, front door and back wall, paneled aluminum side walls and a stainless steel bar floor. The right wall was fitted with two retractable levers that flanked a rectangular port containing a circular fluid receptacle. EtOH delivery into the receptacle occurred via a 20-mL syringe attached to a Hamilton syringe pump located outside the sound-attenuating cubicle. A white chamber light (28 V, 100 mA) located centrally near the ceiling on the left wall remained illuminated during operant sessions. EtOH delivery was controlled by a computer running Med PC IV (Med Associates Inc.) software, which also recorded responses on both levers.

Drugs

The EtOH (10% v/v) was prepared by combining 95% EtOH with tap water. Sweetened EtOH was made by dissolving sucrose (10% w/v) in 10% EtOH. The NAc core and shell were inactivated by intracranial microinfusion of a drug solution containing muscimol (GABA_A agonist; 0.1 mm; Sigma-Aldrich) and baclofen (GABA_B agonist; 1.0 mm; Sigma-Aldrich). The solution was prepared by dissolving 5.71 mg of muscimol and 106.85 mg of baclofen into 500 mL of sterile physiological saline, and has been shown previously to produce selective and dissociable effects on behavior when infused into the NAc core or shell (McFarland & Kalivas, 2001; Fuchs *et al.*, 2004; Floresco *et al.*, 2006).

EtOH pre-exposure

At 7 days after arrival rats were acclimated to the taste and pharmacological effects of EtOH in the colony room. To ensure that they all sampled EtOH, water was removed and rats were given only EtOH for 3 days. They next received concurrent 24-h access to water and sweetened EtOH for 2 weeks, followed by water and EtOH for 4 weeks. Rats were then habituated to limited EtOH access and given EtOH for 1 h/day and water for 23 h/day for 2 weeks. Body weights and liquid volumes consumed were recorded daily. Mean ($\pm$ SEM) EtOH intakes over the last 5 days of limited-EtOH access were 0.61 ± 0.04 and 0.82 ± 0.05 g/kg for the final complement of core- and shell-implanted rats, respectively.

Surgery

Standard stereotaxic procedures were used to implant bilateral, 26-gauge guide cannulae (Plastics One, Roanoke, VA, USA) targeting the NAc core and shell. Rats were anesthetized with isoflurane, their heads shaved and placed in a stereotaxic frame (Kopf, Tujunga, CA, USA). The scalp was incised to expose the skull, and bregma and lambda were used to estimate a flat skull position. The coordinates for cannula implantation were based on pilot surgeries: NAc core, AP +1.2, ML $\pm$ 2.0, DV -3.8 ; NAc shell, AP +1.6, ML $\pm$ 1.0, DV -4.0 . Microinfusions were performed using 33-gauge injectors that protruded 3 mm below the base of the cannulae (final DV: core, -6.8 ; shell, -7.0). Cannulae were anchored to the skull surface with dental cement and five metal screws, and occluded with metal obturators of the same length. Rats were treated postsurgically with buprenorphine (0.05 mg/kg, i.m. single injection) to minimize pain and monitored daily to ensure regular weight gain.

EtOH-SA and extinction

At 7 days after surgery, rats were water-restricted for 16 h and placed into the operant conditioning chambers for a single session wherein responding on the right (active) lever

delivered EtOH (0.1 mL over 3 s) into the fluid receptacle on a continuous reinforcement schedule. Responses on the left (inactive) lever had no consequence. The session ended after rats self-administered 20 mL of EtOH.

Rats were then allowed to self-administer EtOH in daily (Monday to Friday; between 08:00 and 11:00 hours) 60-min sessions. The onset of the white chamber light and the insertion of both levers into the chamber signaled the start of each session. The number of active lever-presses required to produce EtOH increased across sessions from a continuous reinforcement schedule (days 1–12) to a fixed-ratio 3 (FR3) schedule (EtOH delivered upon completion of three responses; days 13–40). Inactive lever-pressing was recorded but had no consequence.

The EtOH-SA sessions occurred in either of two contexts that were distinct across visual, tactile and olfactory domains. One context had clear Plexiglas chamber walls, a green grid floor and a strawberry-scented air freshener, which provided a strong visual and olfactory stimulus, taped to the outside of the chamber door. The second context had black chamber walls, a smooth Plexiglas floor and a single spray of acetic acid (30%) misted into the bedding below the floor. Contexts were kept constant across EtOH-SA sessions and rats were counterbalanced across both contexts based on EtOH intake during prior pre-exposure.

Upon completion of 40 EtOH-SA sessions, lever-pressing was extinguished in the context not used previously. Responding on the active lever activated the pump on an FR3 schedule but did not result in EtOH delivery. All other parameters were as described for prior EtOH-SA sessions. On day 7 of extinction rats were lightly restrained and infused with sterile saline (0.3 μ L/hemisphere; 0.3 μ L/min) into the NAc core or shell to habituate them to the microinfusion procedure. Injectors were left in place for 2 min postinfusion, after which rats were put back into their home-cages (located on carts within the behavioral testing room) for 7–9 min and then placed into the operant conditioning chambers. The same infusion procedure was utilized for each of four subsequent reinstatement tests.

Reinstatement

Testing occurred after a minimum of 12 extinction sessions, once rats achieved a criterion of 8 or less active lever-presses averaged across three consecutive extinction sessions. Using a within-subjects, Latin-square design, rats received intra-NAc saline or muscimol/baclofen (M/B) infusions before tests in either the prior EtOH-SA or the extinction context (resulting in four tests per subject). The start of each test session was signaled by the onset of the white chamber light, insertion of both levers into the chamber and presentation of a 0.2-mL oral EtOH prime that enabled access to the sight, smell and taste of EtOH but was not sufficient to induce a pharmacological effect. Half of the EtOH prime (0.1 mL) was delivered immediately into the fluid receptacle at the start of the test session to ensure that each subject was exposed to EtOH. The remainder (0.1 mL) was delivered upon completion of three active lever responses, in keeping with the method of EtOH delivery during prior SA sessions. Both non-contingent and contingent presentations were used to maximize the likelihood of reinstating lever-pressing (Kruzich *et al.*, 1999; Deroche-Gamonet *et al.*, 2002). During the remainder of the 60-min test session, active lever responses activated the pump on an FR3 schedule but did not deliver EtOH. Successive tests were separated by a minimum of five extinction sessions and conducted after animals had attained the criterion for extinction described above.

Histology

Upon completion of behavioral testing, rats were anesthetized with sodium pentobarbital (390 mg/kg) and perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed, postfixed in formalin and 25% sucrose, sectioned (60 μ m, coronal) and stained with cresyl violet to verify placement of cannulae and injector tips. Rats were excluded if injector

tips were not situated bilaterally in either the NAc core ($n = 2$ subjects excluded) or shell ($n = 2$ subjects excluded) as defined by the atlas of Paxinos & Watson (1997).

Statistical analyses

EtOH-SA was analysed using data averaged across the last full week of EtOH-SA (5 days, FR3) and compared with extinction data averaged across three sessions before the first test (according to the extinction criterion described above). *ANOVA* utilized Lever (Active, Inactive) and Phase (EtOH-SA, Extinction) as within-subjects repeated-measures and Context (see above for descriptions of the two contexts used for EtOH-SA) as the between-subjects factor. There was no main effect of Context in this or any subsequent analysis and therefore all analyses reported in the Results are collapsed across this factor.

Reinstatement was assessed by comparing active lever responding after saline pretreatment to the extinction baseline using planned paired-samples *t*-test comparisons within each Test Context (prior EtOH-SA context, Extinction context). The effect of M/B pretreatment on reinstatement was analysed using repeated-measures *ANOVA* with Treatment (saline, M/B), Test Context and Lever (Active, Inactive) as within-subjects factors. Significant main effects and interactions were further analysed with targeted two-way *ANOVA*s and paired-samples *t*-test comparisons.

Within-session active lever response patterns after saline or M/B pretreatment in the prior EtOH-SA context were examined by averaging responses during three 20-min intervals across the 60-min test session (0–20, 20–40 and 40–60 min). Resulting values were subjected to *ANOVA* with Treatment and Time as within-subjects factors. Latency data were analysed using non-parametric Wilcoxon Signed Ranks Tests. Data from one shell-implanted rat were not included in either response-pattern or latency analyses because time-stamp information for this subject was lost during testing. All analyses were conducted using spss (V11) with a significance level set to $\alpha = 0.05$.

Results

Histology

Figure 1 illustrates the placement of injector tips in either the NAc core or shell. Only rats with cannulae implanted bilaterally into each subregion were included in subsequent analyses (final sample sizes: core, $n = 12$; shell, $n = 11$).

EtOH-SA and extinction by rats with cannulae implanted into the NAc core or shell

Rats learned to self-administer EtOH (see Table 1); responding averaged over the last five sessions on the active lever was significantly higher than the inactive lever for both core- and shell-implanted rats (Lever: core, $F_{1,11} = 65.10$, $P < 0.001$; shell, $F_{1,10} = 165.95$, $P < 0.01$). Active lever-pressing decreased significantly in extinction, with no change in inactive lever responding (see Table 1) (Core: Phase, $F_{1,11} = 63.63$, $P < 0.001$; Lever, $F_{1,11} = 69.60$, $P < 0.001$; Phase $\times$ Lever, $F_{1,11} = 59.09$, $P < 0.001$; Shell: Phase, $F_{1,10} = 174.18$, $P < 0.01$; Lever, $F_{1,10} = 160.57$, $P < 0.001$; Phase $\times$ Lever, $F_{1,10} = 165.00$, $P < 0.001$).

It is very likely that rats in the present study experienced the pharmacological effects of EtOH in the SA context. Average ($\pm$ SEM) EtOH-intake values achieved across the last 5 days of SA on a continuous reinforcement schedule were 0.71 ± 0.07 g/kg for core and 0.62 ± 0.04 g/kg for shell rats. Although subsequent EtOH-intake values were lower on an FR3 schedule (see Table 1), similar doses have been shown to produce detectable levels of EtOH in the NAc (Nurmi *et al.*, 1999; 0.40 g/kg/h, voluntary oral consumption) and stimulate dopamine release in the NAc (Bassareo *et al.*, 2003; 0.33 g/kg, delivered via intraoral infusion) of rats.

Additionally, physiological effects of EtOH *in vitro* have also been demonstrated at low EtOH concentrations (Yin *et al.*, 2007 2–50 mM).

EtOH-seeking triggered by the sensory stimulus properties of EtOH is modulated by context

The saline treatment condition for each experimental group (NAc core and shell) allowed us to examine the reinstatement of EtOH-seeking stimulated by a small non-pharmacological EtOH sample and the modulation of this effect by context. As predicted, significant reinstatement occurred in the prior EtOH-SA context following saline infusion in either the NAc core (Fig. 2A) or shell (Fig. 2B). Compared with extinction baselines, active lever responding at test was significantly elevated in the prior EtOH-SA (core, shell; $P < 0.001$) but not the extinction (core, shell; $P > 0.05$) context.

The contextual modulation of reinstatement was further revealed by difference scores obtained by subtracting test responding in the extinction context from test responding in the prior EtOH-SA context. Individual points in Fig. 2A and B representing the difference score for each subject show that active lever responding elicited by the oral EtOH prime was greater in the prior EtOH-SA than the extinction context for a majority of rats in both the NAc core (Fig. 2A) and shell (Fig. 2B) groups. The mean difference score was also significantly greater than zero for each group (one-way *t*-test; core, $P < 0.05$; shell, $P < 0.01$).

Compared with extinction, responding on the inactive lever did not change significantly after saline infusion in either context for both core- and shell-implanted rats (data not shown).

NAc core inactivation attenuates EtOH-seeking triggered by an oral EtOH prime

The reinstatement of EtOH-seeking induced by an oral EtOH prime in the prior EtOH-SA context was significantly attenuated by inactivating the NAc core (Fig. 3A). ANOVA indicated significant main effects of Test Context ($F_{1,11} = 13.06$, $P < 0.01$) and Treatment ($F_{1,11} = 15.97$, $P < 0.01$), and a significant interaction (Test Context $\times$ Treatment, $F_{1,11} = 5.12$, $P < 0.05$). The EtOH stimulus elicited greater reinstatement in the prior EtOH-SA than the extinction context after saline pretreatment ($P < 0.01$). In the prior EtOH-SA context, active lever responding was significantly lower after M/B compared with saline pretreatment ($P < 0.01$), whereas in the extinction context, responding after M/B was not significantly reduced compared with saline ($P > 0.05$). There was also no difference in responding between the two M/B pretreatments ($P > 0.05$).

The analysis of inactive lever responding (Fig. 3B) indicated a significant main effect of Test Context ($F_{1,11} = 6.85$, $P < 0.05$) but no effect of Treatment ($F_{1,11} = 0.002$, $P > 0.05$) or Test Context $\times$ Treatment interaction ($F_{1,11} = 3.07$, $P > 0.05$). Inactive lever-pressing was higher in the prior EtOH-SA than the extinction context after saline infusion ($P < 0.05$).

In the prior EtOH-SA context, responding was significantly higher on the active compared with the inactive lever in both saline- and M/B-pretreated rats (compare left panels in Fig. 3A and B) (Treatment, $F_{1,11} = 7.50$, $P < 0.05$; Lever, $F_{1,11} = 37.98$, $P < 0.0001$; Treatment $\times$ Lever, $F_{1,11} = 19.99$, $P < 0.01$). Paired-samples *t*-tests verified a significant difference across lever after saline ($P < 0.001$) and M/B ($P < 0.05$) infusion. In the extinction context (compare right panels in Fig. 3A and B) there was no main effect of Treatment ($F_{1,11} = 0.90$, $P > 0.05$) or Lever ($F_{1,11} = 4.19$, $P > 0.05$) but a significant Treatment $\times$ Lever interaction ($F_{1,11} = 5.57$, $P < 0.05$). Paired-samples *t*-tests indicated a significant difference across levers after saline ($P < 0.05$) but not M/B ($P > 0.05$) pretreatment.

NAc shell inactivation does not attenuate EtOH-seeking triggered by an oral EtOH prime

In contrast to findings in the NAc core, rats showed robust reinstatement induced by the oral EtOH prime in the prior EtOH-SA context after either saline or M/B infusion in the NAc shell (Fig. 3C). anova indicated a significant main effect of Test Context ($F_{1,10} = 18.46$, $P < 0.01$) but not Treatment ($F_{1,10} = 0.91$, $P > 0.05$) and there was no significant Test Context $\times$ Treatment interaction ($F_{1,10} = 0.46$, $P > 0.05$). The EtOH stimulus elicited more active lever responding in the prior EtOH-SA than the extinction context after either saline ($P < 0.01$) or M/B ($P < 0.05$) infusion and there was no difference across treatment conditions in either test context ($P > 0.05$ for both comparisons).

The analysis of inactive lever responding (Fig. 3D) indicated a significant main effect of Treatment ($F_{1,10} = 6.01$, $P < 0.05$) and Test Context $\times$ Treatment interaction ($F_{1,10} = 8.45$, $P < 0.05$) but no effect of Test Context ($F_{1,10} = 0.39$, $P > 0.05$). There was no difference between saline and M/B infusion conditions in the prior EtOH-SA context ($P > 0.05$). In the extinction context, inactive lever responding after M/B pretreatment was significantly elevated compared with saline ($P < 0.01$). Additionally, after M/B pretreatment in the NAc shell, inactive lever-pressing was significantly elevated compared with extinction baselines (not shown) in both the prior EtOH-SA (extinction, 3.70 ± 0.52 ; M/B, 8.45 ± 1.49 ; $P < 0.05$) and extinction (extinction, 2.42 ± 0.49 ; M/B, 10.91 ± 2.80 ; $P < 0.01$) contexts.

In the prior EtOH-SA context, responding was significantly higher on the active compared with the inactive lever (compare left panels in Fig. 3C and D) for both infusion conditions (Treatment, $F_{1,10} = 0.13$, $P > 0.05$; Lever, $F_{1,10} = 134.68$, $P < 0.0001$; Treatment $\times$ Lever, $F_{1,10} = 0.01$, $P > 0.05$). Paired-samples *t*-tests verified these differences (saline, $P < 0.0001$; M/B, $P < 0.01$). Within the extinction context (compare right panels in Fig. 3C and D), responding was greater after M/B compared with saline (Treatment, $F_{1,10} = 7.28$, $P < 0.05$). There were no differences across Lever ($F_{1,10} = 1.52$, $P > 0.05$) for either infusion condition (Treatment $\times$ Lever, $F_{1,10} = 1.17$, $P > 0.05$). Interestingly, active lever-pressing was significantly elevated compared with the extinction baseline (not shown) after M/B infusion in the extinction context (extinction, 4.58 ± 0.57 ; test, 11.82 ± 2.15 ; $P < 0.05$).

Analysis of response pattern and latency data

Figure 4 illustrates response patterns on the active lever after either saline or M/B pretreatment in the prior EtOH-SA context. The pattern of extinction responding observed after saline infusion into either the core or shell was similar, i.e. responding decreased over time (0–20 > 20–40 min: $P < 0.01$ both core and shell; 0–20 > 40–60 min, $P < 0.001$ both core and shell). Compared with saline, M/B infusion in the core (Fig. 4A) significantly decreased active lever-pressing during the first 20 min of the test (Time, $F_{2,22} = 14.89$, $P < 0.0001$; Treatment, $F_{1,11} = 9.75$, $P < 0.01$; Time $\times$ Treatment, $F_{2,22} = 11.17$, $P < 0.0001$). In contrast, after M/B pretreatment in the shell, responding remained elevated for a longer time during the session, such that during the last 20 min rats produced more lever-presses after M/B than after saline (Time, $F_{2,18} = 20.35$, $P < 0.001$; Treatment, $F_{1,9} = 0.10$, $P > 0.05$; Time $\times$ Treatment, $F_{2,28} = 6.25$, $P < 0.01$).

At test, rats were exposed to an oral EtOH prime (0.2 mL), half of which was delivered non-contingently at the start of the session and the remainder upon completion of three responses on the active lever. Compared with extinction, presentation of the non-contingent aliquot of EtOH caused a marked reduction in latency to check the fluid receptacle after saline or M/B pretreatment in the NAc core, in the prior EtOH-SA (Fig. 5A, $P < 0.01$ both comparisons) and extinction (Fig. 5B, $P < 0.05$ for both comparisons) contexts. This finding indicates that subjects rapidly accessed the EtOH aliquot regardless of the context or pretreatment received. In contrast, compared with extinction, the latency to the first active lever response decreased

after saline pretreatment in the prior EtOH-SA (Fig. 5C, $P < 0.05$) but not the extinction (Fig. 5D, $P > 0.05$) context. The decrease in latency in the prior EtOH-SA context was attenuated by M/B infusion into the NAc core, such that the latency at test was not significantly different from the latency during extinction (Fig. 5C, $P > 0.05$). There was no difference between extinction and the M/B pretreatment condition in the extinction context (Fig. 5D, $P > 0.05$). This pattern of results obtained for the latency to the first active lever response was consistent with results obtained from the same analysis for the latency to the third active lever response (see Supporting information, Table S1).

As with core-implanted rats, the latency to check the fluid receptacle decreased significantly compared with extinction after saline or M/B pretreatment in the NAc shell, in the prior EtOH-SA (Fig. 6A, $P < 0.05$ for both comparisons) and extinction (Fig. 6B, $P < 0.05$ for both comparisons) contexts. However, in the prior EtOH-SA context the latency to the first active lever response was significantly lower after either saline or M/B pretreatment compared with extinction (Fig. 6C, $P < 0.05$ for both comparisons). There were no differences across extinction and test after either saline or M/B pretreatment in the extinction context (Fig. 6D). The same pattern of results was obtained when the latency to the third active lever response was analysed (in supporting Table S1).

Discussion

These data demonstrate that environmental contexts can modulate EtOH-seeking induced by the sensory stimulus properties of an oral EtOH prime. Robust reinstatement was observed in response to EtOH in a context associated with prior EtOH-SA but not in a context where operant responding had never been reinforced with EtOH. Reinstatement was markedly reduced by inactivating the NAc core. In contrast, inactivating the NAc shell did not prevent reinstatement in the prior EtOH-SA context but did significantly enhance inactive lever-pressing compared with extinction baselines in both test contexts. These findings indicate a role for the NAc in controlling context-specific EtOH-seeking triggered by the orosensory stimulus properties of EtOH, and agree broadly with a critical function for the NAc in the reinstatement of drug-seeking behavior.

EtOH-seeking, reinstatement and environmental context

The taste and smell of preferred alcoholic beverages are likely to acquire considerable incentive value as a function of their temporal contiguity with the eventual pharmacological consequences of EtOH intake. In support of this hypothesis, EtOH taste and smell cues potentiate subjective and physiological reactivity in humans (Kaplan *et al.*, 1985), and activate components of the mesocorticolimbic circuitry that are stimulated by visual EtOH cues (Braus *et al.*, 2001; Grusser *et al.*, 2004; Filbey *et al.*, 2007). Using a preclinical model of relapse, the present study provides strong evidence that the sight, smell and/or taste of EtOH can reinstate EtOH-seeking in rats (see also Chiamulera *et al.*, 1995).

Unlike other reports of reinstatement elicited by comparatively higher doses of experimenter-administered EtOH (Le *et al.*, 1998; Gass & Olive, 2007), the oral EtOH stimulus used to trigger EtOH-seeking in the present study was not sufficient to produce pharmacological effects. Reinstatement was therefore probably a function of the sensory stimulus characteristics of EtOH that had acquired conditioned incentive value through their relationship with the pharmacological effects of EtOH experienced previously during SA.

The EtOH prime may have enabled reinstatement by facilitating the retrieval of an association between the instrumental response (lever-pressing) and the delivery of a reinforcing outcome (EtOH). However, a recent analysis indicates an additional role for outcome/response associations in the appropriate selection of lever-press responding during non-contingent, food

pellet-primed reinstatement (Balleine & Ostlund, 2007). In this view, the discriminative stimulus properties of a non-contingent outcome can trigger responding. Whether response/outcome and/or outcome/response associations initiated reinstatement in the present study, it is clear that this process was gated by the context. As shown in Figs 5 and 6, subjects checked the fluid receptacle after EtOH delivery equally quickly in both the prior EtOH-SA and extinction contexts, but lever-pressing was only initiated in the EtOH-SA context presumably because that is where the EtOH outcome was associated with responding.

That the response-reinstating capacity of the oral EtOH prime was modulated by context is evident in the high degree of active lever responding achieved in the prior EtOH-SA context relative to the extinction context in saline-pretreated rats. This behavioral dissociation highlights the importance of contexts in mediating the formation, recall and execution of conditioned instrumental and Pavlovian associations (Bouton & Bolles, 1979; Bouton, 2004). It also suggests that the most effective trigger for reinstatement may not be the conditioned sensory stimulus properties of EtOH *per se* but the experience of those properties in a relevant environment. This interpretation is critical for alcoholics whose susceptibility to EtOH taste and smell cues may be heightened in an environment linked with past EtOH consumption (Conklin & Tiffany, 2002).

Contributions of the NAc core and shell to oral EtOH-priming-induced reinstatement

Pretreatment with M/B in the NAc core reduced reinstatement triggered by an oral EtOH prime in the prior EtOH-SA context, as revealed by a reduction in active lever-pressing and an increase in the latency to respond on the active lever compared with saline. Despite equally rapid exposure to the first aliquot of the EtOH prime under both treatment conditions and in both test contexts, a quickened onset of active lever-pressing was observed only after saline pretreatment in the prior EtOH-SA context. These observations suggest that after core inactivation, either the EtOH stimulus, environmental context or both were no longer motivationally significant, or that information about their motivational significance could not be translated into a motor outcome. Importantly, it is unlikely that the effects of core inactivation resulted from general locomotor inhibition. The concentration and injection volume of M/B used in the present study have been found to have no significant effects on locomotor activity or food-reinforced instrumental responding after infusion into either the core or shell (Fuchs *et al.*, 2008). Additionally, the robust decrease in latency to check the fluid receptacle for the EtOH prime after both saline and M/B infusion in both test contexts suggests that NAc inactivation did not dampen locomotor activity.

A requirement for the NAc core in behavioral paradigms that employ conditioned cues has been consistently demonstrated, prompting the theory that the core is necessary for the retrieval of conditioned associations or for the utilization of such information in the performance of goal-directed actions (Parkinson *et al.*, 2000; Hall *et al.*, 2001; Hutcheson *et al.*, 2001; Fuchs *et al.*, 2004; Yun *et al.*, 2004; Blaiss & Janak, 2007; Di Ciano *et al.*, 2007). The present finding that core inactivation abolished EtOH-seeking triggered by the stimulus properties of EtOH extends this literature and is the first demonstration that inactivation of this brain region blocks conditioned relapse to EtOH-seeking.

A recent study suggests that reinstatement triggered by environmental contexts may also be mediated by the NAc core. Using contexts similar to those used in the current study, Fuchs *et al.* (2008) found that inactivating either the core or shell reduced context-induced reinstatement of cocaine-seeking. This finding is of interest because no apparent reward-predictive discrete cues contributed to the effects of context on response reinstatement. Further work is necessary to determine whether the core can regulate the effects of contexts *per se* on response reinstatement in addition to its well-described role in mediating the influence of conditioned cues on responding; the anatomical means for such a role exists in that some hippocampal

afferents do innervate the core directly (Brog *et al.*, 1993) and regions that receive hippocampal input, such as the basolateral amygdala (Pitkanen *et al.*, 2000) and prelimbic cortex (Ishikawa & Nakamura, 2006), project to the core (Groenewegen *et al.*, 1996).

However, given the dense, direct hippocampal input to the NAc shell (Kelley & Domesick, 1982; Groenewegen *et al.*, 1987; Thierry *et al.*, 2000), we expected that inactivation of this region would block reinstatement by preventing the retrieval, utilization and/or expression of information that would enable the EtOH-associated and extinction contexts to be disambiguated. However, shell inactivation did not attenuate reinstatement in the prior EtOH-SA context, when measured as increased active lever responses and a decrease in latency to active lever-pressing after saline or M/B pretreatment compared with extinction. This outcome contrasts with findings that context-dependent reinstatement of alcohol-seeking increases c-fos expression in the shell (Hamlin *et al.*, 2007) and that context-dependent reinstatement of cocaine-seeking is blocked by shell inactivation (Fuchs *et al.*, 2008). Interestingly, in both studies, no discrete cue was employed. However, in procedures where a response-contingent cue is present in the conditioning and extinction contexts, shell manipulations also decrease context-dependent reinstatement of heroin-seeking (Bossert *et al.*, 2007). One explanation for why shell inactivation did not attenuate reinstatement in the present study may be that our multi-modal contexts included discrete cues that subjects used to disambiguate the contexts or that entered directly into cue-reward associations. Although the former still appears to rely on the hippocampus (Otto & Poon, 2006), the latter may be mediated by a neural circuit that is independent of the shell but utilizes projections from the basolateral amygdala to NAc core (Ito *et al.*, 2006, 2008). Although we cannot rule out this possibility, it seems unlikely given our previous findings that context-induced reinstatement of EtOH-seeking requires the complete multi-modal stimulus configuration; the olfactory stimulus alone, for example, is not sufficient (Burattini *et al.*, 2006). An alternative explanation for the present findings is that the delivery of the oral EtOH prime engages a distinct circuitry that does not depend upon or can over-ride the processing of contextual information by the shell.

The conclusion from the present findings that functional activity in the shell is not required for contextual modulation of EtOH-seeking must be tempered by the observations that, compared with response levels in extinction, inactivation of the shell significantly increased active lever-pressing at test in the extinction context as well as inactive lever-pressing in both test contexts (see Results). Therefore, one interpretation is that functional activity in the shell enables recognition of, or discrimination between, environmental contexts. Indeed, previous studies on reinstatement of EtOH responding have found that discriminative stimuli that signal EtOH availability elevate the expression of c-fos in both core and shell subregions (Dayas *et al.*, 2007). Accordingly, EtOH in the extinction context could still potentiate responding if that context no longer signified the absence of reinforcement. However, this is unlikely to be the full explanation for the present data, wherein reinstatement after shell inactivation was more robust in the prior EtOH-SA context than in the extinction context, indicating a somewhat intact ability to dissociate between contexts.

Alternatively, functional activity in the NAc shell could mediate the inhibition of incorrect behaviors (Yun *et al.*, 2004; Blaiss, 2007; Fuchs *et al.*, 2008). This hypothesis would account for both the inactivation-induced increase in inactive lever-pressing in the prior EtOH-SA context and the inactivation-induced elevation in responding in the extinction context that was not lever-specific. Further insight relevant to this proposed role for the shell is given by examining the time-course of active lever responding in the prior EtOH-SA context after shell inactivation (Fig. 4). Active lever-pressing is an appropriate (albeit maladaptive) response to make in the EtOH-associated context, at least initially, during the reinstatement test in the absence of EtOH reinforcement. However, whereas vehicle-pretreated rats showed rapid extinction of the unreinforced active lever response, M/B-pretreated rats continued to respond

at high levels throughout the test session. This abnormal pattern of responding after shell M/B infusion suggests that shell inactivation may have impaired recognition that active lever-pressing was no longer appropriate or prevented the inhibition of that response.

Conclusions

The conditioned orosensory stimulus properties of alcohol probably stimulate the initiation and contribute to the maintenance of alcohol consumption by humans. Using a rodent model of relapse to EtOH-seeking, our findings advocate that these cuing effects of alcohol may depend critically on the environmental context in which alcohol is experienced. In addition, they identify the NAc core as necessary for EtOH-seeking triggered by exposure to EtOH in an EtOH-associated context and suggest that the shell may be required to modulate response output. These data enrich a growing literature on the neural targets that mediate alcohol-seeking (Hamlin *et al.*, 2006, 2007; Zhao *et al.*, 2006; Dayas *et al.*, 2007; Bowers *et al.*, 2008). However, the control over behavior exerted by contexts is probably a manifestation of multiple, potentially dissociable associations between contextual stimuli, discrete cues, responses and outcomes. With this in mind, future research should aim to more precisely identify the behavioral targets of NAc inactivation when investigating the modulation of drug-seeking by environmental contexts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by NIAAA grant AA014925. The authors would like to thank Dr Y. Shaham and Dr T.M. Gill for discussions about this work.

Abbreviations

EtOH	ethanol
FR3	fixed-ratio 3
M/B	muscimol/baclofen
NAc	nucleus accumbens
SA	self-administration

References

- Balleine BW, Ostlund SB. Still at the choice-point: action selection and initiation in instrumental conditioning. *Ann. N.Y. Acad. Sci* 2007;1104:147–171. [PubMed: 17360797]
- Bassareo V, De Luca MA, Aresu M, Aste A, Ariu T, Di Chiara G. Differential adaptive properties of accumbens shell dopamine responses to ethanol as a drug and as a motivational stimulus. *Eur. J. Neurosci* 2003;17:1465–1472. [PubMed: 12713649]
- Blaiss, C. Doctoral Thesis. Department of Neurology, University of California at San Francisco; San Francisco, CA: 2007. Formation and maintenance of appetitive Pavlovian associations..
- Blaiss, C.; Janak, P. Program No. 425.9.2007 Neuroscience Meeting Planner. Society for Neuroscience; San Diego, CA: 2007. 2007. The role of the nucleus accumbens in the acquisition and expression of appetitive and aversive Pavlovian conditioning.. Online
- Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. *J. Neurosci* 2007;27:12655–12663. [PubMed: 18003845]

- Bouton ME. Context and behavioral processes in extinction. *Learn Mem* 2004;11:485–494. [PubMed: 15466298]
- Bouton ME, Bolles RC. Role of conditioned contextual stimuli in reinstatement of extinguished fear. *J. Exp. Psychol. Anim. Behav. Process* 1979;5:368–378. [PubMed: 528893]
- Bowers MS, Hopf FW, Chou JK, Guillory AM, Chang SJ, Janak PH, Bonci A, Diamond I. Nucleus accumbens AGS3 expression drives ethanol seeking through G betagamma. *Proc. Natl Acad. Sci. U.S.A* 2008;105:12533–12538. [PubMed: 18719114]
- Braus DF, Wrase J, Grusser S, Hermann D, Ruf M, Flor H, Mann K, Heinz A. Alcohol-associated stimuli activate the ventral striatum in abstinent alcoholics. *J. Neural Transm* 2001;108:887–894. [PubMed: 11515754]
- Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J. Comp. Neurol* 1993;338:255–278. [PubMed: 8308171]
- Burattini C, Gill TM, Aicardi G, Janak PH. The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. *Neuroscience* 2006;139:877–887. [PubMed: 16516392]
- Chiamulera C, Valerio E, Tessari M. Resumption of ethanol-seeking behaviour in rats. *Behav. Pharmacol* 1995;6:32–39. [PubMed: 11224309]
- Collins BN, Brandon TH. Effects of extinction context and retrieval cues on alcohol cue reactivity among nonalcoholic drinkers. *J. Consult. Clin. Psychol* 2002;70:390–397. [PubMed: 11952197]
- Conklin CA, Tiffany ST. Applying extinction research and theory to cue-exposure addiction treatments. *Addiction* 2002;97:155–167. [PubMed: 11860387]
- Davidson D, Tiffany ST, Johnston W, Flury L, Li TK. Using the cue-availability paradigm to assess cue reactivity. *Alcohol. Clin. Exp. Res* 2003;27:1251–1256. [PubMed: 12966318]
- Dayas CV, Liu X, Simms JA, Weiss F. Distinct patterns of neural activation associated with ethanol seeking: effects of naltrexone. *Biol. Psychiatry* 2007;61:979–989. [PubMed: 17098214]
- Deroche-Gamonet V, Piat F, Le Moal M, Piazza PV. Influence of cue-conditioning on acquisition, maintenance and relapse of cocaine intravenous self-administration. *Eur. J. Neurosci* 2002;15:1363–1370. [PubMed: 11994130]
- Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behav. Brain Res* 2002;137:75–114. [PubMed: 12445717]
- Di Ciano P, Robbins TW, Everitt BJ. Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. *Neuropsychopharmacology* 2007;33:1413–1425. [PubMed: 17712353]
- Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat. Neurosci* 2005;8:1481–1489. [PubMed: 16251991]
- Filbey FM, Claus E, Audette AR, Niculescu M, Banich MT, Tanabe J, Du YP, Hutchison KE. Exposure to the taste of alcohol elicits activation of the mesocorticolimbic neurocircuitry. *Neuropsychopharmacology* 2007;33:1391–1401. [PubMed: 17653109]
- Floresco SB, Ghods-Sharifi S, Vexelman C, Magyar O. Dissociable roles for the nucleus accumbens core and shell in regulating set shifting. *J. Neurosci* 2006;26:2449–2457. [PubMed: 16510723]
- Fuchs RA, Evans KA, Parker MC, See RE. Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)* 2004;176:459–465.
- Fuchs RA, Ramirez DR, Bell GH. Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)* 2008;200:545–556. [PubMed: 18597075]
- Gass JT, Olive MF. Reinstatement of ethanol-seeking behavior following intravenous self-administration in Wistar rats. *Alcohol. Clin. Exp. Res* 2007;31:1441–1445. [PubMed: 17666107]
- Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience* 1987;23:103–120. [PubMed: 3683859]
- Groenewegen HJ, Wright CI, Beijer AV. The nucleus accumbens: gateway for limbic structures to reach the motor system? *Prog. Brain Res* 1996;107:485–511. [PubMed: 8782538]

- Grusser SM, Wrase J, Klein S, Hermann D, Smolka MN, Ruf M, Weber-Fahr W, Flor H, Mann K, Braus DF, Heinz A. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology (Berl)* 2004;175:296–302. [PubMed: 15127179]
- Hall J, Parkinson JA, Connor TM, Dickinson A, Everitt BJ. Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. *Eur. J. Neurosci* 2001;13:1984–1992. [PubMed: 11403692]
- Hamlin AS, Blatchford KE, McNally GP. Renewal of an extinguished instrumental response: neural correlates and the role of D1 dopamine receptors. *Neuroscience* 2006;143:25–38. [PubMed: 16949214]
- Hamlin AS, Newby J, McNally GP. The neural correlates and role of D1 dopamine receptors in renewal of extinguished alcohol-seeking. *Neuroscience* 2007;146:525–536. [PubMed: 17360123]
- Heinze M, Wolfing K, Grusser SM. Cue-induced auditory evoked potentials in alcoholism. *Clin. Neurophysiol* 2007;118:856–862. [PubMed: 17307391]
- Hutcheson DM, Parkinson JA, Robbins TW, Everitt BJ. The effects of nucleus accumbens core and shell lesions on intravenous heroin self-administration and the acquisition of drug-seeking behaviour under a second-order schedule of heroin reinforcement. *Psychopharmacology (Berl)* 2001;153:464–472. [PubMed: 11243494]
- Ishikawa A, Nakamura S. Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. *J. Neurophysiol* 2006;96:2134–2138. [PubMed: 16837666]
- Ito R, Robbins TW, McNaughton BL, Everitt BJ. Selective excitotoxic lesions of the hippocampus and basolateral amygdala have dissociable effects on appetitive cue and place conditioning based on path integration in a novel Y-maze procedure. *Eur. J. Neurosci* 2006;23:3071–3080. [PubMed: 16819997]
- Ito R, Robbins TW, Pennartz CM, Everitt BJ. Functional interaction between the hippocampus and nucleus accumbens shell is necessary for the acquisition of appetitive spatial context conditioning. *J. Neurosci* 2008;28:6950–6959. [PubMed: 18596169]
- Kalivas PW, McFarland K. Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology (Berl)* 2003;168:44–56. [PubMed: 12652346]
- Kaplan RF, Cooney NL, Baker LH, Gillespie RA, Meyer RE, Pomerleau OF. Reactivity to alcohol-related cues: physiological and subjective responses in alcoholics and nonproblem drinkers. *J. Stud. Alcohol* 1985;46:267–272. [PubMed: 4033125]
- Kelley AE, Domesick VB. The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. *Neuroscience* 1982;7:2321–2335. [PubMed: 6817161]
- Kruzich PJ, Grimm JW, Rustay NR, Parks CD, See RE. Predicting relapse to cocaine-seeking behavior: a multiple regression approach. *Behav Pharmacol* 1999;10:513–521. [PubMed: 10780257]
- Le AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y. Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. *Psychopharmacology (Berl)* 1998;135:169–174. [PubMed: 9497022]
- Ludwig AM. Pavlov's “bells” and alcohol craving. *Addict. Behav* 1986;11:87–91. [PubMed: 3739808]
- McCusker CG, Brown K. Alcohol-predictive cues enhance tolerance to and precipitate “craving” for alcohol in social drinkers. *J. Stud. Alcohol* 1990;51:494–499. [PubMed: 2270057]
- McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J. Neurosci* 2001;21:8655–8663. [PubMed: 11606653]
- Niaura RS, Rohsenow DJ, Binkoff JA, Monti PM, Pedraza M, Abrams DB. Relevance of cue reactivity to understanding alcohol and smoking relapse. *J. Abnorm. Psychol* 1988;97:133–152. [PubMed: 3290304]
- Nurmi M, Kiianmaa K, Sinclair JD. Brain ethanol levels after voluntary ethanol drinking in AA and Wistar rats. *Alcohol* 1999;19:113–118. [PubMed: 10548154]
- Otto T, Poon P. Dorsal hippocampal contributions to unimodal contextual conditioning. *J. Neurosci* 2006;26:6603–6609. [PubMed: 16775148]
- Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ. Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behav. Neurosci* 2000;114:42–63. [PubMed: 10718261]

- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; New York, NY: 1997.
- Pitkanen A, Pikkarainen M, Nurminen N, Ylinen A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann. N.Y. Acad. Sci* 2000;911:369–391. [PubMed: 10911886]
- Staiger PK, White JM. Cue reactivity in alcohol abusers: stimulus specificity and extinction of the responses. *Addict. Behav* 1991;16:211–221. [PubMed: 1776539]
- Streeter CC, Gulliver SB, Baker E, Blank SR, Meyer AA, Ciraulo DA, Renshaw PF. Videotaped cue for urge to drink alcohol. *Alcohol. Clin. Exp. Res* 2002;26:627–634. [PubMed: 12045470]
- Thierry AM, Gioanni Y, Degenetais E, Glowinski J. Hippo-campo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus* 2000;10:411–419. [PubMed: 10985280]
- Tiffany ST, Conklin CA. A cognitive processing model of alcohol craving and compulsive alcohol use. *Addiction* 2000;95(Suppl. 2):S145–S153. [PubMed: 11002909]
- Tsiang MT, Janak PH. Alcohol seeking in C57BL/6 mice induced by conditioned cues and contexts in the extinction-reinstatement model. *Alcohol* 2006;38:81–88. [PubMed: 16839854]
- Yang CR, Mogenson GJ. Electrophysiological responses of neurones in the nucleus accumbens to hippocampal stimulation and the attenuation of the excitatory responses by the mesolimbic dopaminergic system. *Brain Res* 1984;324:69–84. [PubMed: 6151418]
- Yin HH, Park BS, Adermark L, Lovinger DM. Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum. *Eur. J. Neurosci* 2007;25:3226–3232. [PubMed: 17552991]
- Yun IA, Nicola SM, Fields HL. Contrasting effects of dopamine and glutamate receptor antagonist injection in the nucleus accumbens suggest a neural mechanism underlying cue-evoked goal-directed behavior. *Eur. J. Neurosci* 2004;20:249–263. [PubMed: 15245497]
- Zhao Y, Dayas CV, Aujla H, Baptista MA, Martin-Fardon R, Weiss F. Activation of group II metabotropic glutamate receptors attenuates both stress and cue-induced ethanol-seeking and modulates c-fos expression in the hippocampus and amygdala. *J. Neurosci* 2006;26:9967–9974. [PubMed: 17005860]
- Zironi I, Burattini C, Aicardi G, Janak PH. Context is a trigger for relapse to alcohol. *Behav. Brain Res* 2006;167:150–155. [PubMed: 16256214]

Fig. 1. Placement of injector tips within the NAc core ($n = 12$, filled circles) and shell ($n = 11$, open squares), according to the rat brain atlas of Paxinos & Watson (1997).

Fig. 2. Mean ($\pm$ SEM) responding on the active lever in saline-pretreated rats (filled bars) that were exposed to an oral EtOH prime in either the prior EtOH-SA or extinction context. Extinction baselines (open bars) were obtained using data averaged across three sessions before the test. A difference score (open triangles, average indicated by horizontal line) was obtained by subtracting test responding in the extinction context from test responding in the prior EtOH-SA context. (A) NAC core, (B) NAC shell. Symbols denote significant outcomes from paired-samples t-test comparisons: $**P < 0.0001$ significant difference from corresponding extinction baseline.

Fig. 3.

Mean ($\pm$ SEM) responding on the active (A and C) and inactive (B and D) levers after either saline (filled bars) or M/B (hatched bars) infusions into the NAc core (A and B) or NAc shell (C and D). At test, rats were exposed to an oral EtOH prime in either the prior EtOH-SA or extinction context. Symbols denote significant outcomes from paired-samples t-test comparisons: ^^ $P < 0.01$ significant difference between saline and M/B within context; ## $P < 0.01$, # $P < 0.05$ significant difference across contexts within either saline or M/B infusion condition.

Fig. 4. Mean ($\pm$ SEM) active lever responses in 20-min intervals after a saline or M/B test in the prior EtOH-SA context for core (A) and shell (B) rats. Symbols denote significant outcomes from paired-samples *t*-test comparisons: ** $P < 0.01$, * $P < 0.05$ significant difference between saline and M/B.

Fig. 5.

Mean ($\pm$ SEM) latency to the first port entry into the EtOH-delivery receptacle (A and B) and to the first active lever-press (C and D) after saline (filled bars) or M/B (hatched bars) tests in the prior EtOH-SA (A and C) or extinction (B and D) context for NAc core-implanted rats. Extinction baselines (open bars) were obtained using data averaged across three sessions before the test. Symbols denote significant outcomes from non-parametric Wilcoxon Signed Ranks Test: * $P < 0.05$ significant difference between test and corresponding extinction.

Fig. 6.

Mean ($\pm$ SEM) latency to the first port entry into the EtOH-delivery receptacle (A and B) and to the first active lever-press (C and D) after saline (filled bars) or M/B (hatched bars) tests in the prior EtOH-SA (A and C) or extinction (B and D) context for NAc shell-implanted rats. Extinction baselines (open bars) were obtained using data averaged across three sessions before the test. Symbols denote significant outcomes from non-parametric Wilcoxon Signed Ranks Test: * $P < 0.05$ significant difference between test and corresponding extinction.

Table 1

Behavioral measures (mean $\pm$ SEM) averaged across the final 5 days of EtOH-SA and the final three extinction sessions before the first reinstatement test

	Active lever	Inactive lever	EtOH intake (mL)	EtOH intake (g/kg)
Core ($n = 12$)				
EtOH-SA	78.65 $\pm$ 4.81	4.02 $\pm$ 0.71 **	2.45 $\pm$ 0.15	0.37 $\pm$ 0.02
Extinction	5.25 $\pm$ 0.86 ***	1.78 $\pm$ 0.30 *	–	–
Shell ($n = 11$)				
EtOH-SA	81.05 $\pm$ 3.77	4.20 $\pm$ 0.50 **	2.56 $\pm$ 0.12	0.38 $\pm$ 0.02
Extinction	5.82 $\pm$ 0.60 ***	3.82 $\pm$ 0.57 *	–	–

Symbols denote significant outcomes from paired-samples *t*-test comparisons:

**
 $P < 0.001$

*
 $P < 0.05$ significant difference from corresponding active lever

 $P < 0.001$ significant difference from active lever during EtOH-SA.