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Enhanced alcohol-seeking behavior by nicotine in the posterior ventral tegmental area of female alcohol-preferring (P) rats: modulation by serotonin-3 and nicotinic cholinergic receptors

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Abstract

Rationale—Alcohol and nicotine co-use can reciprocally promote self-administration and drug-craving/drug-seeking behaviors. To date, the neurocircuitry in which nicotine influences ethanol (EtOH) seeking has not been elucidated. Clinical and preclinical research has suggested that the activation of the mesolimbic dopamine system is involved in the promotion of drug seeking. Alcohol, nicotine, and serotonin-3 (5-HT_3) receptors interact within the posterior ventral tegmental area (pVTA) to regulate drug reward. Recently, our laboratory has reported that systemic administration of nicotine can promote context-induced EtOH seeking.

Objectives—The goals of the current study were to (1) determine if microinjections of pharmacologically relevant levels of nicotine into the pVTA would enhance EtOH seeking, (2) determine if coadministration of nicotinic cholinergic receptor antagonist (nACh) or 5-HT_3 receptor antagonists would block the ability of nicotine microinjected into the pVTA to promote EtOH seeking, and (3) determine if 5-HT_3 receptors in the pVTA can modulate EtOH seeking.

Results—Nicotine (100 and 200 μM) microinjected into the pVTA enhanced EtOH seeking. Coinfusion with 200 μM mecamylamine (nACh antagonist) or 100 and 200 μM zacopride (5-HT_3 receptor antagonist) blocked the observed nicotine enhancement of EtOH seeking. The data also indicated that microinjection of 1 μM CPBG (5-HT_3 receptor agonist) promotes context-induced EtOH seeking; conversely microinjection of 100 and 200 μM zacopride alone reduced context-induced EtOH seeking.

Conclusions—Overall, the results show that nicotine-enhanced EtOH-seeking behavior is modulated by 5-HT_3 and nACh receptors within the pVTA and that the 5-HT_3 receptor system within pVTA may be a potential pharmacological target to inhibit EtOH-seeking behaviors.

Keywords

Alcohol-seeking behavior; Serotonin-3 receptors; Nicotinic cholinergic receptors; Alcohol-preferring P rat; Pavlovian Spontaneous Recovery; Zacopride; Mecamylamine

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Conflict of interest I certify that there is no actual or potential conflict of interest in relation to this article.

Introduction

Alcohol and nicotine are also commonly used together, and the co-abuse of both drugs can increase use and relapse more than either drug alone. The severity of nicotine dependency is linked to more severe levels of alcohol relapse (Abrams et al. 1992; Gulliver et al. 1995) and impairs the likelihood that an individual with alcohol dependence will succeed in becoming abstinent if they continue to smoke during this period (Gulliver et al. 1995; Sobell et al. 1995; Daeppen et al. 2000). Preclinical studies have provided evidence that nicotine can enhance relapse behaviors, such as ethanol (EtOH) seeking (Le et al. 2003; Hauser et al. 2012a) and EtOH relapse drinking (Lopez-Moreno et al. 2004; Alen et al. 2009; Hauser et al. 2012a).

The activation of dopamine (DA) neurons in the ventral tegmental area (VTA) plays a critical role in drug reward/reinforcement and compulsive drug-seeking behaviors; both EtOH (Brodie et al. 1990; Brodie et al. 1999) and nicotine (Calabresi et al. 1989; Nisell et al. 1994) can activate VTADA neurons. Nicotinic acetylcholine (nACh) receptors within the VTA are thought to be one of the molecular targets underlying EtOH and nicotine co-abuse. The reinforcing effects of nicotine are modulated via stimulation of nACh receptors within the VTA (Corrigall et al. 1994; Nisell et al. 1994); studies have shown that the reinforcing effects of EtOH may be partially modulated by nACh receptors (Blomqvist et al. 1996; Ericson et al. 2003; Soderpalm et al. 2000). EtOH intake (Le et al. 2000; Hendrickson et al. 2009) and nicotine-stimulated EtOH drinking (Smith et al. 1999; Sajja and Rahman 2012) can be reduced by mecamylamine (Mec), a nonselective nACh receptor antagonist.

A few studies have examined the involvement of nACh receptors in EtOH-seeking behavior. Local infusion of α-conotoxin MII (α3β2, β3, and α6 nicotinic antagonists) into the VTA and systemic administration of Mec can reduce cue-induced EtOH seeking, whereas dihydro-β-erythroidine (α4β2 nicotinic antagonist) did not have an effect (Lof et al. 2007). In addition, varenicline, an α4β2 nicotinic receptor partial agonist, reduced EtOH-seeking behavior (Steensland et al. 2007).

The nACh and serotonin-3 (5-HT_3) receptors are members of the Cys-loop ligand-gated ion channel superfamily; 5-HT_3 receptors share up to 30 % sequence homology of nACh receptors. Nicotine binds with a higher affinity to the 5-HT_3 receptor compared to any nACh receptor (Jackson and Yakel 1995; Breitinger et al. 2001; Gurley and Lanthorn 1998). In addition, the nACh receptor antagonist Mec and epibatidine (nicotinic agonist) have at least a fourfold greater affinity for the 5-HT_3 receptor than the nACh receptor (Drisdel et al. 2008). In contrast, zacopride (Zac), a 5-HT_3 receptor antagonist, does not have an appreciable affinity for nicotinic receptors (Kidd et al. 1993).

The posterior VTA (pVTA) mediates the reinforcing actions of EtOH (Rodd-Henricks et al. 2000) and nicotine (Ikemoto et al. 2006; Hauser et al. 2013). The activation of 5-HT_3 receptors is involved in mediating the reinforcing effects of EtOH (Rodd-Henricks et al. 2003) and nicotine (Hauser et al. 2013) within the pVTA. The pVTA is also involved in mediating context-induced EtOH-seeking behavior via activation of local DA neurons

(Hauser et al. 2011). Because of the similarities in structure between nACh and 5-HT_3 receptors (Mascia et al. 2000; Peters et al. 2006), and the potential interactions of these receptors and of nicotine at the 5-HT_3 receptor (Bianchi et al. 1995; Gurley and Lanthorn 1998; Nayak et al. 2000; Dougherty and Nichols 2009), it is possible that some of the effects of nicotine on EtOH seeking within the pVTA may be mediated in part through activation of 5-HT_3 receptors.

The selectively bred alcohol-preferring (P) line of rats appears to be a useful animal model to study EtOH and nicotine use. P rats will intravenously (i.v.) self-administer more nicotine as well as express greater nicotine-seeking behavior than the alcohol-nonpreferring (NP) rats (Le et al. 2006a). Nicotine also has greater reinforcing effects in P than NP rats (Le at al. 2006a). Intracranial self-administration studies have provided further support that EtOH and nicotine may share common genetic risk factors because P rats are more sensitive to the reinforcing effects of EtOH (Rodd et al. 2003) and nicotine (Hauser et al. 2013) within the pVTA compared to Wistar rats.

Context has been shown to influence extinction learning and reinstatement of previously learned behaviors (Bouton 2002). In a spontaneous recovery paradigm, subjects are allowed to self-administer in a specific environment, the behavior is extinguished in the same environment, the subjects are withheld from that environment for a certain time, and behavior is recorded when the animals are returned to the original environment. Since spontaneous recovery is defined in the alcohol clinical literature as the cessation of alcohol consumption in human alcoholics without treatment intervention, we have altered the term to Pavlovian Spontaneous Recovery (PSR). PSR is a unique phenomenon in that it is time dependent (Bouton 1988), directly correlated to reward saliency (Robbins 1990), and contextual cues associated with first-learned signals and the amount of first- and second-learned associations (Brooks 2000).

The objectives of the current study were to test the hypothesis that local application of nicotine within the pVTA would enhance context-induced EtOH-seeking behavior and that nicotine's stimulating effects are mediated in part via activation of 5-HT_3 and nACh receptors in the pVTA. In addition, we tested the hypothesis that the activation of 5-HT_3 receptors alone in the pVTA is involved in mediating context-induced EtOH-seeking behavior.

Methods

Animals

Adult EtOH-naïve female P rats from the 70th generation weighing 250–325 g at the start of the experiment were used. Female rats were used in the present study because they maintain their body and head size better than male rats for more accurate and reliable stereotaxic placements. Rats were maintained on a 12-h reversed light dark cycle (lights off at 0900 hours). Food and water were available in the home cage ad libitum throughout the experiment. All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the

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

Chemical agents and vehicle

Nicotine HCl (Sigma, St. Louis, MO), Mec (Sigma, St. Louis, MO), Zac (Tocris Bioscience, Ellisville, MO), and 1-(m-chlorophenyl)-biguanide (CPBG, Tocris Bioscience, Ellisville, MO) were dissolved in artificial cerebrospinal fluid (aCSF), and the pH was adjusted to 7.4±0.1. The aCSF consisted of (in millimolar) 120.0 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, and 10.0 D-glucose.

Operant response training

P rats were placed in the standard two-lever operant-conditioning chamber, as previously described (Rodd-Henricks et al. 2002a, b; Rodd et al. 2006; Hauser et al. 2011, 2012a). Operant-conditioning sessions were 60 min in duration and occurred daily for 10 weeks. The EtOH concentration used during self-administration was 15% (*v/v*). During the initial 4 weeks of daily access to operant responding, both solutions (water and EtOH) were reinforced on a fixed ratio 1 (FR1) schedule. The response requirement for EtOH was increased to FR3 for 3 weeks, and then to FR5 for 3 weeks. After the P rats had established stable levels of responding on the FR5 schedule for EtOH and FR1 for water, they underwent seven sessions of extinction training (60 min/session), when neither water nor EtOH was available.

Stereotaxic surgeries

After extinction training, all rats were maintained in the home cages for 14 days. Previous research has shown that 2 weeks of home caging produced robust expression of EtOH seeking (Rodd-Henricks et al. 2002a, b; Rodd et al. 2006). Stereotaxic implantation was performed after 7 days in the home cage. While under isoflurane anesthesia, rats were prepared for bilateral stereotaxic implantation of 22-gauge guide cannula (Plastics One, Roanoke, VA) into the pVTA; the guide cannula was aimed 1.0 mm above the target region. Coordinates (Paxinos and Watson 1998) for placements to target the pVTA were −5.8 mm posterior to bregma, +2.1 mm lateral to the midline, and −8.5 mm ventral from the surface of the skull at a 10° angle to the vertical. A 28-gauge stylet was placed into the guide cannula and extended 0.5 mm beyond the tip of the guide. After surgery, rats were individually housed and allowed to recover for 7 days. Animals were handled for at least 5 min daily beginning on the fourth recovery day and were habituated for 2 consecutive days to the handling procedures necessary for microinjections.

Context-induced EtOH seeking: Pavlovian Spontaneous Recovery (PSR) testing

After 14 days in the home cages, rats were returned to the operant chambers for PSR testing in 60-min sessions. The FR5-FR1 schedule lever contingencies and dipper functioning were maintained, but EtOH and water were absent for the four consecutive PSR sessions (Hauser et al. 2012a; Rodd et al. 2006). There are four consecutive PSR sessions because previous studies have shown that exposure to EtOH odor cues or EtOH priming (Rodd-Henricks et al.

2002a, b) and some drugs (Dhaher et al. 2010) may enhance PSR responding for more than one session.

Experiment 1: effects of nicotine in the pVTA on EtOH seeking—The first set of rats was microinjected bilaterally with vehicle (aCSF) or nicotine (50, 100, or 200 μM, *n*=7–8/group). Nicotine was administered using the electrolytic microinfusion transducer system, as previously described (Hauser et al. 2011). Nicotine was administered consecutively to both sides of the pVTA for 10 min/side using three 5-s pulses per minute; each 5-sec pulse infused 100 nl. These concentrations of nicotine have been previously shown to be self-infused into the pVTA (Hauser et al. 2013). In this and all subsequent experiments, the micro-injections were given prior to only the first PSR session, starting 25–30 min before the session. The use of the electrolytic microinfusion transducer system has been shown to give good neuroanatomical specificity (Ikemoto et al. 2006; Rodd-Henricks et al. 2000, 2002a; Rodd et al. 2007).

Experiment 2: coinfusion of nicotine with Mec into the pVTA on EtOH seeking —The second set of rats was microinjected bilaterally with vehicle (aCSF), 100 μM nicotine +100 μMMec, or 100 μM nicotine+200 μMMec (*n*=6–7/group). The 100-μM nicotine data from experiment 1 were used to compare the effects of Mec+nicotine. The concentrations of Mec used have been reported to effectively reduce EtOH drinking (Ericson et al. 1998) and EtOH-associated cue-induced DA levels within the VTA (Lof et al. 2007).

Experiment 3: coinfusion of nicotine with Zac into the pVTA on EtOH seeking —The third set of rats was microinjected bilaterally with vehicle (aCSF), 100 μM nicotine +100 μM Zac, 100 μM nicotine+ 200 μM Zac (*n*=5–7/group). The 100-μM nicotine data from experiment 1 were used to compare the effects of Zac+nicotine. These concentrations of Zac were previously used in intracranial self-administration (ICSA) studies to inhibit the self-infusion of EtOH into the pVTA (Rodd-Henricks et al. 2003).

Experiment 4: effects of Zac in the pVTA on EtOH seeking—The fourth set of rats (*n*=5–8/group) was microinjected bilaterally with aCSF, or 100 or 200 μM Zac.

Experiment 5: effects of CPBG in the pVTA on EtOH seeking—The fifth set of rats (*n*=4–5/group) was microinjected bilaterally with aCSF, or 1 or 10 μM CPBG. The concentrations of CPBG were selected based on previous research that showed P rats will self-administer 1 and 10 μM CPBG into the pVTA (Rodd et al. 2007).

Histology

At the termination of the experiment, 1 % bromophenol blue (0.5 μl) was injected into the infusion site. Subsequently, the animals were given a fatal dose of pentobarbital and then decapitated. The brains were removed and immediately frozen at −70 °C. The frozen brains were subsequently equilibrated at −15 °C in a cryostat microtome and then sliced into 40-μm sections. Sections were then stained with cresyl violet and examined under a light microscope for verification of the injection site using the rat brain atlas of Paxinos and Watson (1998).

Statistical analysis

Overall operant responding (60 min) data were analyzed with a mixed factorial ANOVA with a between-subjects factor of dose and a repeated measure of "session". For the PSR tests, the baseline measure for the factor of session was the average number of responses on the EtOH or water lever for the last three extinction sessions. Post hoc Tukey's *b* was used to determine individual differences. All analyses with $p \leq 0.05$ were considered significant.

Results

Histology placements

The pVTA is defined as the VTA region at the level of the interpeduncular nucleus at 5.4–6.0 mm posterior to bregma (Rodd-Henricks et al. 2000). Only animals that had correct injector placements were used in data analysis. As seen in Fig. 1, the pVTA injector placements were at 5.4–6.0 mm posterior to bregma. The success rate for dual pVTA placements was around 85 %. Incorrect injection sites were located in the anterior VTA or red nucleus.

Responses on the EtOH and water levers during self-administration and extinction

Average responses on the EtOH lever during the last five sessions of the EtOH self-administration part of the procedure ranged from 168 to 218 responses/session across the five experiments (Table 1). Average responses on the water lever during these sessions were usually less than 20 responses/session. Average EtOH intakes during these five sessions ranged from 1.2 to 1.5 g/kg/session. Responses on the EtOH lever were markedly reduced across extinction sessions, whereas the low responses on the water lever were not significantly altered across extinction sessions (Table 1).

Effects of nicotine in the pVTA on EtOH seeking

Examining the effects of nicotine alone on the number of responses on the lever previously associated with the delivery of EtOH (Fig. 2a) indicated a significant effect of session ($F_{4,13}=30.10$, $p<0.001$), "dose" ($F_{3,16}=13.98$, $p<0.001$), and session by dose interaction ($F_{12,45}=3.18$, $p<0.01$). Post hoc comparisons indicated that P rats given 100 or 200 μM nicotine directly into the pVTA responded more than P rats microinjected with aCSF or 50 μM nicotine during the first PSR session. Comparison to extinction baseline values revealed that responding on the lever previously associated with EtOH was significantly increased during the first PSR session for rats given aCSF or 50,100, or 200 μMnicotine (*p* values <0.05). Nicotine did not significantly alter responses on the lever previously associated with water (Fig. 2b).

Microinjection of nicotine into areas outside of the pVTA (i.e., the anterior VTA and red nucleus) did not appear to significantly alter responses on the EtOH or water levers during the first or subsequent PSR sessions (Fig. 3).

Effects of co-infusion of nicotine with Mec into the pVTA on EtOH-seeking

The number of responses on the lever previously associated with the delivery of EtOH for rats given aCSF, 100 μM nicotine, or 100 μM nicotine with Mec (Fig. 4a) indicated a

significant effect of session ($F_{4,14}$=25.19, p<0.001), dose ($F_{3,16}$= 7.08, p<0.01), and session by dose interaction ($F_{12,45}$=2.71, p<0.01). Post hoc comparisons indicated that P rats given 100 μM nicotine+200 μMMec responded significantly less than P rats microinjected with 100 μMnicotine, 100 μM nicotine+100 μMMec, or aCSF. P rats given 100 μM nicotine or 100 μMnicotine+100 μM Mec responded more on the EtOH lever during the first PSR session compared to the aCSF group. Comparison to extinction baseline values indicated responding on the lever previously associated with EtOH was significantly increased during the first PSR testing for rats given aCSF, 100 μM nicotine and 100 μM nicotine+100 μMMec (p values<0.05). There were no significances in the number of responses on the lever previously associated with the delivery of water during the first PSR session (Fig. 4b).

Effects of coinfusions of nicotine with Zac into the pVTA on EtOH seeking

Analysis of the number of responses on the lever previously associated with the delivery of EtOH for rats microinjected with aCSF, 100 μM nicotine, 100 μMnicotine+100 μM Zac, or 100 μM nicotine+200 μM Zac (Fig. 5a) indicated a significant effect of session ($F_{4,15}$= 11.790, p<0.001), dose ($F_{3,18}$=6.975, p<0.01), and session by dose interaction ($F_{12,51}$=2.788,p<0.005). Post hoc comparisons indicated that P rats given 100 μMnicotine +100 or 200 μM Zac responded significantly less than P rats given aCSF or nicotine alone. P rats given 100 μM nicotine responded more during the first PSR session compared to the aCSF group. P rats given aCSF or nicotine alone responded more during the first PSR session compared to extinction baseline (p value <0.01). Microinjection of 100 μM nicotine +100 μM Zac (but not nicotine+ 200 μM Zac) reduced the number of EtOH lever responses during the first PSR session compared to extinction baseline (p values <0.01). The results indicated that there were no significant group differences for the number of responses on the lever previously associated with water (Fig. 5b).

Effects of Zac in the pVTA on EtOH-seeking

For Zac alone, the number of responses on the lever previously associated with the delivery of EtOH (Fig. 6a) indicated a significant effect of session ($F_{4,12}$= 17.69, p<0.001), dose ($F_{2,15}$=3.70, p=0.049), and session by dose interaction ($F_{8,26}$=4.47,p<0.01). Post hoc comparisons indicated that P rats given 100 and 200 μM Zac responded significantly less than P rats given aCSF. Comparison to extinction baseline values revealed that responding on the lever previously associated with EtOH was significantly increased during the first PSR testing for rats that administered aCSF (p<0.01), where-as there were no differences between 100 or 200 μM Zac compared to extinction baseline. There were no significant group differences in the number of responses on the lever previously associated with water during the first PSR session (Fig. 6b).

Effects of CPBG in the pVTA on EtOH seeking

For CPBG, examining the number of responses on the lever previously associated with the delivery of EtOH (Fig. 7a) indicated a significant effect of session ($F_{4,6}$ = 42.99, p<0.001), dose ($F_{2,9}$=4.07, p=0.055), and session by dose interaction ($F_{8,12}$=6.78, p<0.01). Individual ANOVAs performed for each session indicated a significant effect of dose during the first PSR test session ($F_{2,9}$=68.40,p<0.001). Post hoc comparisons indicated that P rats given 1

μM CPBG directly into the pVTA responded significantly more during the first PSR session than P rats given aCSF, whereas those that administered 10 μM CPBG responded significantly less during the first PSR compared to the aCSF group. In addition, EtOH lever responses were higher in the aCSF and 1-μM CPBG groups during the first PSR test session compared to extinction baseline (p values <0.05). There were no significant differences between 10-μM CPBG and extinction baseline values. There were no significant group differences in the number of responses on the lever associated with water during the first PSR session (Fig. 7b).

Discussion

The major findings of this study are that physiologically relevant levels of nicotine microinjected into the pVTA can promote context-induced EtOH seeking and that 5-HT_3 and nACh receptors are involved. The 5-HT_3 receptor antagonist Zac microinjected into the pVTA reduced responding on the EtOH lever in PSR test at both doses (Fig. 6a), suggesting that activating 5-HT_3 receptors within the pVTA are involved in the expression of context-induced EtOH-seeking behavior in P rats. 5-HT_3 receptors play a role in increasing the activity of VTA DA neurons (Campbell et al. 1996). In addition, there are 5-HT_3 receptors found within the pVTA (Herve et al. 1987). Inhibition of 5-HT_3 receptors within the VTA can inhibit EtOH-induced extracellular DA release in the VTA (Campbell et al. 1996). A previous study (Hauser et al. 2011) indicated that activation of DA neurons in the pVTA, but not the anterior VTA, mediates context-induced EtOH-seeking behavior of P rats. Overall, these results suggest that activation of 5-HT inputs at 5-HT_3 receptors in the pVTA, resulting in the stimulation of VTA DA neurons, may be involved in the expression of EtOH-seeking behavior.

The current results are in agreement with the findings of Le et al. (2006b) who report that intraperitoneal injections of the 5-HT_3 receptor antagonists, ondansetron and tropisetron, reduced footshock-induced EtOH-seeking behavior in Wistar rats. In addition, the current findings also support clinical findings that administration of a 5-HT_3 receptor antagonist can reduce alcohol craving in alcohol-dependent subjects (Johnson 2004; Johnson et al. 2002; Sellers et al. 1994).

In contrast, other studies have found that the 5-HT_3 receptor antagonist ondansetron did not alter nicotine-induced decreases in self-stimulation threshold within the VTA (Ivanova and Greenshaw 1997) nor did the 5-HT_3 antagonists ICS 205–930 and MDL-72222 alter intravenous nicotine self-administration or nicotine-induced stimulation of activity (Corrigall and Coen 1994). The apparent disagreement between the present study and those mentioned above may be due to a combination of factors, including different mechanisms that may underlie the different behaviors that were measured, as well as differences in the drug histories of the animals and the routes of administration of the antagonists.

If activation of 5-HT_3 receptors in the pVTA is involved in regulating context-induced EtOH-seeking behavior, as suggested by the results with zacopride (Fig. 6), then local microinfusion of a 5-HT_3 receptor agonist into the pVTA should increase expression of context-induced EtOH-seeking behavior. The microinjection of 1 μM CPBG into the pVTA

increased responding on the EtOH lever approximately twofold over aCSF levels of responding (Fig. 7a), providing additional support for the involvement of 5-HT_3 receptors in mediating context-induced EtOH-seeking behavior. This effect did not appear to be due to an increase in general motor activity since responses on the water lever were not altered (Fig. 7b). However, in contrast to the effects observed with 1 μM CPBG, the microinjection of 10 μM CPBG into the pVTA significantly reduced responding on the EtOH lever compared to aCSF levels (Fig. 7a). This concentration of CPBG should still be having an effect on DA release as suggested by reverse microdialysis studies (Campbell et al. 1996; Liu et al. 2006), and ICSA results showed that the 10 μM CPBG was the optimal concentration self-infused (Rodd et al. 2007). Therefore, it is possible that 10 μM effects of CPBG, due to its enhanced reinforcing effects and higher DA release in pVTA, are producing a euphoric effect leading to the reduction of context-induced EtOH seeking in the current study. Alternatively, it is possible that the current experimental approach may be producing a local higher-CPBG concentration than the ICSA and reverse microdialysis procedures, resulting in inhibition of DA reuptake and higher concentrations of DA-activating D2 autoreceptors, which would reduce VTA DA neuronal activity and inhibit EtOH-seeking behavior (Hauser et al. 2011).

Varenicline, an α4β2 nACh receptor partial agonist and a potent 5-HT_3 receptor agonist (Lummis et al. 2011), reduced EtOH-seeking behavior in rats (Steensland et al. 2007). The apparent differences between the current 5-HT_3 agonist results and Steensland et al. (2007) findings may be due to differences in the route of administration, e.g., with systemic administration; varenicline may be acting at sites outside the VTA to reduce EtOH seeking.

Nicotinic receptors within the pVTA appear to be involved in mediating EtOH-seeking behavior. Microinfusion of 100 and 200 μM nicotine increased responding on the EtOH lever twofold over control values (Fig. 2a). These concentrations did not alter responding on the water lever (Fig. 2b), indicating that the increased responding on the EtOH lever was not due to an increase in general motor activity. Concentrations of 10 to 200 μM nicotine were self-infused into the pVTA by P rats (Hauser et al. 2013), suggesting that the concentrations of nicotine used in the present study can produce positive effects. Local application of 100 μMnicotine within the VTA has been shown to increase somatodendritic (Rahman et al. 2004) and terminal (Ding et al. 2012) DA release. Overall, the results are consistent with effects of nicotine on EtOH-seeking behavior being mediated by increased VTA DA neuronal activity. These results are in agreement with previous studies that demonstrated (a) the involvement of pVTA DA neuronal activity (Hauser et al. 2011) in regulating EtOH seeking and (b) that systemically administered nicotine significantly enhanced EtOH-seeking behavior in P rats (Hauser et al. 2012a). The nicotine concentrations in the current study approximate levels attained by human smokers (15–150 ng/ml; Benowitz and Jacob 1984) and of P rats consuming nicotine (Hauser et al. 2012b).

The enhancing effect of nicotine on EtOH-seeking behavior was inhibited by coinfusion with Mec (Fig. 4a) and Zac (Fig. 5a), suggesting that nicotine may be acting at both nACh and 5-HT_3 receptors. These results are consistent with the effects of 5-HT_3 receptor antagonists inhibiting the self-infusion of nicotine into the pVTA (Hauser et al. 2013), and with the high affinity of nicotine for the 5-HT_3 receptor (Jackson and Yakel 1995).

Responding on the water lever was not altered by any of the treatments (Figs. 4b and 5b); because of the low responses on the water lever, a floor effect may have prevented observing any reduced responding due to general motor impairment. However, previous studies indicated that microinjection of a 5-HT_3 receptor antagonist into the pVTA did not impair operant responding (Rodd et al. 2010); neither systemic (Ford et al. 2009) nor VTA (Ericson et al. 1998) administration of Mec appeared to impair motor activity. Interestingly, it has been reported that Mec (10 μM) can inhibit 5-HT-activated currents by acting as an antagonist at 5-HT_3 receptors (Drisdel et al. 2008), thus suggesting that Mec may be a 5-HT_3 receptor antagonist as well as a nACh receptor antagonist in the current study on EtOH seeking. However, it is not clear if the results of the present study are selective for EtOH-seeking behavior or if similar effects could also be observed for other drugs.

The α4 nicotinic receptor and 5-HT_3 receptor coexist on striatal nerve terminals (Dougherty and Nichols 2009; Nayak et al. 2000), although it is not known if a similar coexistence also occurs within the VTA. Activation of nACh or 5-HT_3 receptors within the pVTA produces reinforcing effects (Hauser et al. 2013; Rodd et al. 2007) and stimulates DA release (Liu et al. 2006; Rahman et al. 2004), suggesting that both types of receptors may be located on VTADA neurons. Antagonism of nACh receptors with Mec can reduce nicotine-induced DA release within VTA (Nisell et al. 1994; Rahman et al. 2004). Although there are no current reports on Zac's effects on nicotine-induced DA release, it has been reported that the 5-HT_3 antagonist ICS 205–930 can attenuate systemic nicotine-induced DA release in the Acb, thus suggesting that some of nicotine's stimulating effects on DA may be mediated in part by 5-HT_3 receptors (Carboni et al. 1989). The α4 and α6 nicotinic receptors have been shown to be necessary for nicotine-induced DA neuronal activity in the VTA (Zhao-Shea et al. 2011; Gotti et al. 2010); α7 nicotinic receptors located on the presynaptic glutamate terminals (Albuquerque et al. 2009; Gotti and Clementi 2004; Nayak et al. 2000; Wonnacott 1997) are thought to prolong DA neurotransmission by enhancing glutamatergic excitation in the presence of nicotine (Pidoplichko et al. 2004). 5-HT_3 receptors also appear to be involved in mediating the release of glutamate (Dong et al. 2009). Overall, these latter findings suggest that the interactions of nicotinic and 5-HT_3 receptors within the pVTA are likely complex and involve more than just their interactions directly on DA neurons.

In conclusion, the ability of local applications of nicotine into the pVTA to promote context-induced EtOH seeking suggests that the pVTA is a neuroanatomical site in which drugs of abuse can activate seeking behavior for another drug of abuse. The current findings also provide evidence that the actions of nicotine appear to occur through its effects at both nACh and 5-HT_3 receptors in the pVTA and that activation of 5-HT_3 receptors may play a role in mediating context-induced EtOH seeking.

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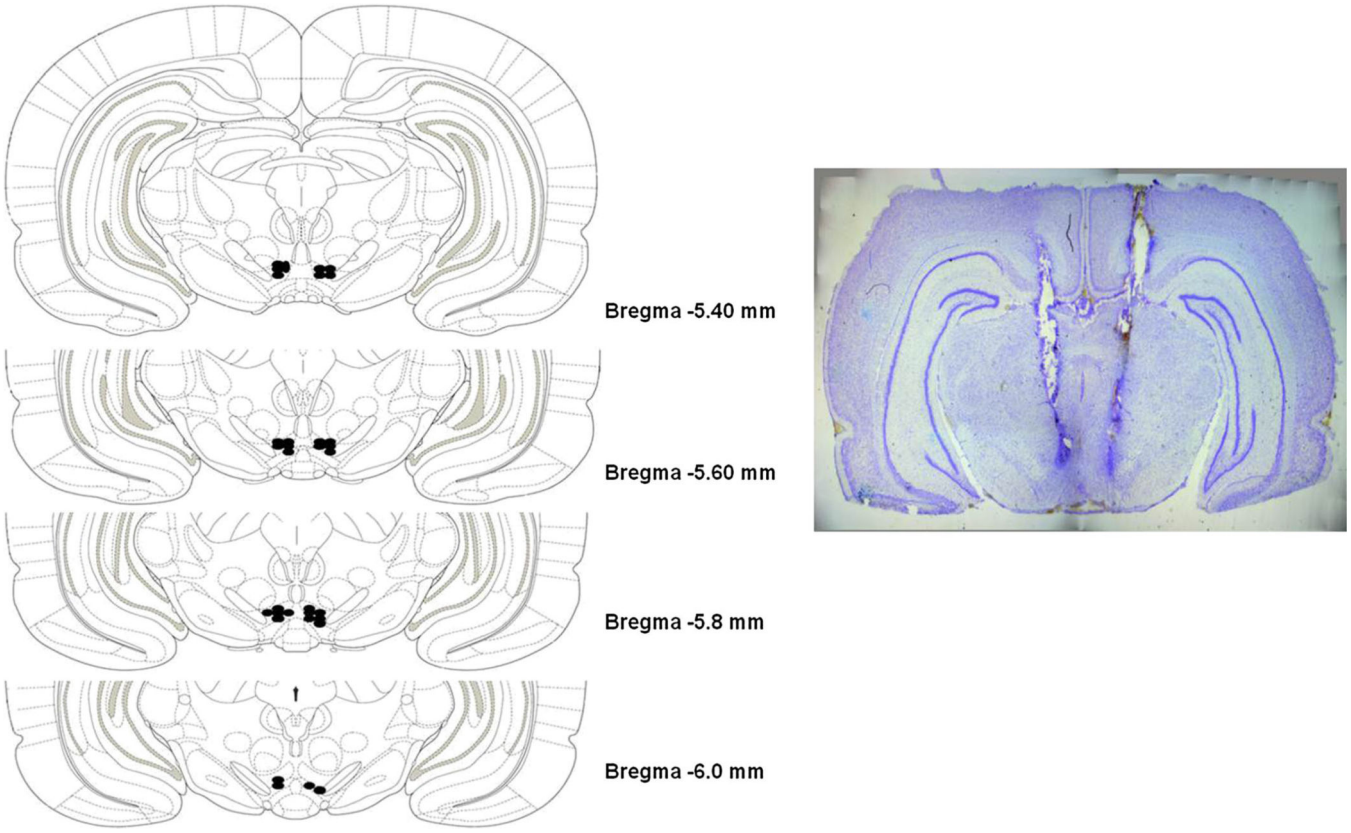

Fig. 1.

Representative dual placements for the microinfusions of aCSF, nicotine, zacopride, or CPBG into pVTA of P rats are shown. Filled circles represent placements of injection sites within the pVTA (defined as −5.4 to −6.0 mm bregma)

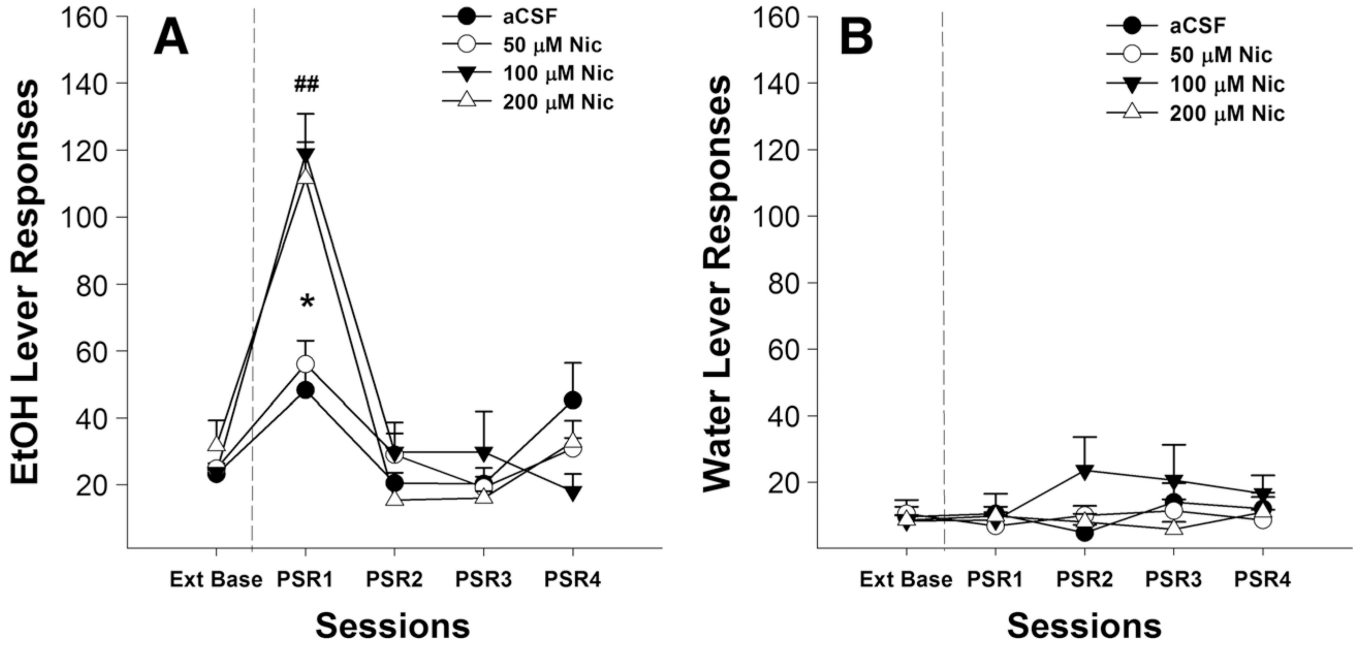

Fig. 2.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (n=6–7/group) microinjected with aCSF or 50, 100, or 200 μM nicotine into the pVTA. Asterisk (*) indicates that rats that administered aCSF or 50, 100, or 200 μM nicotine responded significantly (p<0.05) more on the EtOH lever during the first PSR session compared to extinction baseline levels. Double pound (##) indicates that 100 or 200 μM nicotine increased responding on the EtOH lever during the first PSR session compared to the aCSF and 50 μM nicotine (p<0.05)

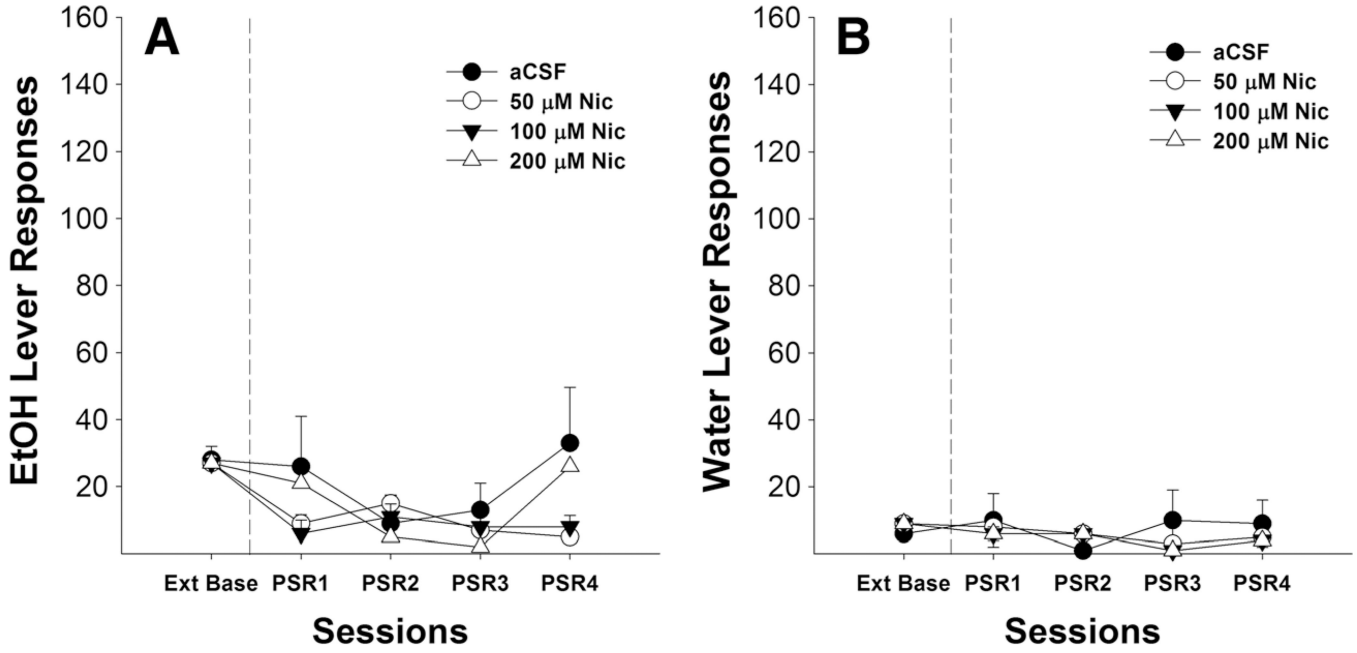

Fig. 3.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (*n*= 1–6/group), with placements in the anterior VTA and red nucleus, microinjected with aCSF or 50, 100, or 200 μM nicotine. No significant differences were detected

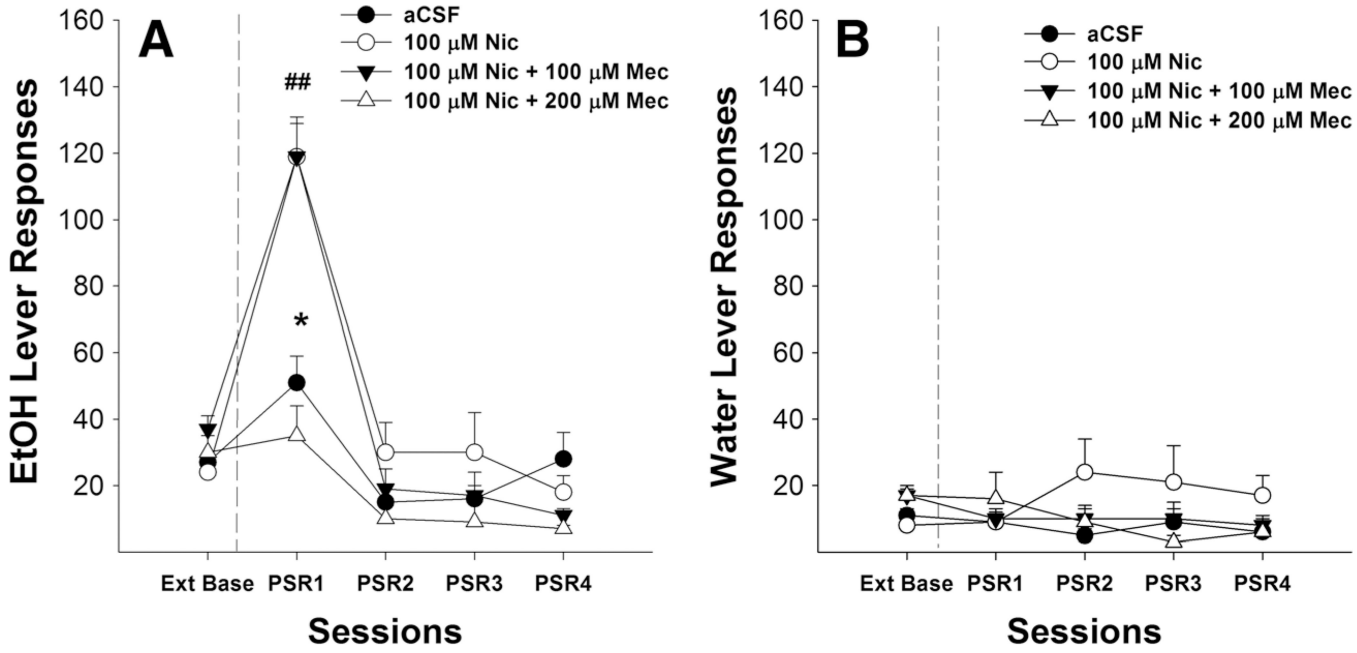

Fig. 4.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (*n*=6–7/group) microinjected with aCSF or 100 μM nicotine, 100 μM nicotine+ 100 μM mecamylamine (a nonselective nicotinic receptor antagonist), or 100 μM nicotine+μM mecamylamine into the pVTA. Asterisk (*) indicates that rats that administered aCSF, 100 μM nicotine, or 100 μM nicotine+100 μM mecamylamine responded significantly ($p<0.05$) more on the EtOH lever during the first PSR session compared to extinction baseline levels. Double pound (##) indicates that 100 μM nicotine or 100 μM nicotine+100 μM mecamylamine increased responding on the EtOH lever during the first PSR session compared to the aCSF, whereas 100 μM nicotine+200 μM mecamylamine decreased responding on the EtOH lever during the first PSR session compared to 100 μM nicotine or 100 μM nicotine+100 μM mecamylamine ($p<0.05$)

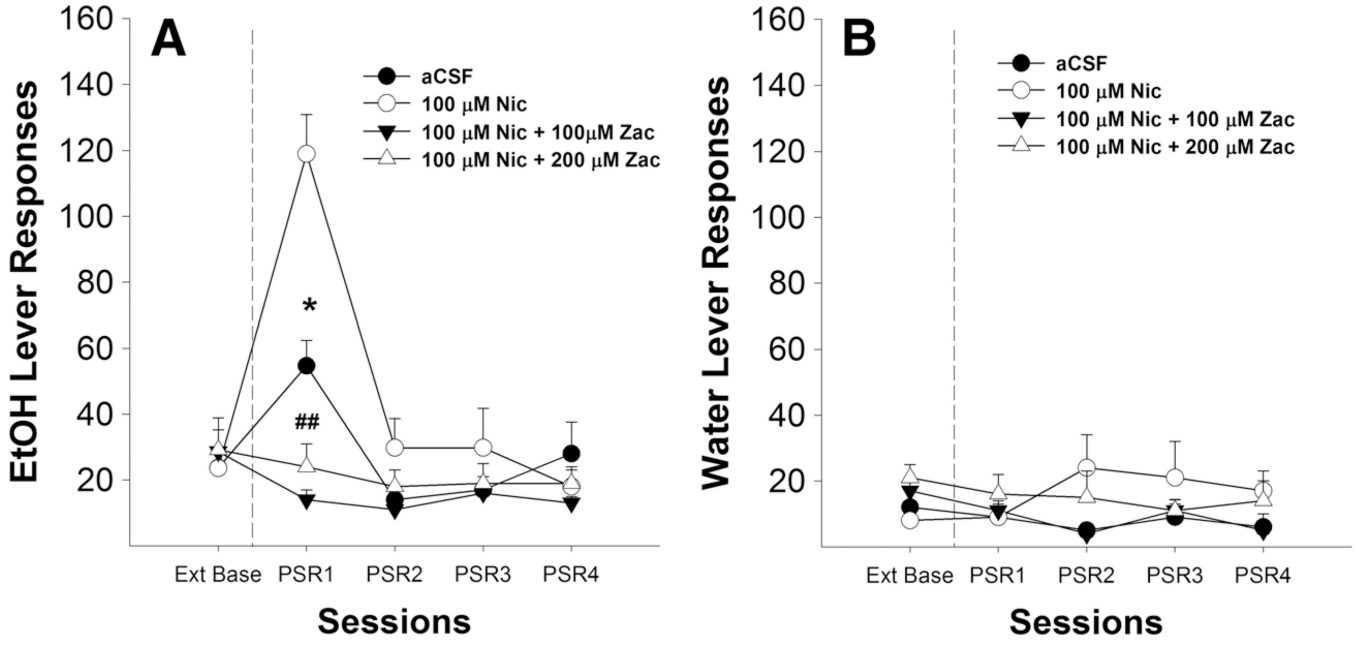

Fig. 5.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (n=5–7/group) microinjected with aCSF, 100 μM nicotine, 100 μM nicotine+ 100 μM zacopride (a 5-HT_3 receptor antagonist), or 100 μM nicotine+ 200 μM zacopride into the pVTA. Asterisk (*) indicates that rats that administered aCSF or 100 μM nicotine responded significantly (p<0.05) more on the EtOH lever during the first PSR session compared to extinction baseline levels. Double pound (##) indicates that 100 μM nicotine+100 μM zacopride, or 100 μM nicotine+200 μM zacopride decreased responding on the EtOH lever during the first PSR session compared to the aCSF or 100 μM nicotine (p<0.05)

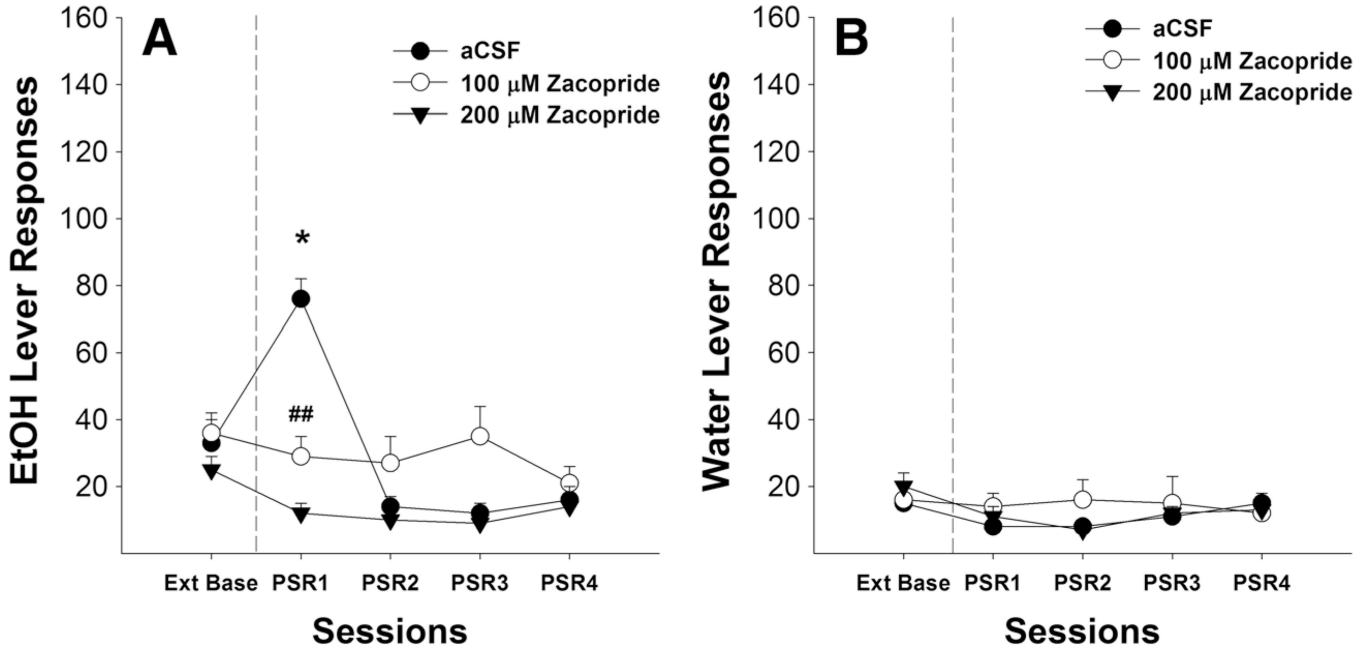

Fig. 6.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (n=5–8/group) microinjected with aCSF, or 100 or 200 μM zacopride (a 5-HT_3 receptor antagonist) into the pVTA. Asterisk (*) indicates that rats that administered aCSF responded significantly (p<0.01) more on the EtOH lever during the first PSR session compared to extinction baseline levels. Double pound (##) indicates that 100 or 200 μM zacopride significantly decreased responding on the EtOH lever during the first PSR session compared to aCSF (p<0.01)

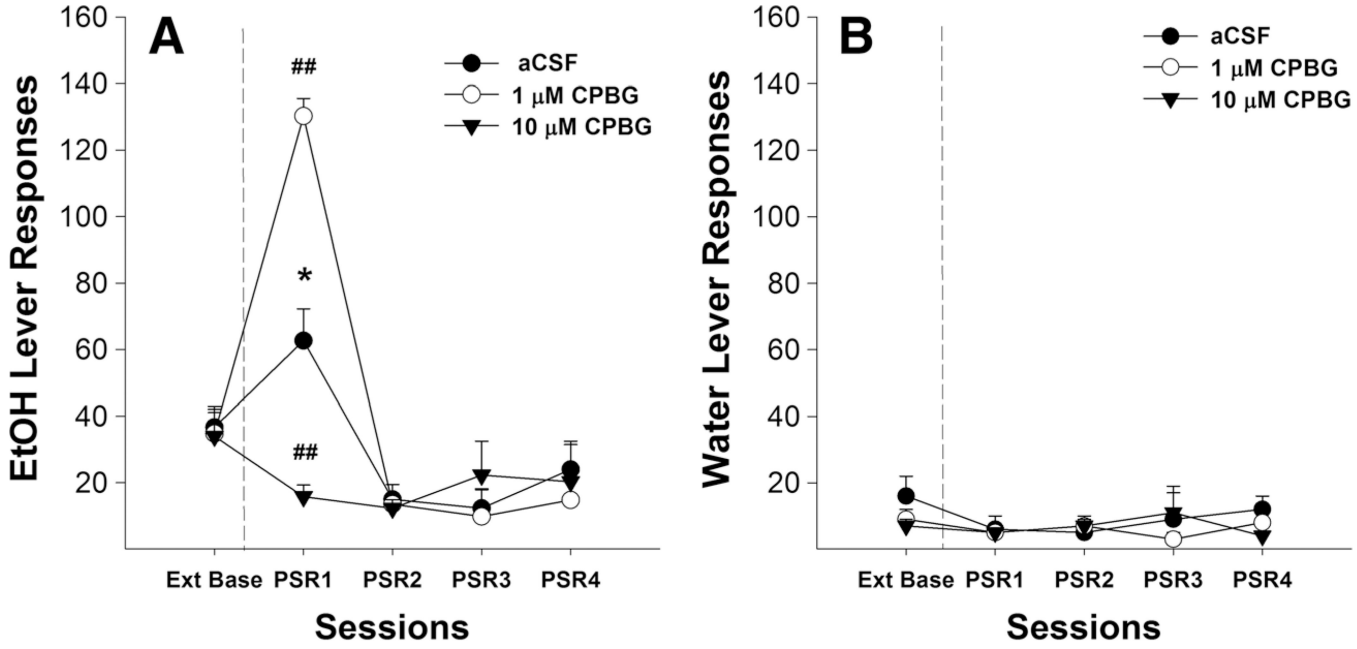

Fig. 7.

Mean (±SEM) responses per session on the lever previously associated with the delivery of EtOH (**a**) or water (**b**) by P rats (n=4–5/group) microinjected with aCSF, or 1 or 10 μM CPBG (a 5-HT_3 receptor agonist) into the pVTA. Asterisk (*) indicates that rats that administered aCSF or 1 μM CPBG responded significantly (p<0.05) more on the EtOH lever during the first PSR session compared to extinction baseline levels. Double pound (##) indicates that 1 μM CPBG increased responding on the EtOH lever, and 10 μM CPBG decreased responding on the EtOH lever during the first PSR session compared to the aCSF (p<0.01)

Table 1

Representation of EtOH and water lever responses (mean± SEM) during the last 5 days of maintenance, the first day of extinction, and last 3 days of extinction that were used for baseline prior to the first PSR session

	EtOH Lever (FR5)	Water Lever (FR1)	EtOH (g/kg)
Experiment 1 (Nicotine)			
EtOH Maintenance	202±22	10±2	1.4±0.2
Extinction (Day 1)	146±11	10±2	
Base Extinction (last 3 days)	26±3	9±3	
Experiment 2 (Nicotine+Mecamylamine)			
EtOH Maintenance	204±15	15±3	1.5±0.1
Extinction (Day 1)	180±11	22±3	
Base Extinction (last 3 days)	29±3	13±2	
Experiment 3 (Nicotine+Zacopride)			
EtOH Maintenance	182±15	19±3	1.3±0.1
Extinction (Day 1)	144±11	8±4	
Base Extinction (last 3 days)	27±3	14±3	
Experiment 4 (Zacopride)			
EtOH Maintenance	168±18	14±3	1.2±0.1
Extinction (Day 1)	151±15	33±9	
Base Extinction (last 3 days)	31±6	17±4	
Experiment 5(CPBG)			
EtOH Maintenance	218±19	9±2	1.5±0.1
Extinction (Day 1)	147±10	12±2	
Base Extinction (last 3 days)	35±7	11±4	