The reinforcing effects of ethanol within the posterior ventral tegmental area depend upon dopamine neurotransmission to forebrain cortico-limbic systems

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Abstract

Ethanol can be self-infused directly into the posterior ventral tegmental area (pVTA) and these effects involve activation of local dopamine neurons. However, the neuro-circuitry beyond the pVTA involved in these reinforcing effects has not been explored. Intra-pVTA micro-injection of ethanol increases dopamine release in the nucleus accumbens (NAC), medial prefrontal cortex (mPFC) and ventral pallidum (VP). The current study tested the hypothesis that the reinforcing effects of ethanol within the pVTA involve activation of dopamine projections from the pVTA to the NAC, VP, and mPFC. Following the acquisition of self-infusions of 200 mg% ethanol into the pVTA, either the dopamine D_2 receptor antagonist sulpiride (0, 10 or 100 μM), or the D_1 receptor antagonist SCH-23390 (0, 10 or 100 μM) was micro-injected into the ipsilateral NAC shell (NACsh), NAC core (NACcr), VP or mPFC immediately prior to the self-infusion sessions to assess the involvement of the different dopamine projections in the reinforcing effects of ethanol. Microinjection of each compound at the higher concentration into the NACsh, VP or mPFC, but not the NACcr, significantly reduced the responses on the active lever (from 40–50 to approximately 20 responses). These results indicate that activation of dopamine receptors in the NACsh, VP, or mPFC, but not the NACcr, is involved in mediating the reinforcing effects of ethanol in the pVTA, suggesting that the 'alcohol reward' neuro-circuitry consist of, at least in part, activation of the dopamine projections from the pVTA to the NACsh, VP and mPFC.

Keywords

Dopamine D1 receptor; Dopamine D2 receptor; Ethanol; Intra-cranial self-administration; Medial prefrontal cortex; Nucleus accumbens; Ventral pallidum; Ventral tegmental area

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Introduction

Identifying brain regions and circuits involved in the reinforcing effect of ethanol is critical toward understanding the neurobiology of alcohol abuse and addiction. A prior study reported ethanol can be self-infused directly into the ventral tegmental area (VTA), suggesting this region as an anatomical site underlying the reinforcing effects of ethanol (Gatto *et al*, 1994). Subsequent studies demonstrated that ethanol is self-infused into the posterior, but not anterior, VTA (Rodd-Henricks *et al*, 2000; Rodd *et al*, 2005). Pharmacological studies indicate that such effects depended on activation of local dopamine neurons (Rodd *et al*, 2004), involving local 5-HT$_3$ and 5-HT$_{2A}$ receptors (Ding *et al*, 2009b; Rodd-Henricks *et al*, 2003), but not local 5-HT$_{1B}$ or GABA$_A$ receptors (Ding *et al*, 2009b, 2011). So far, the neuro-circuits beyond the pVTA underlying this effect have not been explored.

Dopamine neurons in the VTA project to the forebrain coritco-limbic regions, including the nucleus accumbens (NAC), medial prefrontal cortex (mPFC), and ventral pallidum (VP) (Oades and Halliday, 1987; Swanson, 1982). Dopamine neurotransmission in these regions has been suggested to mediate the rewarding and reinforcing effects of ethanol (Koob and Volkow, 2010). Ethanol can increase dopamine release in the NAC following oral self-administration or systemic injection (Doyon *et al*, 2003; Imperato and Di Chiara, 1986; Weiss *et al*, 1993). Inhibition of dopamine receptors in the NAC attenuated oral self-administration of ethanol (Czachowski *et al*, 2001; Hodge *et al*, 1997; Rassnick *et al*, 1992; Samson *et al*, 1993). Similarly, both passive- or self-administration of ethanol increased extracellular dopamine levels in the VP (Melendez *et al*, 2003, 2004). In addition, pharmacological manipulation of dopamine receptors in the VP regulated alcohol drinking in the alcohol-preferring P rat (Melendez *et al*, 2005). In contrast to the NAC and VP, the neurochemical effect of ethanol on dopamine neurotransmission in the mPFC has been controversial. Early studies indicated that systemic injection of ethanol failed to increase extracellular dopamine levels in the mPFC (Engleman *et al*, 2006; Hegarty and Vogel, 1993). However, one recent study demonstrated that passive intravenous injection of ethanol increased extracellular dopamine levels in the mPFC (Schier *et al*, 2013). Microinjection of dopamine receptor agents in the mPFC altered oral alcohol operant self-administration (Hodge *et al*, 1996; Samson and Chappell, 2003). Therefore, the current evidence implicates these three regions in the systemic effects of ethanol. However, the role of dopamine neurotransmission in these regions in the local reinforcing effects of ethanol in the pVTA has not been examined.

Self-infusions of ethanol into the pVTA depend on activation of local dopamine neurons (Rodd *et al*, 2004). Microinjection of ethanol into the pVTA increased dopamine release in the NAC shell (NACsh), VP and mPFC (Ding *et al*, 2009a, 2011). These results suggest that stimulation of dopamine projections from the pVTA to the NACsh, VP and mPFC and subsequent activation of dopamine receptors within these regions may constitute a feed-forward circuitry that is critical to mediating the reinforcing effects of ethanol in the pVTA. Therefore, the current study was designed to examine the effects of local administration of dopamine D$_1$ or D$_2$ receptor antagonists within the NAC, VP and mPFC on ethanol self-infusions into the pVTA. The hypothesis is that activation of dopamine neurotransmission to

these three regions is involved in mediating the local rewarding effects of ethanol in the pVTA.

Materials and methods

Animals

Experimentally-naïve adult female Wistar rats (body weight 270 to 320 g, Harlan Sprague Dawley, Inc., Indianapolis, IN) were used in the present study. Animals were habituated in pairs in a reverse 12-hr light-dark cycle room (light off at 10:00 am) controlled for temperature and humidity. Food and water were available *ad libitum* except during the ICSA test. Female rats were used because these rats maintain their head size better than male rats for more accurate stereotaxic placement (Ding *et al*, 2009b, 2012; Rodd-Henricks *et al*, 2000). The estrous cycle was not monitored. Previous studies suggested that the estrous cycle did not appear to have a significant effect on ICSA behavior (Ding *et al*, 2009b, 2012; Rodd-Henricks *et al*, 2000). Experiments were performed during the dark phase. Protocols used were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine. All experiments were performed in accordance with the principles outlined in the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996).

Chemical agents

The artificial cerebrospinal fluid (aCSF) consisted of 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 2.5 mM CaCl, 10 mM *d*-glucose, pH 7.2–7.4. Ethyl alcohol (190 proof) was obtained from McCormick Distilling, Weston, MO. The dopamine D_2 receptor antagonist (−)-sulpiride and the D_1 receptor antagonist R(+)-SCH23390 were purchased from Sigma-Aldrich (St. Louis, MO). All chemicals were dissolved in the aCSF solution to the desired concentrations.

Stereotaxic surgery procedures

Rats were implanted unilaterally with two 22-gauge cannulae (Plastics One Inc., Roanoke, VA) aimed at the right pVTA (AP −5.6 mm, ML +2.1 mm, DV −8.1 mm) and one of the forebrain regions: NAC shell (AP +1.7 mm, ML +2.3 mm, DV −6.9 mm), VP (AP + 0.1 mm, ML + 2.3 mm, DV −7.0 mm), or mPFC (AP + 3.0 mm, ML + 0.7 mm, DV −3.0 mm), according to Paxinos and Watson (Paxinos and Watson, 1998). Cannulae for the pVTA and NACsh were implanted with a 10° angle to the midline, whereas cannulae for the mPFC and VP were implanted with no angle. Stylets were inserted into cannulae when no experiments were being conducted. Since accumulating evidence suggests that the NAC consists of two distinct sub-regions, shell and core, which differ from each other in various aspects (Zahm, 1999), the current study also examined the involvement of the NAC core (NACcr, AP + 1.6 mm, ML + 2.7 mm, DV − 6.0 mm). Rats were individually housed after surgery and were allowed to recover from surgery for approximately 5–7 days prior to tests during which rats were habituated and handled on a daily basis. Each rat was used for only one ICSA experiment.

General test procedure

The ICSA tests followed procedures previously described (Ding *et al*, 2009b; Rodd-Henricks *et al*, 2000). Briefly, rats were placed into operant conditioning chambers equipped with two levers, one active and one inactive. The active lever was connected to an isolated pulse stimulator (Model 2100) from A-M systems, Inc (Varlsborg, WA) controlled by an operant conditioning control system. The A-M pulse stimulator was connected to two electrodes that were immersed in a solution-filled cylinder container equipped with a 28-gauge injection cannula. Each response on the active lever (FR1 schedule of reinforcement) activated the pulse stimulator that produced a 5-sec infusion current between the electrodes, resulting in an infusion of 100-nl solution into the p-VTA. Each infusion was followed by a 5-sec timeout period. During both the infusion and timeout periods, responses on the active lever were recorded but did not produce further infusions. The responses on the inactive lever were recorded but did not result in any infusions. The assignment of active and inactive lever was counterbalanced among rats. There were a total of seven sessions conducted with each session being 4-hr long and 48–72 hr between sessions.

Experiment 1 examined the involvement of NACsh dopamine receptors in ethanol self-infusions into the pVTA—Rats started with self-infusions of 200 mg% ethanol into the pVTA during the first 4 sessions (acquisition). For sessions 5 and 6, rats received microinjection of aCSF, the dopamine D_2 receptor antagonist sulpiride (10 or 100 μM), or the dopamine D_1 receptor antagonist SCH-23390 (10 or 100 μM) in the NACsh immediately prior to the commencement of ethanol self-infusion in the pVTA. During session 7 (reinstatement), rats self-infused 200 mg% ethanol in the pVTA without treatment in the NACsh. All antagonists were injected in a volume of 0.5 μl with a flow rate at 0.25 μl/min. The injector remained in place for another 2 min before being removed. The 200 mg% ethanol is an optimal dose to produce both behavioral and neurochemical effects in the pVTA (Ding *et al*, 2009a; Rodd-Henricks *et al*, 2000). Since both antagonists showed efficacy at the 100- μM concentration, this concentration was tested in subsequent experiments.

Experiment 2 examined the involvement of NACcr dopamine receptors in ethanol self-infusions into the pVTA—The ICSA procedure was the same as in experiment 1 except sulpiride (100 μM) or SCH-23390 (100 μM) was microinjected into the NACcr immediately before sessions 5 and 6.

Experiment 3 examined the involvement of VP dopamine receptors in ethanol self-infusions into the pVTA—The ICSA procedure was the same as in experiment 1 except that the microinjection of aCSF, sulpiride (100 μM) or SCH-23390 (100 μM) into the VP was given immediately before sessions 5 and 6.

Experiment 4 examined the involvement of mPFC dopamine receptors in ethanol self-infusions into the pVTA—The ICSA procedure was the same as in experiment 1 except that the microinjection of aCSF, sulpiride (100 μM) or SCH-23390 (100 μM) into the mPFC was given immediately before sessions 5 and 6.

Histology

At the end of experiments, rats were euthanized and bromophenol blue was injected into the target regions, and placement of injection sites were verified following procedures previously described (Ding *et al*, 2009b).

Statistical analysis

For each individual group, lever discrimination was determined by 'lever (active vs inactive) x session' mixed ANOVA with repeated measures on session; t-tests were used to compare responses between active and inactive lever following a significant main effect of lever ($p < 0.05$). *F*or determination of drug effect, paired t-tests were used to compare the responses on the active lever during sessions 5 and 6 to average responses on the active lever during acquisition sessions 3 and 4 following significant effects of session and interaction ($p < 0.05$).

Results

Figure 1 depicts the representative non-overlapping placements of injection sites within the pVTA, mPFC, VP, NACsh and NACcr. The pVTA is defined as the VTA region from −5.3 mm to −6.0 mm relative to bregma (Ding *et al*, 2009a; Rodd-Henricks *et al*, 2000). The injection sites in the mPFC were mostly within the prelimbic sub-region of the mPFC. The injection sites in the VP were mostly within the ventral portion of the VP.

The involvement of NACsh dopamine receptors in ethanol ICSA in the pVTA

Fig. 2 depicts the lever responses in groups that received ethanol self-infusions into the pVTA and microinjection of aCSF or sulpiride in the NACsh. The "lever × session" repeated ANOVAs were conducted in each group. The aCSF-treated group (Fig. 2A) demonstrated a significant effect of lever ($F_{1, 14} = 21.5$, $p < 0.001$), but no effect of session ($F_{6, 9} = 1.1$, $p = 0.42$) or interaction ($F_{6,9} = 0.9$, $p = 0.55$). Lever discrimination was observed during sessions 3 to 7. The 10 μM sulpiride-treated group demonstrated significant effects of session ($F_{6, 7} = 37.7$, $p < 0.001$), lever ($F_{1, 12} = 17.8$, $p = 0.001$), and interaction ($F_{6, 7} = 10.1$, $p = 0.004$). Lever discrimination was observed during the last two acquisition sessions and the reinstatement session. Microinjection of 10 μM sulpiride significantly reduced responses on the active lever only during the second treatment (Fig. 2B, $p < 0.05$). The 100 μM sulpiride-treated group (Fig. 2C) demonstrated significant effects of session ($F_{6, 13} = 18.4$, $p < 0.001$), lever ($F_{1, 18} = 16.5$, $p = 0.001$), and interaction ($F_{6, 13} = 6.8$, $p = 0.002$). Lever discrimination was developed during acquisition and reinstatement sessions. Microinjection of 100 μM sulpiride into the NACsh significantly depressed responses on the active lever during both treatment sessions (ps < 0.05). Responses on the active lever during the reinstatement session returned toward the acquisition levels in both sulpiride-treated groups (Fig. 2B&C). In addition, response patterns in 30-min bins (Fig. 2D) indicated that the highest responding on active lever occurred during the first 30 min of session 4, with continued but lower responding occurring throughout the 4-hr session. In contrast, responses on the active lever following microinjection of sulpiride into the NACsh during session 5 were observed essentially only during the first 30-min period.

Fig. 3 shows the effects of microinjection of SCH-23390 into the NACsh on ethanol self-infusion into the pVTA. The repeated measures ANOVA revealed significant effect of lever ($F_{1, 8} = 49.1$, $p < 0.001$), but no effect of session ($F_{6, 3} = 2.5$, $p = 0.25$) or interaction ($F_{6, 3} = 1.0$, $p = 0.54$) in the 10 μM SCH-23390 treated group (Fig. 3A). Lever discrimination was observed during sessions 2 to 7. Microinjection of 10 μM SCH-23390 did not alter responses on the active lever. However, the 100 μM SCH-23390-treated group (Fig. 3B) demonstrated significant effects of session ($F_{6, 5} = 11.6$, $p = 0.008$), lever ($F_{1, 10} = 37.8$, $p < 0.001$), and interaction ($F_{6, 5} = 9.2$, $p = 0.014$). Lever discrimination was seen during acquisition sessions 2–4. Microinjection of 100 μM SCH-23390 significantly reduced responses on the active lever during both treatment sessions, but lever discrimination remained. Responses on the active lever returned toward acquisition levels during the reinstatement session. In addition, response patterns (Fig. 3D) indicate that the highest responding on the active lever in session 4 occurred during the 1st and 3rd hr; whereas responses on the active lever were reduced throughout with almost no responding observed after the 1st hr during session 5 following microinjection of SCH23390 into the NACsh.

The involvement of NACcr dopamine receptors in ethanol ICSA in the pVTA

The effects of microinjection of sulpiride or SCH-23390 into the NACcr on lever responses are shown in Fig. 4. The repeated measures ANOVAs revealed a significant effect of lever, but no effect of session or interaction for both the sulpired-treated (Fig. 4A: session $F_{6, 9} = 0.9$, $p = 0.52$; lever $F_{1, 14} = 13.4$, $p = 0.003$; interaction $F_{6, 9} = 2.6$, $p = 0.1$) and SCH-23390-treated (Fig. 4B: session $F_{6, 5} = 0.6$; $p = 0.70$; lever $F_{1, 10} = 25.1$, $p = 0.001$; interaction $F_{6, 5} = 0.7$, $p = 0.65$) groups. Lever discrimination was observed in sessions 2 to 7 in both groups. Microinjection of either sulpiride (100 μM) or SCH-23390 (100 μM) did not alter responses on the active lever.

The involvement of VP dopamine receptors in ethanol ICSA in the pVTA

In the aCSF-treated group (Fig. 5A), repeated ANOVA revealed significant effects of session ($F_{6, 3} = 89.2$, $p = 0.002$), lever ($F_{1, 8} = 33.2$, $p < 0.001$), and interaction ($F_{6, 3} = 34.1$, $p = 0.007$). Lever discrimination was observed during sessions 3 to 7. The significant effect of session appeared to be due to lower responses on the active lever during sessions 1 and 2. However, microinjection of aCSF did not alter responding for ethanol. The 100 μM sulpiride-treated group (Fig. 5B) demonstrated significant effects of session ($F_{6, 5} = 8.9$, $p = 0.015$), lever ($F_{1, 10} = 15.9$, $p = 0.003$), and interaction ($F_{6, 5} = 10.1$, $p = 0.011$). Lever discrimination developed during acquisition sessions 2–4. Sulpiride significantly reduced responses on the active lever during the first treatment session compared with those during sessions 3 and 4, but not during the second treatment session. In the 100 μM SCH-23390-treated group, repeated measures ANOVA showed significant effects of session ($F_{6, 3} = 45.3$, $p = 0.005$), lever ($F_{1, 8} = 5.37$, $p = 0.049$), and interaction ($F_{6, 3} = 61.8$, $p = 0.003$). Lever discrimination developed during the acquisition sessions 3 and 4. Microinjection of SCH-233920 significantly reduced responses on the active lever during both treatment sessions to levels seen on the inactive lever. Responses returned toward acquisition levels during the reinstatement session.

The involvement of mPFC dopamine receptors in ethanol ICSA in the pVTA

Repeated measures ANOVA in the aCSF-treated group (Fig. 6A) revealed a significant effect of lever ($F_{1, 8} = 27.2$, $p = 0.001$), but not session ($F_{6, 3} = 1.5$, $p = 0.39$) or interaction ($F_{6, 3} = 1.9$, $p = 0.32$). Lever discrimination was observed in sessions 2 to 7. Microinjection of aCSF did not alter responses on the active lever. The 100 μM sulpiride-treated group (Fig. 6B) demonstrated significant effects of session ($F_{6, 5} = 14.9$, $p = 0.005$), lever ($F_{1, 10} = 27.6$, $p < 0.001$), and interaction ($F_{6, 5} = 5.4$, $p = 0.042$). Lever discrimination developed during acquisition sessions 2–4. Sulpiride significantly reduced responses on the active lever during both treatment sessions. However, lever discrimination remained during both treatment sessions. Responses on the active lever returned toward the acquisition levels during the reinstatement session. In the 100 μM SCH-23390-treated group (Fig. 6C), repeated ANOVA shows significant effects of session ($F_{6, 3} = 22.8$, $p = 0.013$), lever ($F_{1, 8} = 16.4$, $p = 0.004$), and interaction ($F_{6, 3} = 36.6$, $p = 0.007$). Lever discrimination developed during acquisition sessions 2–4. Microinjection of SCH-233920 significantly reduced responses on the active lever during both treatment sessions to levels seen on the inactive lever. Active lever responses returned toward the acquisition levels during the reinstatement session.

Discussion

The major findings of the current study are that microinjection of either a dopamine D_1 or D_2 receptor antagonist into the NACsh, the prelimbic sub-region of the mPFC, or ventral portion of the VP, but not the NACcr, reduced ethanol self-infusions into the pVTA. These results suggest that concurrent activation of dopamine receptors in the NACsh, mPFC and VP are required to maintain the rewarding actions of ethanol within the pVTA. Although the activation of dopamine receptors in these regions may involve multiple mechanisms, previous studies found that local application of ethanol into the pVTA increased dopamine release in the NACsh, mPFC and VP (Ding *et al*, 2009a, 2011), suggesting that activation of dopamine projections from the pVTA to these forebrain regions is one important mechanism mediating the reinforcing effects of ethanol in the pVTA. In addition, the feed forward process of the reinforcing effects of ethanol appears to require concurrent activation of both D_1 and D_2 receptors, since microinjection of either antagonist was effective in reducing ethanol self-infusions into the pVTA. These findings suggest that the neuro-circuitry underlying the rewarding effects of ethanol include, at least in part, the dopamine projections from the pVTA to the NACsh, ventral VP and prelimbic mPFC, but not the dopamine projections to the NACcr.

Numerous studies have indicated that the NAC is critical for mediating the reinforcing and rewarding effects of ethanol (Gonzales *et al*, 2004; Koob *et al*, 1998). Systemic and intra-NAC administration of the non-specific dopamine receptor antagonist fluphenazine attenuated oral operant self-administration of ethanol in rats (Rassnick *et al*, 1992). The intra-NAC administration of the D_1 antagonist SCH 23390 or the D_2 receptor antagonist raclopride reduced responding for oral ethanol self-administration (Hodge *et al*, 1997; Samson *et al*, 1993). In addition, microinjection of SCH23390 or raclopride into the NAC reduced ethanol seeking behavior (Chaudhri *et al*, 2009; Czachowski *et al*, 2001). The

current study agrees with these studies and further indicates the involvement of NAC in the reinforcing effects of ethanol in the pVTA.

In the NACsh, both sulpirde and SCH23390 reduced ethanol self-infusions into the pVTA (Figs. 2 & 3), suggesting that a concerted activation of both D_1 and D_2 receptors within the NACsh is required for processing the rewarding information of ethanol in the pVTA. Both doses of sulpiride appeared to be more effective than doses of SCH23390 (Figs. 2 & 3). The pattern of responding on the active lever following sulpiride indicated it was effective within the first 30 min (Fig. 2D). Since SCH23390 and sulpiride have nM affinity to their respective receptors, their differences on the ICSA of ethanol into the pVTA may be due to differences in the cellular distribution of D_1 and D_2 receptors on median spiny projection neurons within the NACsh (Lu *et al*., 1998; Meredith 1999).

On the other hand, inhibition of either D_1 or D_2 receptors in the NACcr did not alter ethanol self-infusions in the pVTA (Fig. 4). Although it remains possible that concurrent inhibition of both receptors may alter ethanol self-infusions into the pVTA, the present results suggest that activation of each individual receptor may not be involved in the reinforcing effects of ethanol in the pVTA. A previous study suggests that D_2 receptors in the NACcr may be involved in systemic reinforcing effects of ethanol, because local injection of a D_2 receptor antagonist raclopride into the NACcr reduced operant responding for oral self-administration of ethanol in rats (Samson *et al*, 2003). It is possible that different behavioral paradigms used (oral operant self-administration vs ICSA) may contribute to such difference.

The differential involvement of the NACsh and NACcr in the reinforcing effects of ethanol in the pVTA agrees with the findings that activation of dopamine receptors in the NACsh, but not NACcr, produced reward (Ikemoto *et al*, 1997). The mechanisms for this difference remain unknown, but may be associated with substantial difference between these two sub-regions in their afferent and efferent projections (Brog *et al*, 1993; Zahm, 2000). For example, the NACsh projects mainly to the ventro-medial VP, which feeds to the mediodorsal thalamus, whereas the NACcr projects mainly to the dorsal lateral VP, which projects to the subthalamic nucleus (Zahm, 2000). In addition, different dopamine projections to the NACsh and NACcr may also contribute to this difference. The pVTA projects mainly to the NACsh, while the NACcr receives projections mainly from the anterior VTA (Ikemoto 2007).

Locomotor activity was not monitored in the current study. Several studies have examined the effects of intra-NAC administration of SCH23390 or sulpiride on locomotor activity. The general consensus among these studies is that intra-NAC treatment did not alter locomotor activity at concentrations up to 1.5 mM for SCH23390 (Coccurello *et al*, 2000; Gerrits *et al*, 1994; Taslimi *et al*, 2012; Zarrindast *et al*, 2012), or up to 700 µM for sulpiride (Coccurello *et al*, 2000; Taslimi *et al*, 2012; Zarrindast *et al*, 2012). These studies suggest that the reduction of ethanol self-infusions into the pVTA by SCH23390 or sulpiride (100 µM each) infusion in the NACsh were not likely due to impairment of general locomotor activity.

Administration of dopamine receptor antagonists into the ventral portion of the VP also reduced self-infusions of ethanol into the pVTA, suggesting that inhibition of dopamine D_1 or D_2 receptor-mediated transmission in the VP prevented the feed forward information needed for maintaining the reinforcing effects of ethanol within the pVTA (Fig. 5). The reduction of self-infusions of ethanol is unlikely due to impairment to general locomotor activity because the concentrations used in the current study were within the range that was shown not to alter motor activity. Microinjection of SCH23390 (0.5 mM) or sulpiride (58 mM) into the VP did not produce motor responses (Napier, 1992). Microinjection of SCH23390 (1.5–25 mM) or sulpiride (1.2 mM–12 mM) into the VP did not reduce water or ethanol consumption in rats, suggesting no alteration in general locomotor activity (Melendez *et al*, 2005; Shimura *et al*, 2006). In addition, the treatments with SCH23390 or sulpiride did not alter responses on the inactive lever (Fig. 5).

These results are consistent with the dopamine-stimulating effects of ethanol on the meso-pallidal pathway, following either systemic or intra-pVTA administration of ethanol (Ding *et al*, 2011; Melendez *et al*, 2003). These results also agree with studies suggesting a general role of the VP in mediating the reinforcing and rewarding effects of ethanol (Kemppainen *et al*, 2012; Melendez *et al*, 2005). Findings from the current study are in contrast to a previous study that examined the involvement of VP D_1 and D_2 receptors in ethanol drinking (Melendez *et al*, 2005). Microinjection of sulpiride into the VP significantly increased ethanol drinking, whereas microinjection of SCH23390 into the VP produced a small, but non-significant decrease of ethanol intake (Melendez *et al*, 2005). The different animal models may explain the different results in these two studies.

A concerted activation of D_1 and D_2 receptors in the prelimbic mPFC seems to be critical to the reinforcing effects of ethanol within the pVTA, as evidenced by the results that either sulpiride or SCH23390 was able to attenuate self-infusions of ethanol into the pVTA (Fig. 6). These effects were not likely due to locomotor impairment. A number of studies have shown that microinjection of SCH23390 into the mPFC did not alter locomotor activity at concentrations up to 6 mM (Hall *et al*, 2009; St Onge *et al*, 2011), and that microinjection of sulpiride into the mPFC did not alter locomotor activity at concentrations up to 3 mM (Beyer and Steketee, 2001; Steketee and Walsh, 2005). These results are consistent with previous findings that intra-pVTA administration of ethanol increased dopamine release in the mPFC (Ding *et al*, 2011). In addition, passive intra-venous infusion of ethanol was shown to increase dopamine overflow in the mPFC (Schier *et al*, 2013). These neurochemical data suggest a role of mPFC dopamine neuro-transmission in the effects of ethanol. Chemical lesion of the mPFC with 6-OHDA altered alcohol drinking behavior (Nielsen *et al*, 1999). Infusion of the D_2 receptor antagonist raclopride into the mPFC reduced oral operant responding for ethanol in rats (Hodge *et al*, 1996; Samson *et al*, 2003). So far, the role of D_1 receptors in the mPFC in oral operant self-administration or voluntary drinking of ethanol has received little attention.

During reinstatement, there appeared to be a difference in the recovery of responding in the VP compared to the other two regions. It is not clear why differences were observed between the reinstatement following sulpiride in the NACsh (Fig. 2) and VP (Fig. 5), and between the reinstatement in the mPFC (Fig. 6) and VP (Fig. 5) following SCH23390

administration. The differences may be due to a combination of factors, such as the unique neuronal circuitry within the VP, the interactions of the VP with the NACSh and pVTA, and differences in the recovery of the VP neuronal circuits to the dopamine antagonists.

In conclusion, the results of the current study provide evidence for an 'alcohol reward circuit' involving the pVTA, NACsh, prelimbic mPFC and ventral VP. The anterior VTA (Rodd-Henricks et al., 2000) and the NACcr (Fig. 4) do not appear to be involved in this circuit. The first step in processing the rewarding effects of ethanol within the pVTA is the concurrent activation of local dopamine neurons projecting to the NACsh, prelimbic mPFC and ventral VP to forward the rewarding signal to circuits that initiate and maintain goal-directed behavior. This activation of pVTA dopamine neurons is mediated, at least in part, by excitatory 5-HT$_3$ receptors (Ding et al., 2011; Rodd-Henricks et al., 2003). The activation of local dopamine neurons results in increased dopamine release in the NACsh, mPFC and VP (Ding et al., 2009a, 2011). Following the release of dopamine, a concerted activation of both D$_1$ and D$_2$ receptors in these three regions appear to be needed to process the rewarding information forward. The major feed forward effects of the dopamine inputs into the (a) NACsh are to regulate GABA outputs to the ventral-medial VP; (b) ventral VP are to regulate GABA outputs to the medio-dorsal thalamus; and (c) the prelimbic mPFC to regulate glutamate outputs to the premotor cortex. The net results of these actions are to initiate and maintain the goal-directed behavior to obtain self-infusions of ethanol into the pVTA. This hypothetical simple scheme does not take into account the multiple interactions these limbic regions have with other structures, nor does it take into account the complex neuronal interactions that occur within each region.

Acknowledgements

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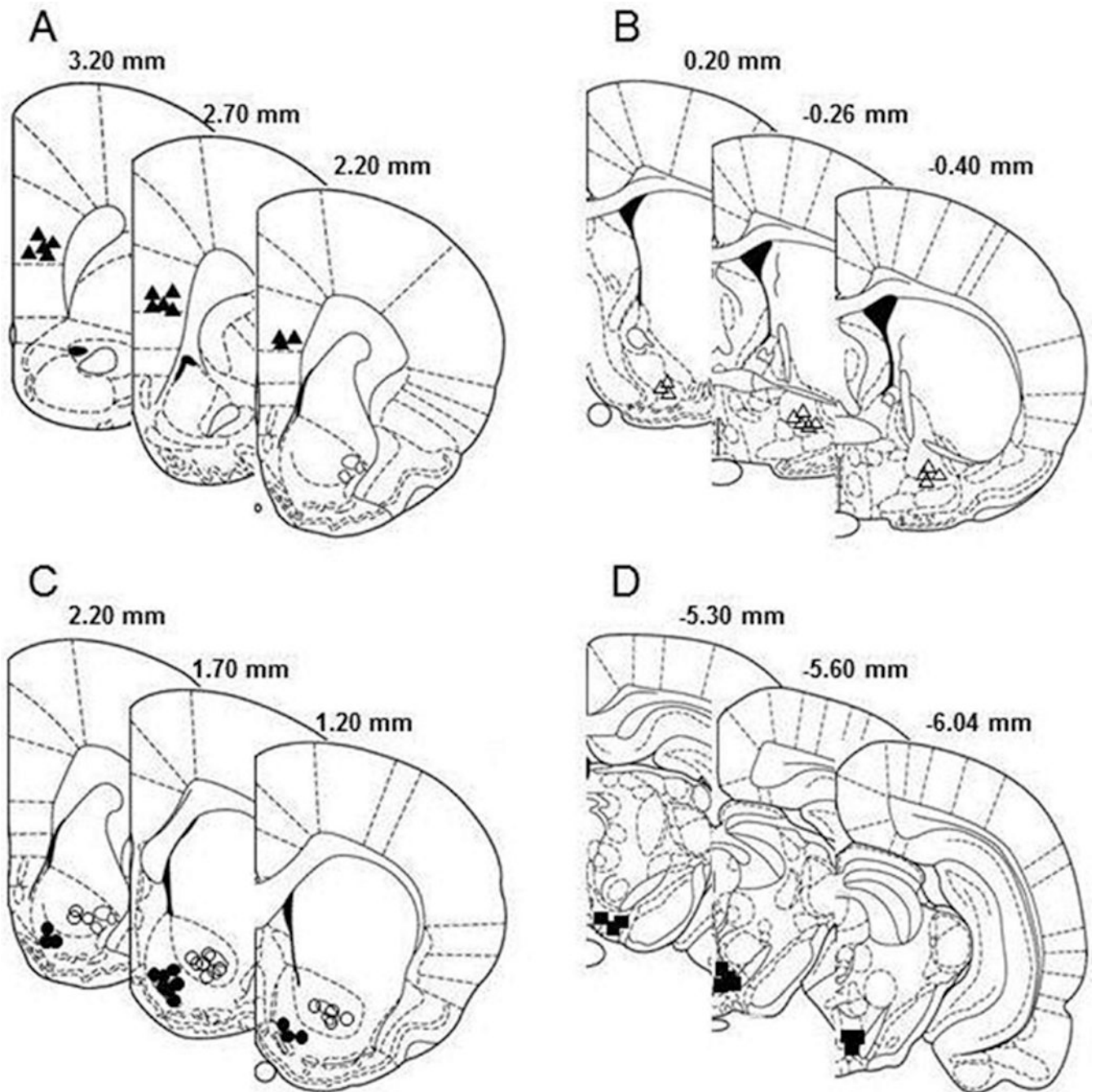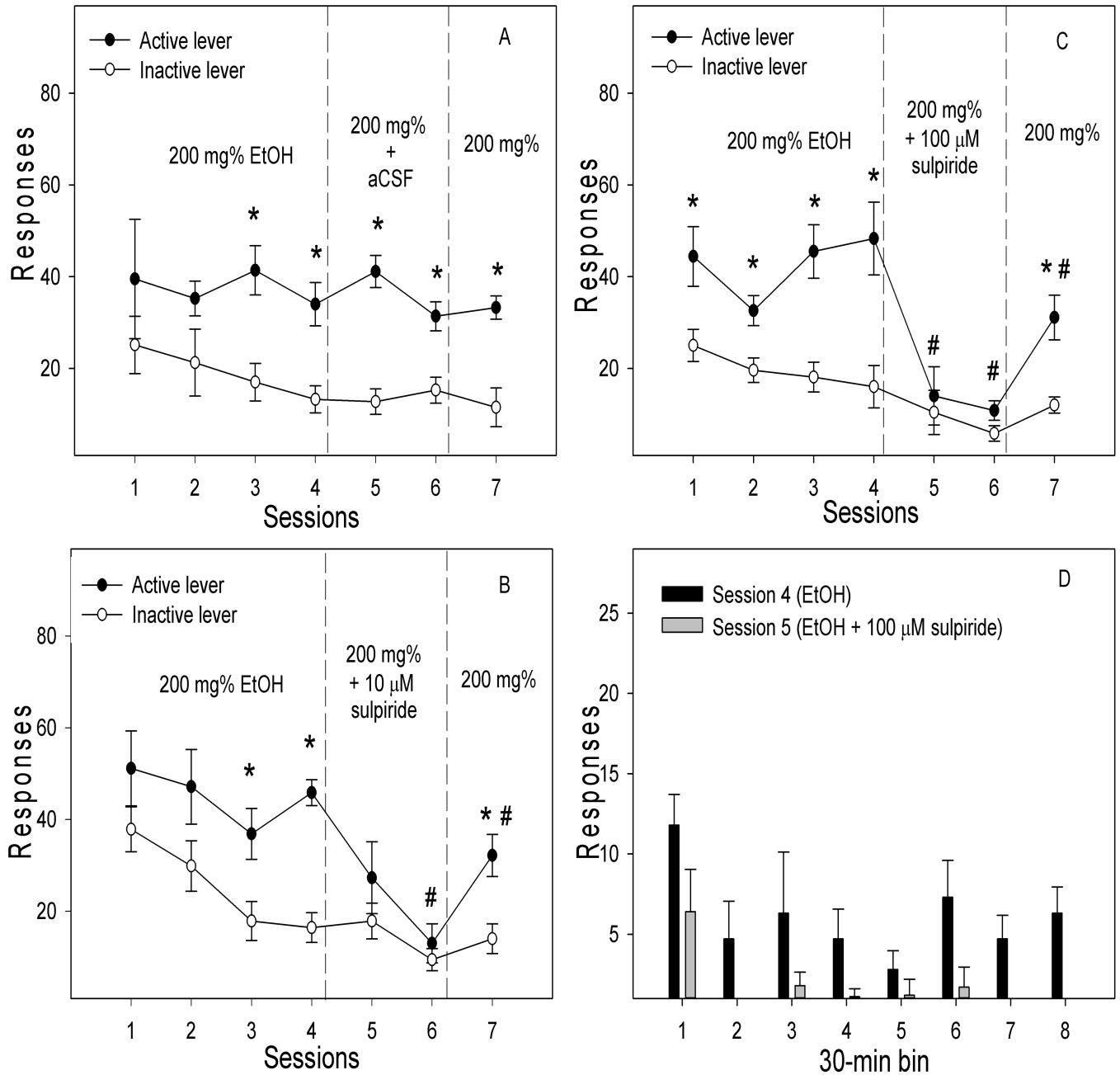
Figure 1.
Representative non-overlapping placements of the injection sites within the medial prefrontal cortex (A, filled triangle), ventral pallidum (B, open triangle), nucleus accumbens (C, filled circle for shell sub-region and open circle for core sub-region), and posterior ventral tegmental area (D, filled square), according to the brain atlas of Paxinos and Watson (1998). Placements within the medial prefrontal cortex were mainly within the prelimbic subregion. Placements within the ventral pallidum were mainly in the ventral portion.

Figure 2.
Effects of microinjection of vehicle (A, n = 8) or the D$_2$ receptor antagonist sulpiride (B & C, 10 and 100 μM, n = 7 and 10 respectively) into the nucleus accumbens shell on responses (Mean ± SEM) on the active and inactive lever for self-infusions of 200 mg% ethanol into the posterior ventral tegmental area. All rats received self-infusion of 200 mg% ethanol into the posterior ventral tegmental area in all sessions; the microinjection of sulpiride was given immediately prior to sessions 5 and 6. * Responses on the active lever were significantly higher than responses on the inactive lever ($p < 0.05$). # Responses on the active lever were significantly lower than average basal responses during sessions 3 and 4 ($p < 0.05$).

Response on the active lever in 30-min bins during acquisition session 4 and following treatment with 100 μM sulpiride in session 5 (panel D).
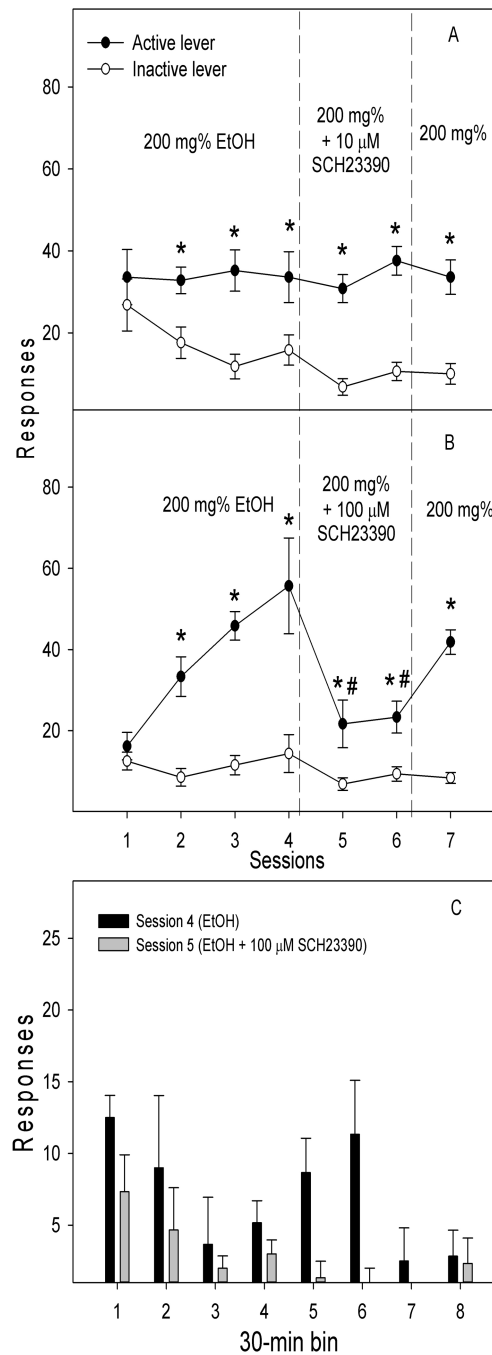
Figure 3.
Effects of microinjection of the D_1 receptor antagonist SCH-23390 (10 and 100 μM, n = 5 and 6, respectively) into the nucleus accumbens shell on the responses (Mean ± SEM) on the active and inactive lever for self-infusions of 200 mg% ethanol into the posterior ventral tegmental area. All rats received self-infusions of 200 mg% ethanol into the posterior ventral tegmental area in all sessions; the microinjection of SCH23390 was given immediately prior to sessions 5 and 6 (panels A and B). * Responses on the active lever were significantly higher than responses on the inactive lever ($p < 0.05$). # Responses on the active lever were

significantly lower than average basal responses during sessions 3 and 4 ($p < 0.05$). Responses on the active lever in 30-min bins during acquisition session 4 and following treatment with 100 μM SCH 23390 in session 5 (panel C).
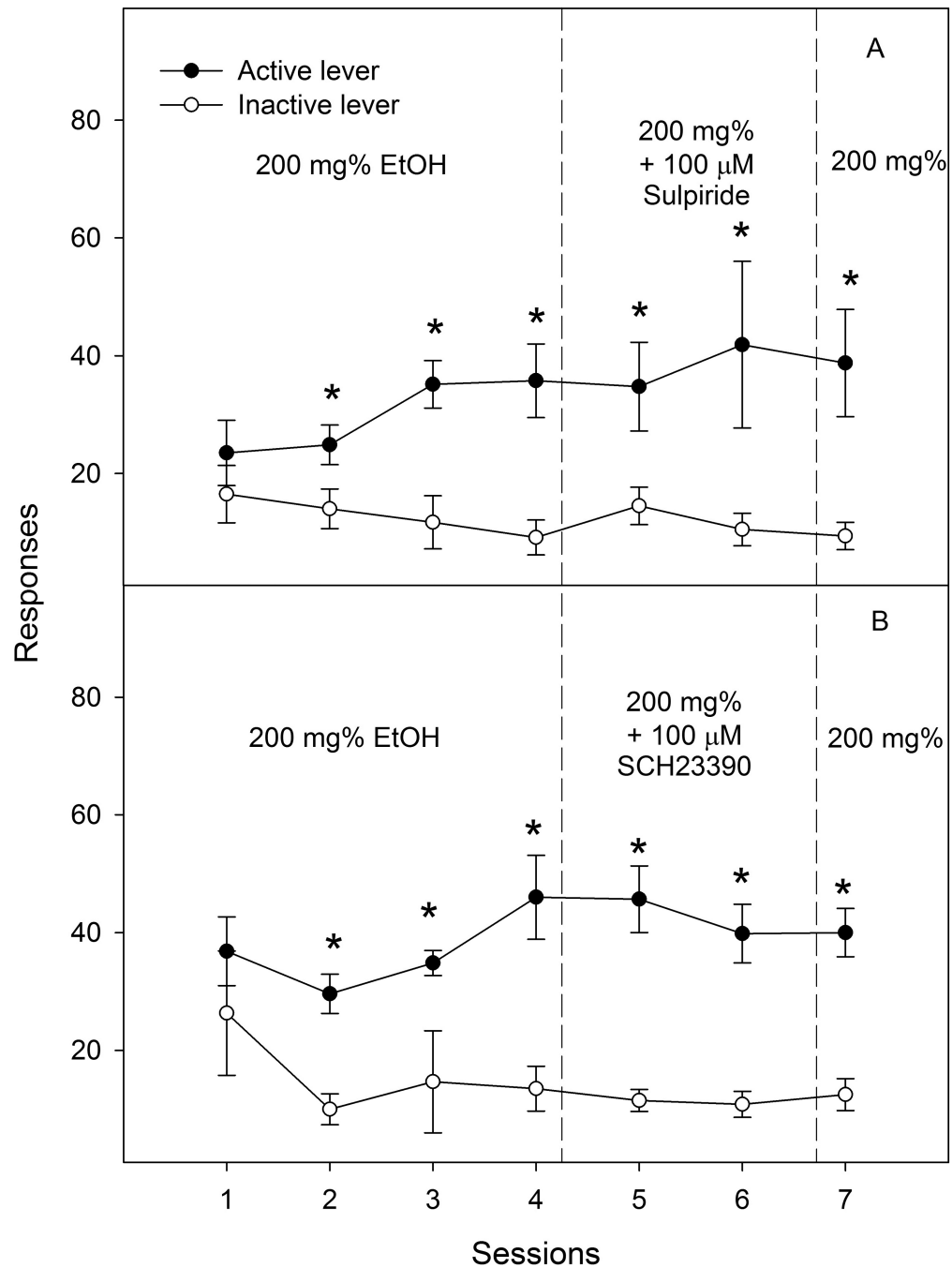
Figure 4.
Effects of microinjection of the D_2 receptor antagonist sulpiride (100 μM, n = 8) or the D_1 receptor antagonist SCH23390 (100 μM, n = 6) into the nucleus accumbens core on responses (Mean ± SEM) on the active and inactive lever for self-infusions of 200 mg% ethanol into the posterior ventral tegmental area. All rats received self-infusions of 200 mg% ethanol in the posterior ventral tegmental area in all sessions; the microinjection of sulpiride (panel A) or SCH-23390 (panel B) was given immediately prior to sessions 5 and 6. *

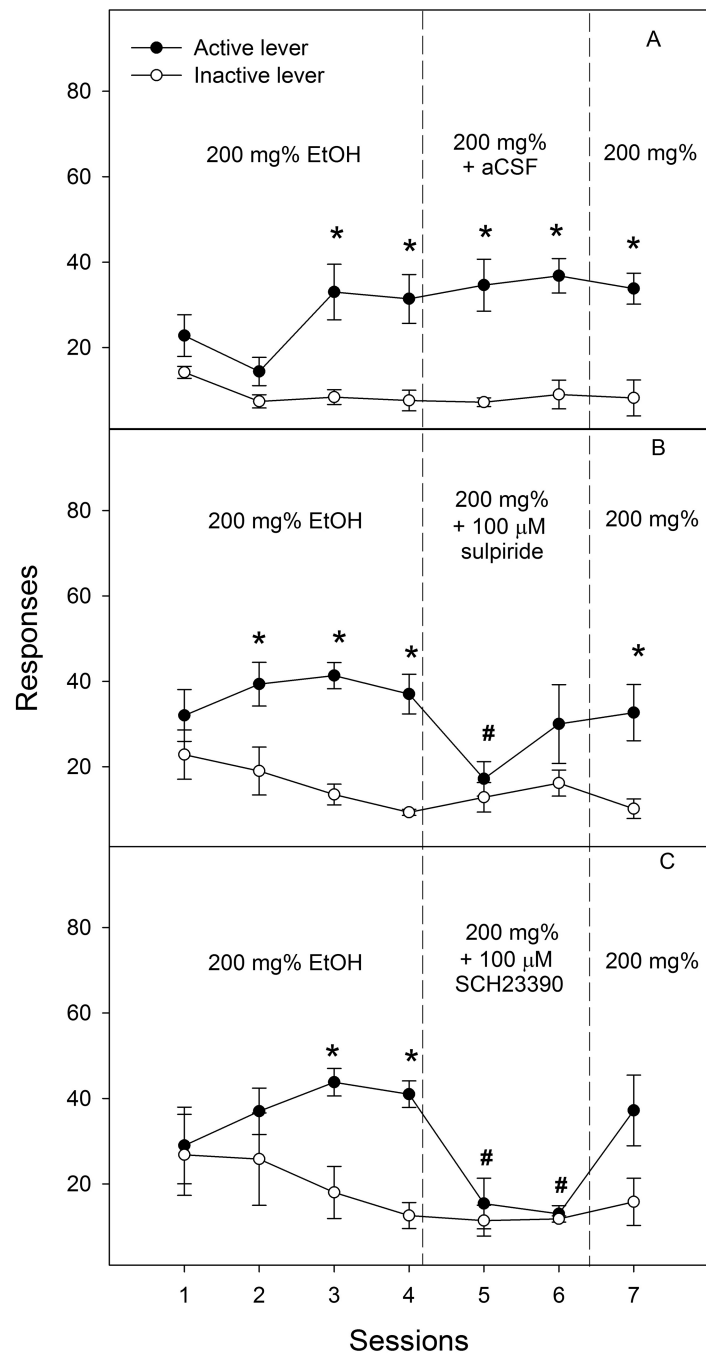Responses on the active lever were significantly higher than responses on the inactive lever ($p < 0.05$).

Figure 5.
Effects of microinjection of aCSF (n = 5), the D_2 receptor antagonist sulpiride (100 μM, n = 6) or the D_1 receptor antagonist SCH23390 (100 μM, n = 5) into the ventral portion of the ventral pallidum on the responses (Mean ± SEM) on the active and inactive lever for self-infusions of 200 mg% ethanol into the posterior ventral tegmental area. All rats received self-infusions of 200 mg% ethanol into the posterior ventral tegmental area in all sessions; the microinjection of aCSF (panel A), sulpiride (panel B) or SCH23390 (panel C) was given immediately prior to sessions 5 and 6. * Responses on the active lever were significantly

higher than responses on the inactive lever ($p < 0.05$). # Responses on the active lever were significantly lower than average basal responses during sessions 3 and 4 ($p < 0.05$).
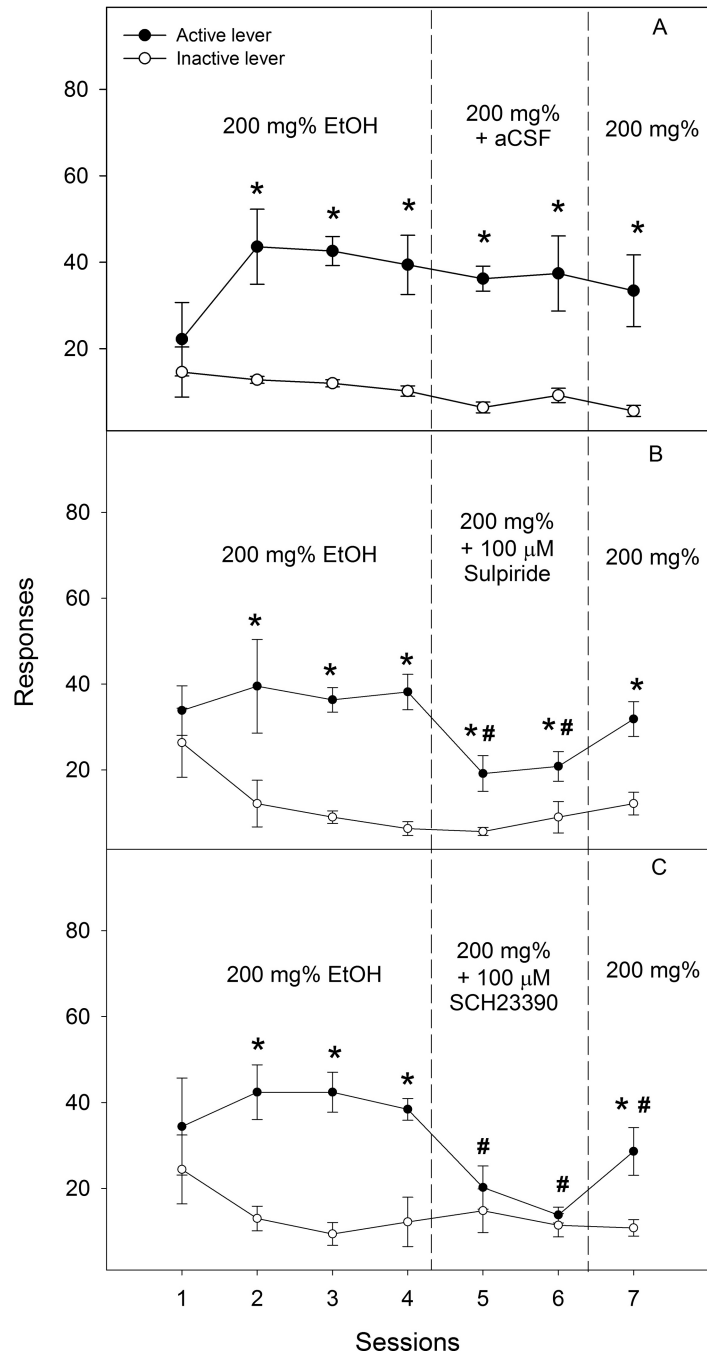
Figure 6.
Effects of microinjection of aCSF (n = 5), the D_2 receptor antagonist sulpiride (100 μM, n = 6) or the D_1 receptor antagonist SCH23390 (100 μM, n = 5) into the prelimbic sub-region of the medial prefrontal cortex on responses (Mean ± SEM) on the active and inactive lever for self-infusions of 200 mg% ethanol into the posterior ventral tegmental area. All rats received self-infusions of 200 mg% ethanol into the posterior ventral tegmental area in all sessions; the microinjection of aCSF (panel A), sulpiride (panel B) or SCH23390 (panel C) was given immediately prior to sessions 5 and 6. * Responses on the active lever were significantly

higher than responses on the inactive lever ($p < 0.05$). # Responses on the active lever were significantly lower than average basal responses during sessions 3 and 4 ($p < 0.05$).