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Involvement of local serotonin-2A but not serotonin-1B receptors in the reinforcing effects of ethanol within the posterior ventral tegmental area of female Wistar rats

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Abstract

Rationale—Previous studies indicated that ethanol could be self-infused into the posterior ventral tegmental area (p-VTA) and that activation of local serotonin-3 (5-HT_3) receptors was involved. 5-HT_{1B} and 5-HT_{2A} receptors are involved in the effects of 5-HT and ethanol on VTA dopamine neurons.

Objective—The current study used the intracranial self-administration (ICSA) procedure to determine the involvement of local 5-HT_{1B} and 5-HT_{2A} receptors in the self-infusion of ethanol into the p-VTA.

Materials and methods—Female Wistar rats were implanted *unilaterally* with a guide cannula aimed at the p-VTA. Seven days after surgery, rats were placed into the two-lever operant conditioning chambers for ICSA tests. The tests consisted of four acquisition sessions with self-infusion of 200 mg% ethanol alone, two or three sessions with co-infusion of the 5-HT_{1B} antagonist GR 55562 (10, 100, or 200 μM) or the 5-HT_{2A} antagonist R-96544 (10, 100, or 200 μM) with 200 mg% ethanol, and one final session with 200 mg% ethanol alone.

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Results—During the acquisition sessions, all rats readily self-infused ethanol and discriminated the active from inactive lever. Co-infusion of GR 55562, at all three doses, had no effect on the self-infusion of ethanol. In contrast, co-infusion of R-96544, at the two higher doses, attenuated responding on the active lever for ethanol infusion ($p<0.05$).

Conclusion—The results suggest that the reinforcing effects of ethanol within the p-VTA are modulated, at least in part, by activation of local 5-HT_{2A}, but not 5-HT_{1B}, receptors.

Keywords

Intracranial self-administration; Ventral tegmental area; Ethanol; Serotonin-1B receptor; Serotonin-2A receptor; GR 55562; R-96544

Introduction

The mesolimbic dopamine (DA) system, originating from the mesencephalic ventral tegmental area (VTA), mediates, at least in part, the reinforcing and rewarding effects of ethanol (Koob 1992; Koob et al. 1998; McBride and Li 1998; Wise 1996). A series of studies, using the intracranial self-administration (ICSA) technique, demonstrated that ethanol can be directly self-infused into the posterior VTA (p-VTA) of both Wistar and alcohol-preferring rats, identifying the p-VTA as a neurosubstrate for the reinforcing effects of ethanol (Gatto et al. 1994; Rodd-Henricks et al. 2000;Rodd et al. 2004, 2005b). The self-infusion of ethanol, which was dependent on activation of local DA neurons (Rodd et al. 2004, 2005b), was prevented by co-infusion of serotonin-3 (5-HT_3) receptor antagonists, suggesting that the reinforcing effects of ethanol within the p-VTA are modulated partially by activation of local 5-HT_3 receptors (Rodd-Henricks et al. 2003; Rodd et al. 2005b).

Evidence indicates that 5-HT transmission within the VTA can regulate local DA neuronal activity and the effects of ethanol. Projections from 5-HT neurons in raphe nuclei terminate in the VTA and form synapses on VTA DA neurons (Azmitia and Segal 1978; Herve et al. 1987; Parent et al. 1981; Van Bockstaele et al. 1994). Electrophysiological and pharmacological studies demonstrated that 5-HT activated a large proportion of VTA DA neurons (Pessia et al. 1994), facilitated DA release from the VTA slice (Beart and McDonald 1982), and enhanced DA release in the nucleus accumbens when infused into the VTA in vivo (Guan and McBride 1989). In addition, 5-HT has been shown to potentiate the excitation of ethanol on VTA DA neurons (Brodie et al. 1995). The effects of 5-HT can be mediated by 5-HT_3 receptors within the VTA. Local administration of a 5-HT_3 receptor agonist stimulated VTA DA neuronal activity (Campbell et al. 1996; Liu et al. 2006), whereas 5-HT_3 receptor antagonists decreased spontaneous firing rates of VTA DA neurons (Minabe et al. 1991; Rasmussen et al. 1991) and prevented ethanol-induced DA release (Campbell et al. 1996).

In addition to 5-HT_3 receptors, evidence also indicated that the effects of 5-HT within the VTA could be *modulated* by 5-HT_{1B} and 5-HT_{2A} receptors (Brodie et al. 1995; Pessia et al. 1994; Yan and Yan 2001; Yan et al. 2005). 5-HT_{1B} receptors are located within the VTA (Bruinvels et al. 1993; Pazos and Palacios 1985) and these receptors were suggested to *modulate* the DA-releasing effects of intra-VTA infusion of 5-HT (Guan and McBride 1989). Systemic or intra-VTA administration of 5-HT_{1B} agonists increased DA release in the nucleus accumbens (Boulenguez et al. 1996; Yan and Yan 2001), which could be blocked by 5-HT_{1B} antagonists (Hallbus et al. 1997). Furthermore, intra-VTA administration of a 5-HT_{1B} agonist prolonged, whereas an antagonist attenuated, ethanol-induced DA release in the nucleus accumbens (Yan et al. 2005). 5-HT_{2A} receptors are also found in the VTA (Doherty and Pickel 2000; Nocjar et al. 2002). The stimulating effect of

5-HT on VTA DA neurons was mimicked by 5-HT_{2A} agonists in vitro, whereas 5-HT_{2A} antagonists blocked the effects (Pessia et al. 1994). Moreover, 5-HT_{2A} agonists were shown to potentiate ethanol-induced excitation on VTA DA neurons (Brodie et al. 1995). Given the effects of 5-HT_{1B} and 5-HT_{2A} receptors in the VTA, the objective of the current study was to determine the effects of antagonism of these receptors on the self-infusion of ethanol into the p-VTA.

Materials and methods

Animals

Experimentally naïve adult female Wistar rats (body weight 270 to 320 g, Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) were used in the present study. Animals were doubly housed in a reverse 12-h light–dark cycle room (light off at 10:00 A.M.), with controlled temperature and humidity, for at least 2 weeks before the beginning of the experiment. Food and water were available *ad libitum* except during the ICSA test. Although both female and male Wistar rats exhibited similar self-infusion of ethanol into the p-VTA (Rodd-Henricks et al. 2000; Rodd et al. 2004), female rats were used in the present study because they maintain their head size better than male rats for more accurate stereotaxic surgery (Rodd-Henricks et al. 2000). The estrous cycle was not monitored in the present study. Previous studies suggested that estrous cycle did not appear to have a significant effect on ICSA behavior (Ikemoto et al. 1998). 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, and 10 mM *d*-glucose, pH 7.2–7.4. Ethyl alcohol (190 proof) was obtained from McCormick Distilling, Weston, MO, USA. The 5-HT_{1B} receptor antagonist GR 55562 dihydrochloride {3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride} (Walsh et al. 1995) and the 5-HT_{2A} receptor antagonist R-96544 hydrochloride {(2R,4R)-5-[2-[2-[2-(3-methoxyphenyl)ethyl] phenoxy]ethyl]-1-methyl-3-pyrrolidine hydrochloride} (Ogawa et al. 2002; Tanaka et al. 2000) were purchased from Tocris (Ellisville, MO, USA). All chemicals were dissolved in the aCSF solution to the desired concentrations prior to use.

Stereotaxic surgery procedures

Rats were implanted unilaterally with a 22-gauge cannula (Plastics One Inc., Roanoke, VA, USA) aimed at the right p-VTA (AP −5.6 mm, ML +2.1 mm, DV −8.5 mm), as described previously (Rodd-Henricks et al. 2003). Stylets were inserted into the cannulae when no experiments were being conducted. Rats were singly housed after surgery and were allowed to recover from surgery for at least 7 days prior to tests. Rats were habituated and handled on a daily basis during the 7-day period.

General test procedure

The ICSA tests followed procedures previously described (Bozarth and Wise 1981; Gatto et al. 1994). Briefly, during test days, rats were placed into operant conditioning chambers equipped with two levers, one active and one inactive. The active lever was connected to an electrolytic microinfusion transducer system (EMIT) controlled by an operant conditioning control system. The EMIT system is a current generator and is connected to two electrodes

that are immersed in a solution-filled cylinder container equipped with a 28-gauge injection cannula. Each response on the active lever (FR1 schedule of reinforcement) produced a 5-s infusion current between the electrodes, resulting in an infusion of 100 nl of solution into the p-VTA. Each infusion was followed by a 5-s 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. The duration of each ICSA session was 4 h, and sessions were conducted every other day. The attrition rate was about 10% mostly due to the loss of the cannula during testing.

The effects of co-infusion of GR 55562 on ethanol ICSA—For the ICSA experiments, rats were randomly assigned to one of five groups (n=5–8 per group). Two groups served as controls and self-infused aCSF or 200 μM GR 55562 alone for seven consecutive sessions. Three co-infusion groups self-infused 200 mg% ethanol for the first four sessions (acquisition). During sessions 5 and 6 (co-infusion), rats self-infused GR 55562 (10, 100, or 200 μM) and 200 mg% ethanol. During session 7 (reinstatement), rats self-infused 200 mg% ethanol. Previous findings indicated that 10 μM GR 55562 could attenuate ethanol-induced dopamine increase in the nucleus accumbens (Yan et al. 2005). This procedure of four acquisition sessions, two co-infusion sessions, and one reinstatement session has previously been used to examine ethanol self-infusion into the p-VTA (Rodd-Henricks et al. 2003). In the past, an additional control group (200 mg% ethanol for seven consecutive sessions) was used, and the data indicate that Wistar rats developed stable responding for 200 mg% ethanol for seven sessions (Rodd-Henricks et al. 2003; Rodd et al. 2004). Therefore, this 200 mg% group was not included in the current study. The 200 mg% ethanol has been demonstrated to be an optimal concentration for self-infusion into the p-VTA by Wistar rats (Rodd-Henricks et al. 2000). The 200 mg% ethanol is also physiologically relevant and can be readily obtained in rats under certain drinking conditions (Murphy et al. 1986; Waller et al. 1984).

The effects of co-infusion of R-96544 on ethanol ICSA—For the ICSA experiments, rats were randomly assigned to one of five groups (n=5–8 per group). Two groups served as controls and self-infused aCSF or 200 μM R-96544 alone for eight consecutive sessions. Three co-infusion groups self-infused 200 mg% ethanol for the first four sessions. During sessions 5–7, rats co-infused R-96544 (10, 100, or 200 μM) and 200 mg% ethanol. During session 8, rats self-infused 200 mg% ethanol. The reason for three co-infusion sessions with R-96544 was that three co-infusion sessions were mistakenly performed in the first group of rats; to keep consistency, all following groups were conducted with three co-infusion sessions.

Histology

At the end of experiments, rats were euthanized and bromophenol blue was injected into the p-VTA, as described previously (Gatto et al. 1994; Ikemoto et al. 1998). Brains were quickly removed and frozen at −20°C. Brain sections (40 μm thick) were sliced in a cryostat microtome and stained with cresyl violet for the verification of the placement of injection sites according to the rat brain atlas of Paxinos and Watson (1998).

Statistical analysis

The “group × session” mixed analyses of variance (ANOVAs) with repeated measures on the session were performed on responses on the active lever and the number of the infusions. For each individual group, lever discrimination was determined by “lever (active vs inactive) × session” mixed ANOVA with repeated measures on session. Post hoc Tukey’s b tests were followed when a significant main effect was found (p<0.05). Drug effects were

determined by comparing the responses on the active lever during antagonist co-infusion sessions to the average responses on the active lever during acquisition sessions 3 and 4.

Results

The p-VTA is the VTA region at the level of the interpeduncular nucleus from −5.3 to −6.0 mm relative to bregma (Rodd-Henricks et al. 2000). Figure 1 depicts the representative non-overlapping placements of injection sites within the p-VTA. Only rats with correct placements in the p-VTA were included in analysis. Approximately 80% of animals had injection sites within the p-VTA.

The effects of co-infusion of GR 55562 on ethanol ICSA

The lever responses for three GR 55562 co-infusion groups are shown in Fig. 2. Lever discrimination was determined by "lever × group" repeated ANOVA conducted in each individual group. The analysis demonstrated that lever discrimination developed for all co-infusion groups (all F values>17.59, all p values<0.005; Fig. 2) but not for the groups that self-infused aCSF or 200 μM GR 55562 (all F values<4.83, all p values>0.09; Table 1). The "group × session" repeated ANOVA performed on the responses on the active lever in the five groups revealed significant effect of group ($F_{4, 26}$=6.94, p=0.001) but no significant effect of session or session × group interaction (all F values<1.16, all p values>0.33). The lack of a significant effect of session (p>0.05) suggested that co-infusion of GR-55562, at all three concentrations, did not alter the responses on the active lever for 200 mg% ethanol during co-infusion sessions (Fig. 2).

The "session × group" repeated ANOVA performed on the number of infusions (data not shown) in all five groups indicated a significant group effect ($F_{4, 26}$=8.61, p<0.001) but no significant effect of session or session × group interaction (all F values<1.98, all p values>0.07). Rats had relatively low numbers of infusions for aCSF and 200 μM GR 55562 (Table 1), which was significantly lower than the infusions in the three co-infusion groups (p<0.05). The lack of a significant effect of session suggested that co-infusion of GR 55562 did not alter the number of infusions of 200 mg% ethanol into the p-VTA.

The effects of co-infusion of R-96544 on ethanol ICSA

Lever responses for three R-96544 co-infusion groups are shown in Fig. 3. Lever discrimination was determined by "lever × session" repeated ANOVA conducted in each different group. The analysis demonstrated that lever discrimination developed for the three co-infusion groups (all F values>15.85, all p values<0.01; Fig. 3) but not for the control groups (all F values<2.43, all p values>0.17; Table 1).

The "session × group" repeated ANOVA performed on the responses on the active lever in all five groups revealed significant effects of session and group and a significant session × group interaction (all F values>1.96, all p values< 0.01). Post hoc comparisons of the responses on the active lever during the first four acquisition sessions among five different groups indicated that the responses for the three co-infusion groups during sessions 2–4 (Fig. 3) were significantly higher than those in the two control groups (all p values<0.05). Furthermore, the responses in the three co-infusion groups during each acquisition session were within the range reported previously (Rodd-Henricks et al. 2000,2003) and were not statistically different from one another (all p values>0.05).

The significant session × group interaction term was decomposed by holding the "group" constant and examining the "session" effect for the last two acquisition sessions and the three co-infusion sessions (sessions 3–7) in each co-infusion group separately, which allowed an assessment of the effects of co-infusion of R-96544 on ethanol self-infusion. Co-

infusion of 10 μM R-96544 did not alter the responses on the active lever ($F_{4, 16}$=0.64, p>0.05; Fig. 3, top panel), whereas co-infusion of 100 or 200 μM R-96544 significantly altered the responses on the active lever (all $F_{4, 28}$>5.59, all p values<0.002; Fig. 3, middle and bottom panels). Post hoc analysis indicated significantly lower responses on the active lever during co-infusion sessions 5–7 compared to the average responses during session 3 and 4 (all p values<0.05) in the 100 and 200 μM R-96544 co-infusion groups (Fig. 3, middle and bottom panels).

The "session × group" repeated ANOVA performed on the number of infusions in the five groups (data not shown) indicated significant effects of session ($F_{7, 196}$=2.75, p=0.01) and group ($F_{4, 28}$=4.96, p=0.004) but no significant effect of session × group interaction ($F_{28, 196}$=1.23, p=0.21). Rats had relatively low numbers of infusions for aCSF and 200 μM R-96544 (Table 1). Co-infusion of 10 or 100 μM R-96544 did not significantly alter the number of infusions of 200 mg% ethanol into the p-VTA (all F values<2.20, all p values>0.05). However, 200 μM R-96544 significantly reduced the number of infusions of 200 mg% ethanol into the p-VTA (p<0.05) during all three co-infusion sessions (baseline 26±3, fifth session 19±3, sixth session 17±3, and seventh session 16±3).

Discussion

The major finding of the current study is that activation of local 5-HT_{2A} receptors, but not 5-HT_{1B} receptors, may modulate the reinforcing effects of ethanol within the p-VTA. This conclusion is supported by results showing that (a) co-infusion of the 5-HT_{2A} receptor antagonist R-96544 attenuated operant responses for ethanol (Fig. 3) and reduced the number of infusions of ethanol into the p-VTA and (b) co-infusion of the 5-HT_{1B} receptor antagonist GR 55562 had no effect on the self-infusion of ethanol into the p-VTA (Fig. 2).

R-96544 is a potent and selective 5-HT_{2A} receptor antagonist (K_i=1.6 nM; Ogawa et al. 2002). The behavioral effects of R-96544 in reducing ethanol self-infusion into the p-VTA are consistent with the localization of 5-HT_{2A} receptors within the VTA (Cornea-Hebert et al. 1999; Doherty and Pickel 2000; Nocjar et al. 2002; Pazos et al. 1985). The antagonist effect on ethanol self-infusion could be a result of R-96544 blocking the excitation of VTA DA neurons modulated by 5-HT_{2A} receptors directly on DA neurons and/or indirectly through excitatory inputs to the VTA.

The 5-HT_{2A} receptor is one subtype of the G-protein-coupled 5-HT_2 receptor family and mediates major excitatory effects of 5-HT (Hoyer et al. 2002). Some in vitro studies suggest that activation of 5-HT_{2A} receptors in the brain depolarizes neurons. Perfusion of VTA slices with 5-HT_{2A} agonists directly depolarized a large portion of VTA DA neurons, which was prevented by administration of 5-HT_{2A} antagonists (Pessia et al. 1994). Activation of 5-HT_{2A} receptors mediated 5-HT-induced depolarization of neurons in the nucleus accumbens, possibly by reducing potassium conductance (North and Uchimura 1989). Moreover, activation of 5-HT_{2A} receptors within the VTA potentiated the excitatory effects of ethanol on DA neurons (Brodie et al. 1995). These studies suggest that activation of 5-HT_{2A} receptors within the VTA may increase DA neuronal activity and blockade of 5-HT_{2A} receptors may decrease DA neuronal activity. Because activation of local DA neurons is involved in the reinforcing effects of ethanol within the p-VTA (Rodd et al. 2004, 2005b), co-infusion of the 5-HT_{2A} antagonist R-96544 could result in reduced DA neuronal activity and decreased self-infusion of ethanol (Fig. 3). In support of the idea that activation of 5-HT_{2A} receptors increases DA neuronal activity, single intravenous injection of the 5-HT_{2A} antagonist M100907 significantly decreased burst firing of VTA DA neurons; repeated intravenous injections of M100907 decreased both burst firing of DA neurons and the number of spontaneously active VTA DA neurons (Minabe et al. 2001). However, some

earlier studies reported the opposite effect with a different 5-HT_{2A} antagonist, i.e., ritanserin (Shi et al. 1995; Ugedo et al. 1989).

The 5-HT_{2A} receptor has long been associated with ethanol-related behavior. Polymorphisms in the 5-HT_{2A} receptor gene are associated with alcohol abuse and dependence (Hwu and Chen 2000; Nakamura et al. 1999). Systemic administration of 5-HT_{2A} antagonists decreased voluntary ethanol intake and preference and reduced operant responses for ethanol in rats (Myers et al. 1992, 1993; Overstreet et al. 1997; Roberts et al. 1998). The current study suggests that the reinforcing effects of ethanol might be modulated, in part, by activation of 5-HT_{2A} receptors within the p-VTA. In addition to ethanol, recent studies have associated 5-HT_{2A} receptors with the effects of other drugs of abuse. 5-HT_{2A} receptor antagonists attenuated DA increase in the nucleus accumbens induced by amphetamine and morphine (Auclair et al. 2004a, b). Antagonism of 5-HT_{2A} receptors also decreased the discriminative stimulus effects of cocaine (Filip et al. 2006), attenuated locomotor hyperactivity and development of behavioral sensitization induced by psychostimulants and morphine (Fletcher et al. 2002; Auclair et al. 2004a, b; Filip et al. 2004), and reduced cocaine-primed reinstatement of operant responding (Fletcher et al. 2002). Taken together, the 5-HT_{2A} receptor plays an important role in the neurochemical and behavioral effects of drugs of abuse.

Although selective for the 5-HT_{2A} receptor subtype, R-96544 exhibits affinity for 5-HT_2 receptors (IC_{50}=2.2 nM; Tanaka et al. 2000), which also includes the 5-HT_{2C} subtype, in addition to the 5-HT_{2A} subtype (Hoyer et al. 2002). Evidence shows that 5-HT_{2C} receptors are located in the VTA and regulate DA neuronal activity (Bubar and Cunningham 2007; Clemett et al. 2000). However, the antagonism of 5-HT_{2C} receptors with R-96544 may not be involved in the behavioral effects of R-96544 on self-infusion of ethanol in the p-VTA (Fig. 3). Previous research suggests that activation of VTA 5-HT_{2C} receptors may exert tonic inhibition of VTA DA neurons. Systemic administration of selective 5-HT_{2C} receptor agonists decreased, whereas antagonists increased, basal and evoked VTA DA neuronal activity (Di Matteo et al. 1999; Pierucci et al. 2004). The intra-VTA administration of a 5-HT_{2C} receptor agonist attenuated cocaine- and stress-induced DA overflow (Navailles et al. 2008; Pozzi et al. 2002), whereas local perfusion of a 5-HT_{2C} receptor antagonist into the VTA enhanced 3,4,-methylenedioxymethamphetamine-induced DA overflow in the nucleus accumbens (Bankson and Yamamoto 2004). If R-96544 was acting at 5-HT_{2C} receptors, then its administration should increase DA neuronal activity and further enhance ethanol excitation of DA neurons, resulting in increased, but not decreased (Fig. 3), self-infusion of ethanol. Therefore, the effects of R-96544 within the p-VTA on ethanol self-infusion may not involve 5-HT_{2C} receptors.

In the current study, the R-96544 achieved its effects at relatively high concentrations (100 and 200 μM, Fig. 3). This range of concentrations has been typically used in the ICSA studies with other agents. For example, the DA D_2 receptor antagonist sulpiride (1–3 mM) was tested on the self-infusion of the DA reuptake inhibitor nomifensine and *phencyclidine* (Carlezon and Wise 1996). 5-HT_3 receptor antagonists (10–200 μM) or DA D_2 agonist quinpirole (10–100 μM) was used to prevent the self-infusion of ethanol and cocaine (Rodd-Henricks et al. 2003; Rodd et al. 2004,2005a). Co-administration of DA D_1 or D_2 receptor antagonists (mM range) was used to decrease the self-infusion of cocaine or a mixture of D_1 and D_2 receptor agonists (Ikemoto 2003;Ikemoto et al. 1997). Even so, it should be remembered that R-96544, at micromolar levels, may have nonspecific effects, e.g., antagonism of DA D_2 receptors (IC_{50}=2.4 μM) or 5-HT_3 receptors (IC_{50}>5 μM; Tanaka et al. 2000), which has been shown to be involved in the reinforcing effects of ethanol within the p-VTA (Rodd-Henricks et al. 2003; Rodd et al. 2004,2005b). Therefore, it is possible

that high concentrations of R-96544, although given in a very small volume, may be blocking D_2 and/or 5-HT_3 receptors in addition to 5-HT_{2A} receptors.

After removing R-96544, the responses on the active lever during session 8 did not recover to the preantagonist levels in the 100 and 200 μM R-96544 co-infusion groups (Fig. 3). Although previous studies (Rodd-Henricks et al. 2003;Rodd et al. 2004) reported a recovery of responses, the current study did not observe such a recovery in the reinstatement session. It should be noted that only two co-infusion sessions were conducted in the previous studies (Rodd-Henricks et al. 2003;Rodd et al. 2004), whereas there were three co-infusion sessions in the current study. Perhaps, with the three co-infusion sessions, it will require more than one reinstatement session for responding to return to baseline levels.

The results with the 5-HT_{1B} antagonist GR 55562 indicated that blockade of local 5-HT_{1B} receptors does not alter the self-infusion of ethanol into the p-VTA (Fig. 2), suggesting that activation of local 5-HT_{1B} receptors may not be involved in the reinforcing effects of ethanol in the VTA. In addition, it is possible that 5-HT_{1B} receptors in the p-VTA may not be tonically activated. The 5-HT_{1B} receptors are primarily localized on axon terminals and function as presynaptic autoreceptors and heteroreceptors to regulate neurotransmitter release (Boschert et al. 1994;Maroteaux et al. 1992;Sari et al. 1999). Although intra-VTA application of 5-HT_{1B} agonists increased DA release in the nucleus accumbens (Yan and Yan 2001), reverse microdialysis of 5-HT_{1B} antagonists into the VTA did not alter extracellular dopamine levels in the nucleus accumbens (O'Dell and Parsons 2004;Yan et al. 2005), suggesting that 5-HT_{1B} receptors in the VTA may not tonically modulate DA activity.

The results suggest that 5-HT_{1B} receptors in the p-VTA do not modulate the local reinforcing effects of ethanol. However, the results do not exclude the involvement of 5-HT_{1B} receptors in modulating the rewarding and other effects of ethanol. Systemic administration of 5-HT_{1B} receptor agonists suppressed oral ethanol self-administration (Tomkins and O'Neill 2000; Wilson et al. 1998) and the conditioned reinforcing effects of ethanol (Wilson et al. 2000), as well as ethanol-induced aggressive behavior (Miczek and De Almeida 2001), suggesting that activation of 5-HT_{1B} receptors would reduce ethanol effects. Consistent with this idea, 5-HT_{1B} receptor knockout mice have higher ethanol intakes than wild-type mice (Crabbe et al. 1996). However, contrary to these findings, overexpression of 5-HT_{1B} receptors in the nucleus accumbens increased voluntary ethanol drinking (Hoplight et al. 2006). This inconsistency may suggest that 5-HT_{1B} receptors in different brain regions (e.g., the nucleus accumbens, ventral pallidum, etc.) may be differentially involved in regulating ethanol consumption.

In addition to ethanol, 5-HT_{1B} receptors are also involved in effects of psychostimulants. Activation of 5-HT_{1B} receptors potentiated the cocaine-induced increase in extracellular levels of DA in the nucleus accumbens (Parsons et al. 1999; O'Dell and Parsons 2004) and facilitated locomotor stimulation and sensitization of psychostimulants (Neumaier et al. 2002; Przegalinski et al. 2004). On the other hand, antagonism of 5-HT_{1B} receptors decreased the discriminative stimulus effects and locomotor stimulation of psychostimulants (Papla et al. 2002; Filip et al. 2003), reduced self-administration of cocaine (David et al. 2004), and attenuated cocaine-induced sensitization (Przegalinski et al. 2004). However, 5-HT_{1B} receptor knockout mice exhibited enhanced self-administration of cocaine (Rocha et al. 1998), contrary to the antagonist effects (David et al. 2004). The different findings with knockout mice may be due to the compensatory mechanisms. Overall, the 5-HT_{1B} receptor appears to play an important role in the reinforcing effects of ethanol and the psychostimulants.

One limitation of the current study was that the experimental design only tested one antagonist to each receptor type. In general, it is difficult to prove receptor modulation of specific effects due to relative specificity of the antagonist and relatively large doses applied. Thus, the conclusions would be more convincing if more than one antagonist to each of the two receptors had been tested in the current study. Nonetheless, the current study provided evidence that activation of local 5-HT_{2A} receptors, but not 5-HT_{1B} receptors, may be one of the mechanisms modulating the response-contingent behavior reinforced by ethanol in the p-VTA, further confirming the role of 5-HT transmission in the p-VTA in regulating the reinforcing effects of ethanol.

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Abbreviations

aCSF	artificial cerebrospinal fluid
DA	dopamine
EMIT	electrolytic microinfusion transducer
5-HT	serotonin
ICSA	intracranial self-administration
p-VTA	posterior ventral tegmental area
VTA	ventral tegmental area

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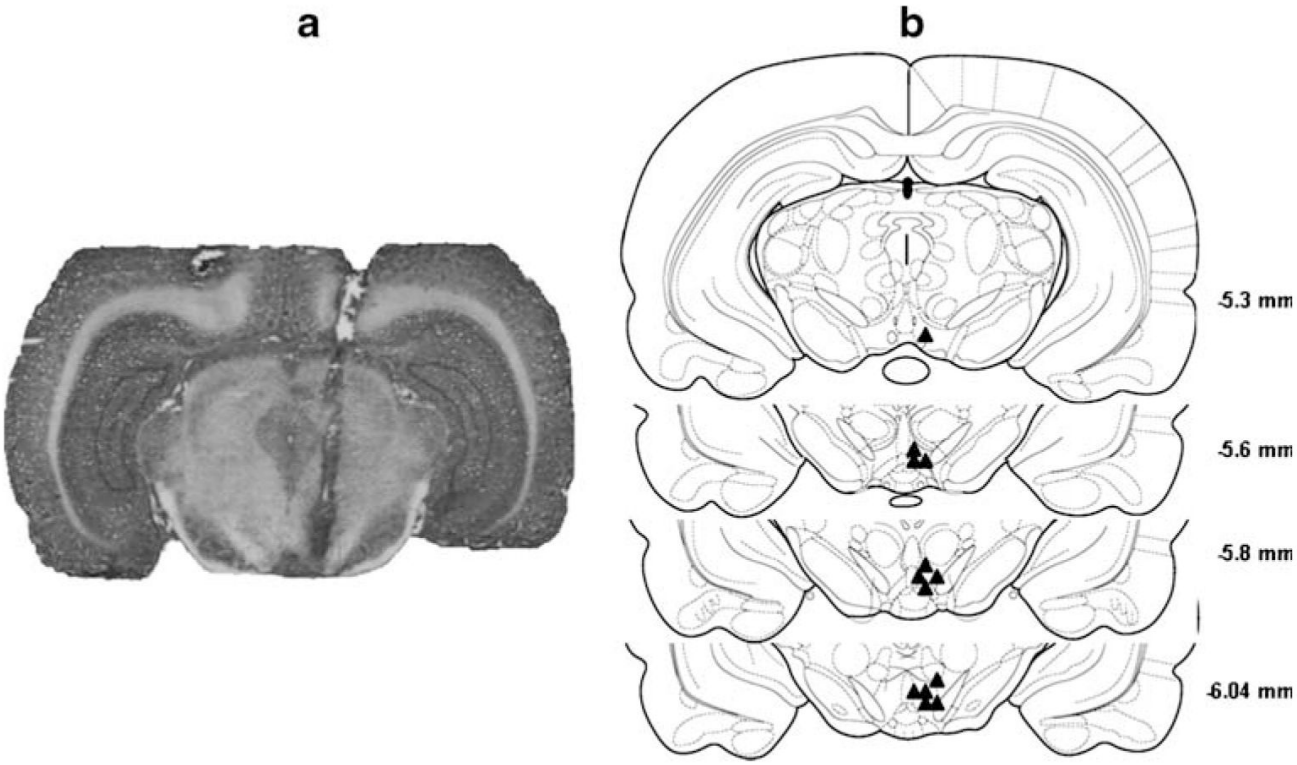

Fig. 1.

a A photomicrograph of a representative brain slice with an injection site within the p-VTA. **b** Representative non-overlapping placements of the injection sites within the p-VTA. The p-VTA corresponds to coronal sections from 5.3 to 6.0 mm posterior to bregma (Rodd-Henricks et al. 2000). The *filled triangles* represent the injection sites

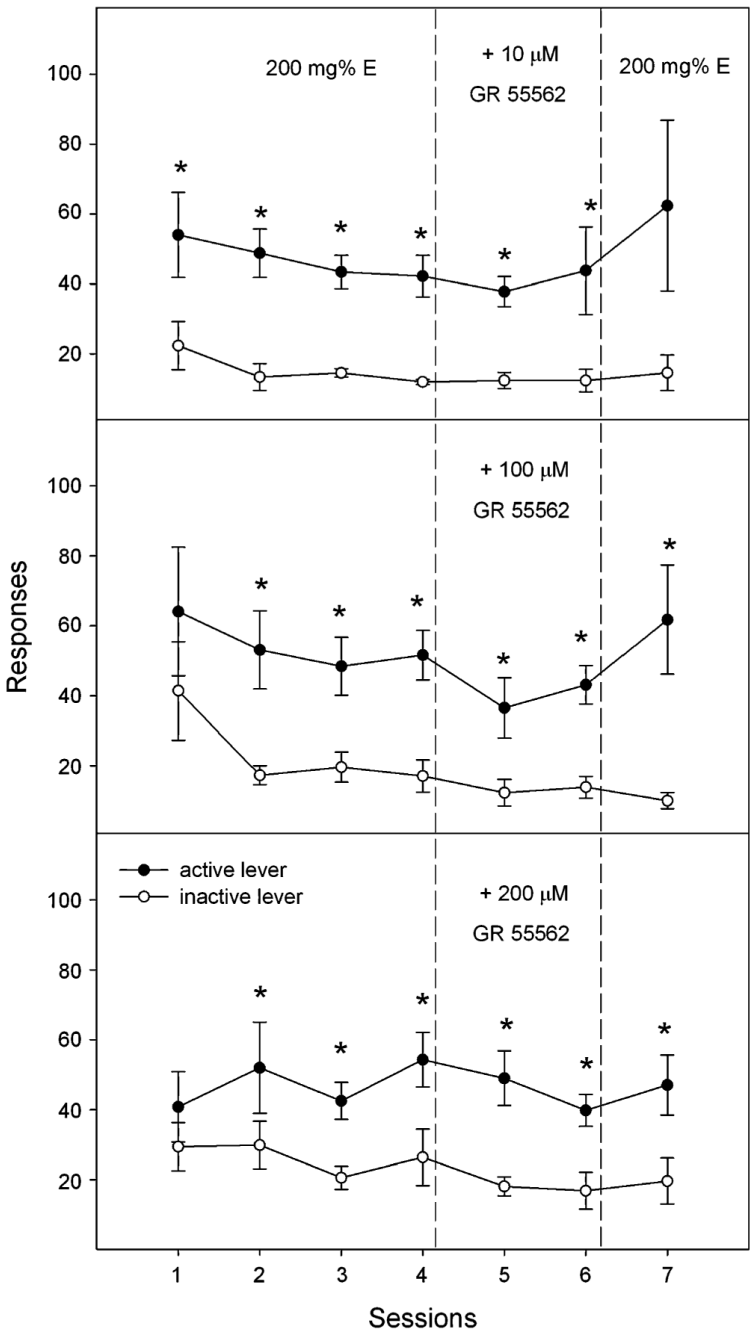

Fig. 2.

Mean responses (±SEM) on the active and inactive lever by female Wistar rats for self-infusion of 200 mg% ethanol in the first four sessions, 200 mg% ethanol plus 10 μM (*top*, *n*=5), 100 μM (*middle*, *n*=8), or 200 μM GR 55562 (*bottom*, *n*=8) in sessions 5 and 6, and 200 mg% ethanol alone in session 7 into the p-VTA. *Asterisks*, responses on the active lever were significantly higher than responses on the inactive lever ($p<0.05$). There was no effect of co-administration of GR 55562 on lever responses for ethanol

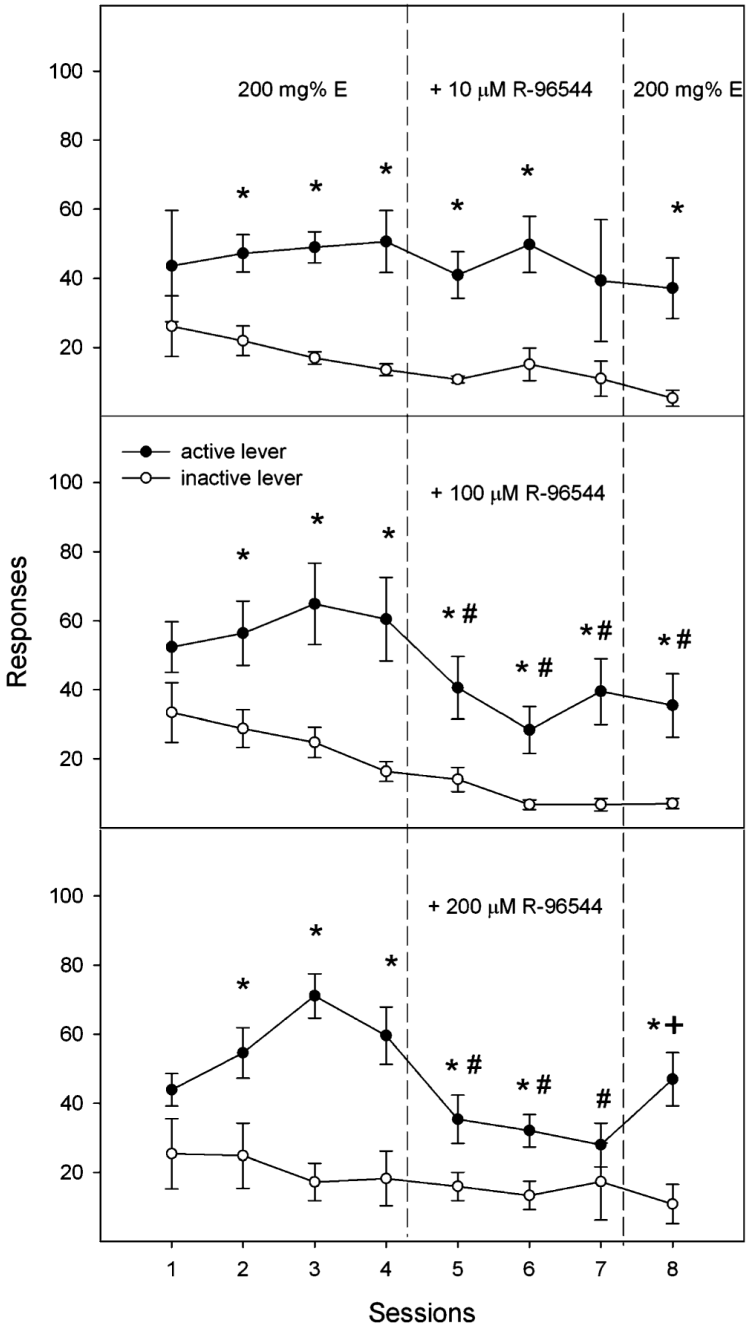

Fig. 3.

Mean responses (±SEM) on the active and inactive lever by female Wistar rats for self-infusion of 200 mg% ethanol in the first four sessions, 200 mg% ethanol plus 10 μM (*top*, *n*=5), 100 μM (*middle*, *n*=8), or 200 μM R-96544 (*bottom*, *n*=8) in sessions 5, 6, and 7, and 200 mg% ethanol alone in session 8 into the p-VTA. *Asterisks*, responses on the active lever were significantly higher than responses on the inactive lever ($p<0.05$); *number signs*, responses on the active lever are significantly different from the average responses during sessions 3 and 4 ($p<0.05$); *plus signs*, responses on the active lever in session 8 are significantly different from the responses during session 7 ($p<0.05$)

Table 1

Average lever responses and numbers of infusions per session (mean±SEM) for self-infusion of aCSF, GR 55562, and R-96544 alone

Groups	N	Responses		Infusions
		Active	Inactive	
aCSF[a]	10	14±2	9±2	7±1
200 μM GR 55562	5	18±9	12±6	7±3
200 μM G-96544	7	20±7	12±3	8±3

[a]Pooled data from aCSF groups since no difference was found between these two groups