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Ethanol Is Self-Administered Into the Nucleus Accumbens Shell, But Not the Core: Evidence of Genetic Sensitivity

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Abstract

Background—A previous study indicated that selectively bred alcohol-preferring (P) rats self-administered ethanol (EtOH) directly into the posterior ventral tegmental area at lower concentrations than Wistar rats. The present study was undertaken to determine involvement of the nucleus accumbens (Acb) with EtOH reinforcement, and a relationship between genetic selection for high alcohol preference and sensitivity of the Acb to the reinforcing effects of EtOH.

Methods—Adult P and Wistar rats were assigned to groups that self-infused 0 to 300 mg% EtOH into the Acb shell (AcbSh) or Acb Core (AcbC). Rats were placed into 2-lever (active and inactive) operant chambers and given EtOH for the first 4 sessions (acquisition), artificial cerebrospinal fluid (aCSF) for sessions 5 and 6 (extinction), and EtOH again in session 7 (reinstatement). Responding on the active lever produced a 100-nl injection of the infusate.

Results—Alcohol-preferring rats self-infused 75 to 300 mg% EtOH, whereas Wistar rats reliably self-infused 100 and 300 mg% EtOH into the AcbSh. Both P and Wistar rats reduced responding on the active lever when aCSF was substituted for EtOH, and reinstated responding in session 7 when EtOH was restored. EtOH was not self-infused into the AcbC by P or Wistar rats.

Conclusions—The present results indicate that the AcbSh, but not AcbC, is a neuroanatomical structure that mediates the reinforcing actions of EtOH. The data also suggest that, compared to Wistar rats, the AcbSh of P rats is more sensitive to the reinforcing effects of EtOH.

Keywords

Intracranial Self-Administration; Nucleus Accumbens; Reinforcement; Alcohol-Preferring Rats; Ethanol

The mesolimbic dopamine (DA) system is implicated in alcohol drinking, and the rewarding properties of ethanol (EtOH) and other drugs of abuse (Ikemoto, 2007; Koob et al., 1998). EtOH self-administration increases DA release in the nucleus accumbens (Acb) of alcohol-preferring (P) and Wistar rats (Gonzales and Weiss, 1998; Melendez et al., 2002; Weiss et al., 1993, 1996). Microinjection of D₂ receptor antagonists into the Acb of Long-Evans or Wistar rats reduced EtOH self-administration (Rassnick et al., 1992; Samson et al., 1993), whereas microinjection of amphetamine increased EtOH intake in P and Long-Evans rats

(McBride et al., 1993; Samson et al., 1993), suggesting that activation of the mesolimbic DA projection to the Acb may be involved in regulating alcohol drinking. In contrast to the microinjection studies, results with the DA neurotoxin 6-hydroxydopamine (6-OH-DA) suggest that the DA projections from the ventral tegmental area (VTA) to the Acb may not be necessary to maintain alcohol drinking. Lesions of the Acb with 6-OH-DA did not alter operant EtOH self-administration or 24-hour EtOH intakes of Wistar rats (Fahlke et al., 1994; Rassnick et al., 1993). However, 6-OHDA lesions of the Acb of alcohol-preferring (P) rats reduced EtOH intake during acquisition of EtOH drinking, although, similar to the studies with Wistar rats, 6-OH-DA under maintenance conditions had no significant effect on alcohol drinking (Ikemoto et al., 1997a). The latter results suggest that the Acb DA system plays an important role in the acquisition of alcohol drinking. The apparent disagreement between the 6-OH-DA lesion studies and the microinjection studies conducted under maintenance conditions may be a result of compensatory mechanisms occurring in the Acb over the time-course of degeneration of the DA projections, which maintains EtOH intake in the 6-OH-DA experiments. Therefore, it is possible that if the latter conditions exist, the Acb could have an important role in regulating alcohol drinking and the reinforcing effects of EtOH.

The intracranial self-administration (ICSA) of substances directly into specific brain areas is a method to elucidate the reinforcing properties of drugs in neuroanatomically distinct pathways. Previous studies, focusing on the mesolimbic DA system have demonstrated that both the VTA and some of its projection regions, support self-administration of EtOH and/or drugs of abuse (Bozarth and Wise, 1981; Gatto et al., 1994; Goeders and Smith, 1984; McKinzie et al., 1999; Rodd-Henricks et al., 2000, 2002b). The Acb has been shown to support the self-administration of amphetamine (Chevrette et al., 2002; Hoebel et al., 1983; Phillips et al., 1994a,b), cocaine (Ikemoto, 2003; McKinzie et al., 1999), ($\pm$)-3,4-methylenedioxymethamphetamine (MDMA; Shin et al., 2008), DA agonists (Ikemoto et al., 1997b) and the DA uptake inhibitor nomifensine (Carlezon et al., 1995).

Close investigation of this phenomenon provided evidence of anatomical and functional heterogeneity for self-administration of EtOH and/or other drugs of abuse within mesolimbic areas that was dependent on DA neurotransmission (McKinzie et al., 1999; Rodd-Henricks et al., 2000). Additionally, alcohol-preferring (P) rats were more sensitive than outbred Wistar rats to the reinforcing effects of EtOH in the posterior VTA (Rodd et al., 2004), suggesting a genetic component for direct EtOH self-administration into the VTA.

Anatomical heterogeneity for self-administration has been demonstrated in both the VTA and Acb. The posterior, but not the anterior, VTA supports the self-administration of EtOH (Rodd-Henricks et al., 2000), cocaine (Rodd et al., 2005a), the 5-HT₃ receptor agonist *m*-chlorophenylbiguanide (CPBG) (Rodd et al., 2007), acetaldehyde (an EtOH metabolite) (Rodd-Henricks et al., 2002a), and salsolinol, a condensation product of acetaldehyde and DA (Rodd et al., 2008a). Within the Acb, the shell (AcbSh), but not the core (AcbC), subregion supports the self-administration of cocaine (McKinzie et al., 1999), salsolinol (Rodd et al., 2003), the DA uptake inhibitor nomifensine (Carlezon et al., 1995), phencyclidine, dizoclipine (MK-801) or 3-($\pm$)-2-carboxypiperazin-4-yl propyl-1-phosphate (Carlezon and Wise, 1996), and a combination of DA D₁ and D₂ receptor agonists (Ikemoto et al., 1997b). In a recent report, the medial AcbSh, but not the AcbC or the olfactory tubercle, supported the ICSA of MDMA, which could be disrupted by coadministration of D₁ or D₂ receptor antagonists (Shin et al., 2008). However, no published reports have investigated the self-administration of EtOH into the Acb. The current work evaluates the effects of self-administration of EtOH into the AcbSh or AcbC in female Wistar and P rats. Based on previous work, the hypothesis to be tested was that the AcbSh, but not the AcbC,

would support the self-administration of EtOH, and that P rats would demonstrate greater sensitivity than Wistar rats to the rewarding properties of EtOH.

MATERIALS AND METHODS

Animals

Experimentally naïve, female P rats, from the 46th and 47th generations, and Wistar rats (Harlan, Indianapolis, IN) weighing 250 to 320 g at time of surgery were used. Female rats were used in the present study because (1) female rats were used in previous studies involving the ICSA of EtOH (Gatto et al., 1994; Rodd-Henricks et al., 2000, 2003) and (2) female rats appear to maintain their body weights and head size better than male rats for more accurate stereotaxic placements (Ikemoto et al., 1997a,b; Rodd-Henricks et al., 2000, 2002a, 2003). Rats were double-housed upon arrival and maintained on a 12-hour reverse light–dark cycle (lights off at 9 AM). Although not systematically studied, the estrus cycle did not appear to have a significant effect on ICSA behavior in the present study, or in previous ICSA studies (Gatto et al., 1994; Ikemoto et al., 1997a,b; Rodd-Henricks et al., 2000, 2002a,b, 2003), as indicated by no obvious fluctuations in ICSA behavior in female rats given similar doses of the same agent for 4 or more baseline sessions. Food and water were freely available except in the test chamber. Animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. 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, NIH, and the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Drug and Vehicle

The artificial cerebrospinal fluid (aCSF) consisted of (in mM): 120.0 NaCl, 4.8 KCl, 1.2 KH_2PO_4 , 1.2Mg SO_4 , 25.0 NaHCO_3 , 2.5 CaCl_2 , and 10.0 D-glucose. Ethyl alcohol (190 proof; McCormick Distilling Co., Weston, MO) was dissolved in the aCSF solution. When necessary, 0.1 N NaOH was added to adjust the pH to 7.4 ± 0.1 .

Apparatus

The test chambers ($30 \times 30 \times 26$ cm; $w \times h \times d$) were situated in a sound-attenuating cubicle ($64 \times 60 \times 50$ cm; Coulbourn Instruments, Allentown, PA) and illuminated by a dim house light during testing. Two identical levers (3.5×1.8 cm) were mounted on a single wall of the test chamber, 15 cm above a grid floor, and were separated by 12 cm. Levers were raised to this level to avoid accidental brushing against the lever and to reduce responses as a result of general locomotor activation. Directly above each lever was a row of 3 different colored cue lights. The light (red) to the far right over the active bar was illuminated during resting conditions. A desktop computer equipped with an operant control system (L2T2 system; Coulbourn Instruments) recorded the data and controlled the delivery of infusate in relation to lever response.

An electrolytic microinfusion transducer system (see Bozarth and Wise, 1980) was used to control microinfusions of drug or vehicle. Briefly, 2 platinum electrodes were placed in an infusate-filled cylinder container (28 mm in length $\times$ 6 mm in diameter) equipped with a 28-gauge injection cannula (Plastics One, Roanoke, VA). The electrodes were connected by a spring-coated cable (Plastics One) and a swivel (Model 205; Mercotac, Carlsbad, CA) to a constant current generator (MNC, Shreveport, LA) that delivered 6 μA of quiescent current and 200 μA of infusion current between the electrodes. Depression of the active lever delivered the infusion current for 5 seconds, which led to the rapid generation of H_2 gas (raising the pressure inside the airtight cylinder), and, in turn, forcing 100 nl of the infusate

through the injection cannula. During the 5-second infusion and additional 5-second time-out period, the house light and right cue light (red) were extinguished and the left cue light (green) over the active lever flashed on and off at 0.5-second intervals.

Animal Preparation

While under isoflurane anesthesia, a unilateral 22-gauge guide cannula (Plastics One) was stereotactically implanted in the right hemisphere of each subject, and aimed 1.0 mm above the target region. Coordinates (Paxinos and Watson, 1998) for placements into the AcbSh were 1.7 mm anterior to bregma, 2.4 mm lateral to the midline, and 7.5 mm ventral from the surface of the skull at a 10° angle to the vertical. Coordinates for placements into the AcbC were 1.7 mm anterior to bregma, 2.7 mm lateral to the midline, and 6.5 mm ventral from the surface of the skull at a 10° angle to the vertical. To ensure that tissue did not clog the injector when it was inserted for the self-administration experiments, a 28-gauge stylet that extended 0.5 mm beyond the tip of the guide was placed into the guide cannula between experimental sessions. Following surgery, all rats were individually housed and allowed to recover 7 to 10 days. Animals were handled for at least 5 minutes daily following the fourth recovery day. Subjects were not acclimated to the test chamber prior to the commencement of data collection, nor were they trained on any other operant paradigm.

General Test Condition

For testing, subjects were brought to the testing room, the stylet was removed, and the injection cannula screwed into place. The injection cannula was removed after each session and replaced by the stylet. To avoid trapping air at the tip of the injection cannula, the infusion current was delivered for 5 seconds during insertion of the injector, which resulted in a single noncontingent administration of infusate at the beginning of the session. Injection cannulae extended 1.0 mm beyond the tip of the guide. The test chamber was equipped with 2 levers. Depression of the “active lever” (fixed ratio 1 [FR1] schedule of reinforcement) caused the delivery of a 100- μ l bolus of infusate over 5 seconds followed by a 5-second time-out period. During both the 5-second infusion period and 5-second timeout period, responses on the active lever did not produce further infusions. Responses on the “inactive lever” were recorded, but did not result in infusions. The assignment of active and inactive lever with respect to the left or right position was counterbalanced among subjects. The active and inactive levers remained the same for each rat throughout the experiment. No shaping technique was used to facilitate the acquisition of lever responses. The number of infusions and responses on the active and inactive lever were recorded. The duration of each test session was 4 hours, and sessions occurred every other day to maximize site viability.

Dose–Response

Wistar rats with guide cannula placements in the AcbSh or AcbC were randomly assigned to 1 of 7 EtOH concentration groups ($n = 6$ to 8/group/injector location). A vehicle group received infusions of aCSF for all 7 sessions. The other groups received infusions of 50, 75, 100, 150, 200, or 300 mg% EtOH (which equates to 11, 16, 22, 33, and 65 mM EtOH) for the first 4 sessions. During the fifth and sixth sessions, all animals received infusions of aCSF. On the seventh session, rats were allowed to respond for their originally assigned infusate. A previous study indicated that stable responding on the EtOH lever was attained by sessions 3 and 4, extinction was reached within 2 sessions, and responding on the active lever was reinstated within 1 session when EtOH was restored (Rodd-Henricks et al., 2000).

Alcohol-preferring rats with guide cannula placements in the AcbSh were randomly assigned to 1 of 7 EtOH concentration groups ($n = 6$ to 7/group). The groups were allowed to self-administer 0 (aCSF), 50, 75, 100, 150, 200, or 300 mg% EtOH. P rats with guide cannula placements within the AcbC were allowed to self-administer 0 (aCSF), 75, 100,

150, 200, or 300 mg% ($n = 4$ to 6/group). Rats were allowed to self-infuse the assigned infusate for the initial 4 sessions, aCSF during sessions 5 and 6, and the original infusate for session 7.

Histology

At the termination of the experiment, 1% bromophenol blue ($0.5 \mu\text{l}$) was injected into the infusion site. Subsequently, the animals were given a fatal dose of Nembutal and then decapitated. Brains were removed and immediately frozen at -70°C . Frozen brains were subsequently equilibrated at -15°C in a cryostat microtome and then sliced into $40 \mu\text{m}$ 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

Data analysis consisted of a line $\times$ concentration $\times$ session mixed ANOVA, with a repeated measure of "session," performed on the number of infusions. Additionally, for each individual group, lever discrimination was determined by type (active or inactive) $\times$ day mixed ANOVA with a repeated measure of "session." Post hoc analyses were conducted using the Tukey's b test.

RESULTS

Cannula placements in and around the AcbC and AcbSh are depicted in Fig. 1. Cannula placements outside the Acb were ventral to the AcbSh. Primary locations of the cannula tips were between $+1.0$ to $+1.6$ mm relative to bregma. Additionally, approximately 97% of all subjects successfully completed the experimental paradigms and had cannula tip locations within the AcbC, AcbSh, or area immediately ventral to the AcbSh. There was no apparent evidence of toxicity from a gross histological examination of the tissue sections. Also, the fact that the rats show reinstatement of EtOH self-administration behavior on day 7 suggests that the systems mediating this behavior were still intact on the final day of the experiment. Wistar and P rats failed to self-administer EtOH into the area ventral to the AcbSh ($n = 8$ and 7, respectively).

EtOH concentrations of 0 to 300 mg% were tested in the present study to determine the response-contingent behaviors of Wistar and P rats to self-administer EtOH in the AcbSh and AcbC (Figs. 2 and 3). In Wistar rats, the overall analysis examined the number of infusions received across all 7 sessions and indicated that there was a significant effect of cannula location ($F_{1,82} = 29.9$; $p < 0.0001$), EtOH concentration ($F_{6,82} = 8.3$; $p < 0.0001$), session ($F_{6,77} = 20.2$; $p < 0.0001$), and a cannula location $\times$ concentration $\times$ session interaction ($F_{36,492} = 2.6$; $p < 0.0001$). Reducing the analysis to the average number of infusions received during the initial 4 sessions (acquisition; Fig. 2) revealed that there was a significant effect of cannula location ($F_{1,82} = 41.4$; $p < 0.0001$), concentration ($F_{6,82} = 8.1$; $p < 0.0001$), and a cannula location $\times$ concentration interaction ($F_{7,94} = 9.9$; $p < 0.0001$). Decomposing the interaction term by holding the cannula location constant revealed that, for Wistar rats with guide cannula placements within the AcbSh, there was a significant effect of EtOH concentration on the average number of self-infusions during the first 4 sessions ($F_{6,44} = 13.8$; $p < 0.0001$). Post hoc analysis indicated that rats self-administering 100, 150, 200, and 300 mg% EtOH into the AcbSh self-infused more than rats administering aCSF, 50 or 75 mg% EtOH (which did not differ from each other). In addition, Wistar rats given 300 mg% EtOH received fewer infusions than rats given 150 mg% EtOH. In contrast, with guide cannula placements into the AcbC, there was no significant effect of EtOH concentration on self-infusion levels of Wistar rats ($F_{6,38} = 0.2$; $p = 0.975$).

For P rats, the overall analysis performed on the number of infusions received across all 7 sessions indicated that there was a significant effect of cannula location ($F_{1,65} = 32.5$; $p < 0.0001$), EtOH concentration ($F_{6,65} = 9.3$; $p < 0.0001$), session ($F_{6,60} = 15.2$; $p < 0.0001$), and a cannula location $\times$ concentration $\times$ session interaction ($F_{30,320} = 2.7$; $p < 0.0001$). Reducing the analysis to the average number of infusions received during the initial 4 sessions (acquisition; Fig. 3) revealed that there was a significant effect of cannula location ($F_{1,65} = 39.9$; $p < 0.0001$), concentration ($F_{6,65} = 6.6$; $p < 0.0001$), and a cannula location $\times$ concentration interaction ($F_{5,65} = 5.2$; $p < 0.0001$).

Decomposing the interaction term by holding the cannula location constant revealed that P rats, with guide cannula placements within the AcbSh, showed a significant effect of EtOH concentration on the average number of self-infusions during the first 4 sessions ($F_{6,38} = 12.5$; $p < 0.0001$). Post hoc analysis indicated that rats given 75, 100, 150, 200, and 300 mg % EtOH received more infusions into the AcbSh than rats given aCSF or 50 mg% EtOH (which did not differ from each other). In addition, P rats given 150 mg% received more infusions than P rats given 75, 100, or 300 mg% and that rats given 200 mg% EtOH received more infusions than rats given 100 or 300 mg% EtOH. In contrast, with placements in the AcbC, there was no significant effect of EtOH concentration on the self-infusion levels of P rats ($F_{5,27} = 1.3$; $p = 0.28$).

Throughout the sessions, the number of lever presses for aCSF by both P and Wistar rats did not differ ($p = 0.76$) on the active and inactive levers. In Wistar rats, the overall statistical analysis of the active lever responses indicated there was a significant effect of concentration ($F_{6,82} = 10.8$; $p < 0.0001$), session ($F_{6,77} = 29.1$; $p < 0.0001$), cannulae location ($F_{1,82} = 41.4$; $p < 0.0001$), and a cannulae location $\times$ concentration $\times$ session interaction ($F_{36,492} = 3.1$; $p < 0.0001$). In Wistar rats with placements in the AcbSh, active lever responses (Figs. 4 and 5; left panels) were higher in the 100, 150, and 200 mg% EtOH groups compared to aCSF or 50 mg% EtOH groups during sessions 2 to 4 ($F_{6,44} > 11.3$; $p < 0.0001$; Tukey's b $p < 0.05$). In Wistar rats given 300 mg% EtOH, responding on the active lever was higher than observed for aCSF rats only during the third session; these rats did not display lever discrimination ($p > 0.08$). In addition, comparing within each group, Wistar rats given 100 to 200 mg% EtOH discriminated between active and inactive levers during sessions 2 to 4 ($p < 0.03$). In sessions 5 and 6, when aCSF was substituted for EtOH, Wistar rats in the 100 to 200 mg% EtOH groups displayed reduced responding on the active lever to the level of the inactive lever. In Wistar rats given 100 to 200 mg% EtOH (Figs. 4 and 5), there was a significant effect of session when contrasting the active lever responses among sessions 4, 5, and 6 ($F_{2,6} > 15.6$; $p < 0.004$). Post hoc comparisons indicated that active lever responses were lower during session 5 and 6 than during session 4. When the original infusate was returned (session 7), Wistar rats given 100 to 200 mg% EtOH returned to levels found in session 4, and were significantly higher than responses in sessions 5 and 6 ($F_{2,6} > 26.8$; $p < 0.001$). In addition, in Wistar rats that self-administered 200 mg% EtOH, the number of responses during session 7 was significantly higher than that observed during the fourth session ($p < 0.001$).

In contrast, Wistar rats with placements in the AcbC (Figs. 4 and 5, right panels), did not display differences in responding on the active lever between groups, failed to display lever discrimination, and there was no observed alteration in responding during aCSF substitution. Statistically, there was no effect of concentration ($F_{6,38} = 0.43$; $p = 0.85$), session ($F_{6,33} = 0.52$; $p = 0.79$), or concentration $\times$ session interaction ($F_{36,228} = 0.48$; $p = 0.99$).

For P rats, the overall statistical analysis of the active lever responses indicated there was a significant effect of concentration ($F_{6,65} = 10.4$; $p < 0.0001$), session ($F_{6,60} = 23.1$; $p < 0.0001$), cannulae location ($F_{1,65} = 41.8$; $p < 0.0001$), and a cannulae location $\times$

concentration $\times$ session interaction ($F_{30,320} = 3.1$; $p < 0.0001$). For P rats with placements in the AcbSh, active lever responses (Figs. 6 and 7; left panels) were higher in the 75, 100 (not shown), 150, 200, and 300 mg% EtOH groups than for P rats given aCSF or 50 mg% EtOH during sessions 1 to 4 ($F_{6,48} > 8.5$; $p < 0.0001$; Tukey's b $p < 0.05$). In addition, comparing within each group, P rats given 75 to 300 mg% EtOH discriminated between the active and inactive levers during sessions 1 to 4 ($p < 0.02$). In sessions 5 and 6, when aCSF was substituted for EtOH, P rats in the 75 to 300 mg% EtOH groups displayed reduced responding on the active lever ($p < 0.012$). Of note, responding on the inactive lever increased during session 5 for P rats given 75 to 200 mg% ($p < 0.001$), and during session 6 for P rats given 150 and 200 mg% EtOH ($p < 0.01$). For P rats given 75 to 300 mg% EtOH (Figs. 5 and 6; left panels), there was a significant effect of session when contrasting the active lever responses among sessions 4, 5, and 6 ($p < 0.03$). Post hoc comparisons indicated that active lever responses were lower during session 5 and 6 than during session 4, except for the 150 mg% EtOH group during session 5. When the original infusate was returned (session 7), P rats given 75 to 300 mg% EtOH returned to the level of responding in session 4; moreover, responses in session 7 were higher than in sessions 5 and 6 ($p < 0.001$).

Similar to Wistar rats, P rats with placements in the AcbC (Figs. 6 and 7, right panels) did not display differences in responding on the active lever between groups, failed to display lever discrimination, and there was no observed alteration in responding during aCSF substitution. Statistically, there was no effect of concentration ($F_{7,43} = 0.9$; $p = 0.54$), session ($F_{6,22} = 0.3$; $p = 0.90$) or concentration $\times$ session interaction ($F_{30,120} = 0.47$; $p = 0.99$).

A dose–response plot was constructed comparing the average number of self-infusions in the AcbSh received during sessions 1 to 4 between P and Wistar rats (Fig. 8). There was an effect of line ($F_{1,82} = 9.2$; $p < 0.001$), concentration ($F_{6,82} = 26.1$; $p < 0.001$), and a line $\times$ concentration interaction ($F_{6,82} = 8.7$; $p < 0.0001$). Examining the self-infusions by Wistar and P rats for each concentration of EtOH revealed that the P rats received more infusions than Wistar rats at 75, 150, and 200 mg% ($p < 0.009$).

In session 1, Wistar rats given aCSF or 150 mg% EtOH responded on the active lever a small number of times in the early part of the session, but responded very little thereafter (Fig. 9). A similar pattern of responding on the active lever by Wistar given aCSF was also evident in sessions 4, 6, and 7. In contrast, Wistar rats given 150 mg% EtOH showed high levels of responding on the active lever in session 4, with the highest number of responses occurring in the first and fourth hours of the session, with moderate levels of responding during the middle 2 hours (Fig. 9). During session 6 (extinction), the pattern of responding on the active lever resembled the pattern observed in session 4, except the number of responses were markedly lower in session 6. When EtOH was returned in session 7, Wistar rats reinstated responding on the active lever with a shift to higher responding in the first three 30-minute blocks (compared to the first two 30-minute blocks in session 4).

The pattern of responding for aCSF by P rats was similar to the pattern observed for Wistar rats in sessions 1, 4, 6, and 7 (Fig. 9). However, unlike the pattern observed for Wistar rats in session 1 for 150 mg% EtOH, P rats increased responding on the active lever for the self-infusion of EtOH after the first hour, which was sustained for the remainder of the session. In subsequent sessions, P and Wistar rats exhibited somewhat similar patterns of responding on the active lever for self-infusions of 150 mg% EtOH (sessions 4 and 7) and during extinction (session 6).

DISCUSSION

The results of this study indicate that Wistar and P rats will initiate and maintain the self-infusion of EtOH into the AcbSh but not the AcbC (Figs. 2–7). The self-infusion of EtOH into the AcbSh does not appear to be a result of a general increase in behavioral activity because rats in this study learned to discriminate the active from the inactive lever for the self-infusion of 100 to 200 mg% EtOH for Wistar rats and 75 to 300 mg% EtOH for P rats (Figs. 4 to 7). Additionally, in the same EtOH groups, rats decreased responding on the active lever when aCSF was substituted for EtOH and reinstated responding when the EtOH was restored (Figs. 4 to 7). A genetic effect was also apparent as the P rat demonstrated greater sensitivity to EtOH, as indicated by self-infusion of 75 mg% EtOH into the AcbSh, whereas Wistar rats did not demonstrate self-infusions until 100 mg% EtOH was given (Figs. 2 and 3). Moreover, P rats obtained significantly more EtOH self-infusions than Wistar rats at the 75, 150, and 200 mg% concentrations (Fig. 8). Overall, the data suggest that EtOH is reinforcing in the AcbSh and selective breeding for high alcohol intake is associated with increased sensitivity of this brain region to the reinforcing properties of EtOH.

A nonspecific effect of EtOH on cell membranes in the AcbSh is not likely responsible for EtOH self-infusion because dose-related infusions and responses were obtained with EtOH concentrations that are pharmacologically relevant and within the range of blood alcohol concentrations achieved by alcohol-preferring P rats during oral (Murphy et al., 1986) or intragastric (Waller et al., 1984) self-administration. In the Acb, the 75 to 300 mg% doses may have selective effects on brain mechanisms mediating reinforcement, because within this range of EtOH concentrations, Wistar and P rats (1) readily discriminate the active from the inactive lever during EtOH acquisition, (2) extinguish lever discrimination and responding on the active lever when aCSF is substituted for EtOH, and (3) reinstated lever discrimination and responding on the active lever when EtOH is restored (Figs. 4 to 7).

Although the neurobiological mechanisms underlying the self-infusion of EtOH in the AcbSh are unknown, previous studies have shown that other substances known to increase DA neurotransmission are also self-administered into the AcbSh. The DA uptake inhibitor nomifensine (Carlezon et al., 1995), salsolinol (Rodd et al., 2003), cocaine (Carlezon et al., 1995; McKinzie et al., 1999; Rodd-Henricks et al., 2002b), MDMA (Shin et al., 2008), and a combination of DA D₁ and D_{2/3} receptor agonists (Ikemoto et al., 1997b) are all self-administered into the AcbSh. Self-administration of either salsolinol (Rodd et al., 2003), MDMA (Shin et al., 2008), or cocaine (Rodd-Henricks et al., 2002b) is inhibited by coinjection with a DA D_{2/3} receptor antagonist (sulpiride or raclopride). Moreover, the self-administration of the D₁ and D_{2/3} receptor agonist combination was blocked by the addition of a D₁ or D_{2/3} receptor antagonist and each agonist alone was not self-administered (Ikemoto et al., 1997b). These studies suggest that substances that act to increase DA neurotransmission support self-administration into this brain area, and activation of both D₁ and D_{2/3} receptors may be necessary. Further studies are needed to determine if these DA receptors are also involved in the ICSC of EtOH into the AcbSh.

Ethanol could affect DA systems in the AcbSh via a number of possible mechanisms. EtOH has been shown to enhance DA neurotransmission through the serotonin system by activation of 5-HT₃ receptors (Lovinger, 1999). These receptors have been shown to mediate DA release in the Acb and mesocortical brain areas (Alex and Pehek, 2007; Campbell and McBride, 1995) and have been implicated as playing a role in the development of alcohol and drug abuse (Engleman et al., 2008). Thus, the ICSC of EtOH into the AcbSh may result from its effect at 5-HT₃ receptors to locally increase extracellular DA levels. Additional studies with 5-HT₃ receptor agents are needed to examine this

possibility. EtOH administration also increases release of γ -aminobutyric acid (GABA) and induces neuroadaptive changes in GABAergic systems (see Criswell and Breese, 2005; Krystal et al., 2006). Recent evidence indicates that oral EtOH self-administration has been shown to reduce the expression of the glutamic acid decarboxylase 1 gene and the GABA_A receptor subunit $\beta 2$ gene in the Acb of inbred alcohol-preferring rats (Rodd et al., 2008b). This suggests that EtOH self-administration may result in reduced transmission at certain GABA_A receptors in the Acb. If ICSA of EtOH in the Acb also results in reduced GABAergic transmission, the reinforcing effects observed in the current study could result from the local disinhibition of systems associated within the reward pathway. In addition, GABA_A receptors containing the $\alpha 4$ subunit in the AcbSh appear to be involved in the preference and consumption of low to moderate amounts of EtOH in Long-Evans rats (Rewal et al., 2009). EtOH has also been shown to affect nicotinic cholinergic receptors (nAChRs), N-methyl-D-aspartate (NMDA) glutamate receptors, as well as L-type Ca²⁺ channels and G protein-activated inwardly rectifying K⁺ channels (see Vengeliene et al., 2008, for recent review). Together, these studies indicate that there are a number of neurotransmitter systems, receptors, and effector systems that could mediate the self-administration of EtOH into the AcbSh.

A direct reinforcing effect of EtOH in the Acb could also help explain why 6-OH-DA lesions of the Acb do not reduce EtOH intakes under maintenance conditions (Fahlke et al., 1994; Ikemoto et al., 1997a; Rassnick et al., 1993), and only partially reduce EtOH intakes under acquisition conditions (Ikemoto et al., 1997a). If EtOH can have reinforcing effects within multiple sites involving multiple transmitter systems then disrupting 1 system may not be sufficient to significantly reduce EtOH intakes, especially if the EtOH reinforcing site is “downstream” of the lesion. The current study also produced an inverted-U-shaped concentration-response curve for both Wistar and P rats (Figs. 2 and 3). For the 300 mg% concentration, the number of infusions were still above those of aCSF for both strains (Figs. 2 and 3), and responses on the active lever were greater than aCSF for the P rats (Fig. 7) but not for the Wistar rats (Fig. 5). A similar inverted-U-shaped concentration-response relationship was observed previously for EtOH self-administration into the posterior VTA by Wistar and P rats (Rodd et al., 2004; Rodd-Henricks et al., 2000). The tendency for subjects to respond and/or infuse less EtOH at high concentrations may be reflective of the recruitment of receptor systems that oppose the reinforcing mechanisms that mediate EtOH self-infusion observed at lower concentrations. High concentrations (300 mg% and higher) of EtOH might also affect membrane fluidity and the functioning of ion channels involved in EtOH self-administration. Also, as these animals have control over the amount of EtOH infused throughout the session by adjusting their response rate, they may attempt to “titrate” their EtOH dose at higher concentrations by reducing response rates and number of infusions. From the current data, in the AcbSh, EtOH has a larger range of concentrations at which there is clear lever discrimination, extinction, and reinstatement behavior in P rats (75 to 300 mg%) when compared with Wistar rats (100 to 200 mg%). If EtOH drinking is in part mediated through EtOH effects in the AcbSh and/or the posterior VTA, the enhanced rewarding properties of EtOH at lower and higher EtOH concentrations may result in increased EtOH intake in the P versus the Wistar rat. Thus, this expansion of the EtOH dose-response curve in these brain areas of P rats compared to Wistars may be an important factor in the expression of the alcohol-preferring phenotype (Murphy et al., 2002).

The regional heterogeneity of EtOH self-administration observed in the current study has also been demonstrated in the VTA (Rodd-Henricks et al., 2000), where EtOH was self-administered into the posterior, but not the anterior VTA. The dose-response effects for the ICSA of EtOH into the AcbSh observed for Wistar and P rats in the present study were similar to the concentration range reported for the ICSA of EtOH into the posterior VTA of Wistar (Rodd-Henricks et al., 2000) and P rats (Gatto et al., 1994; Rodd et al., 2004). In

addition, similar to the differences in sensitivity to the reinforcing effects of EtOH observed in the AcbSh between P and Wistar rats, the posterior VTA was more sensitive to the reinforcing effects of EtOH in P than Wistar rats. Both Wistar and P rats also showed a similar time-course of responding as observed in previous for EtOH self-infusion into the posterior VTA (Rodd et al., 2004; Rodd-Henricks et al., 2000) during acquisition, extinction, and reinstatement (Fig. 9). The posterior VTA also supports the self-administration of cocaine, acetaldehyde, salsolinol, and the 5-HT₃ agonist CPBG, all of which are reduced with the co-infusion of the DA D_{2/3} receptor agonist quinpirole or a 5-HT₃ receptor antagonist (Rodd et al., 2005a,b, 2007, 2008a). Also consistent with current work, and suggestive of regional heterogeneity in mesolimbic systems, systemic (i.v.) EtOH administration was found to induce a greater elevation of extracellular DA levels in the AcbSh than in the AcbC of Long–Evans rats (Howard et al., 2008). A recent review (Ikemoto, 2007) of the neuroanatomical projections of the VTA DA system and their role in addictions stated that the medial accumbens shell is a “drugreward trigger zone” that receives “strong dopaminergic innervation from the posteromedial VTA and central linear nucleus (drug-reward trigger zones in the ventral midbrain), but with little innervation from anteromedial or lateral VTA. This arrangement of dopaminergic neurons can provide an explanation for the behavioral data, suggesting that the anterior and posterior VTA are functionally heterogeneous.” The current study further implicates the mesolimbic system, possibly involving GABA neurons in the AcbSh, in mediating the reinforcing properties of EtOH and other drugs of abuse. In addition, the results suggest that increased sensitivity of this pathway to the reinforcing effects of EtOH is associated with a genetic predisposition for alcohol preference and high EtOH intake.

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Fig. 1. Representative injection sites in the AcbSh and AcbC of P and Wistar rats (overlapping sites are excluded). Circles represent sites of injection within the AcbSh and squares represent sites of injection within the AcbC. For illustrative purposes, injection sites for P and Wistar rats are depicted on the left and right sides of the figures, respectively.

Fig. 2.

Dose-response graph for number of infusions averaged over the first 4 sessions for the self-infusion of 0, 50, 75, 100, 150, 200, and 300 mg% EtOH into the AcbSh and AcbC of Wistar rats. Asterisks indicate that the number of infusions administered by the Wistar rat was significantly greater ($p < 0.05$) than aCSF or 50 mg. Plus symbol indicates that Wistar rats self-administering 150 mg% EtOH was significantly greater ($p < 0.05$) compared to aCSF, 50 and 300 mg% EtOH groups.

Fig. 3.

Dose-response graph for number of infusions averaged over the first 4 sessions for the self-infusion of 0, 50, 75, 100, 150, 200, and 300 mg% EtOH into the AcbSh and AcbC of P rats. Asterisks indicate that the number of infusions administered by the P rat was significantly greater ($p < 0.05$) than aCSF or 50 mg% EtOH. Plus symbol indicates that the number of infusions of 150 mg% EtOH was significantly greater ($p < 0.05$) compared all groups, except the 200 mg% EtOH group. Hash symbol indicates that the number of infusions of 200 mg% EtOH was significantly greater ($p < 0.05$) than aCSF, 50 and 100 mg% EtOH.

Fig. 5.

Responses on the active and inactive lever by Wistar rats for the self-infusion of 150, 200, or 300 mg% EtOH for the first 4 sessions (acquisition), aCSF for sessions 5 and 6 (extinction), and EtOH again in session 7 (reinstatement). Asterisks indicate responses on the active lever were significantly higher ($p < 0.05$) than responses on the inactive lever for that session and greater than aCSF controls.

Fig. 6.

Responses on the active and inactive lever by P rats for the self-infusion of 0, 50, or 75 mg% EtOH for the first 4 sessions (acquisition), aCSF for sessions 5 and 6 (extinction), and EtOH again in session 7 (reinstatement). Asterisks indicate responses on the active lever were significantly higher ($p < 0.05$) than responses on the inactive lever for that session and greater than aCSF controls.

Fig. 7.

Responses on the active and inactive lever by P rats for the self-infusion of 150, 200, or 300 mg% EtOH for the first 4 sessions (acquisition), aCSF for sessions 5 and 6 (extinction), and EtOH again in session 7 (reinstatement). Asterisks indicate responses on the active lever were significantly higher ($p < 0.05$) than responses on the inactive lever for that session and greater than aCSF controls.

Fig. 8. Dose-response graph for number of infusions averaged over the 4 acquisition sessions for the self-infusion of 0, 50, 75, 100, 150, 200, and 300 mg% EtOH into the AcbSh in P and Wistar rats. Asterisks indicate significant differences between P and Wistar rats.

Fig. 9.

Intrasection time-course of responses on the active lever for Wistar and P rats self-infusing aCSF (sessions 1, 4, 6, and 7) or 150 mg% EtOH in sessions 1 and 4 (acquisition), aCSF in session 6 (extinction), and 150 mg% EtOH in session 7 (reinstatement). Data are mean $\pm$ SEM active lever responses in 30-minute blocks.