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Ethanol-Induced Conditioned Taste Avoidance:

Reward or Aversion?

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Abstract

Background—Rats avoid intake of a palatable taste cue when paired with all drugs of abuse tested. Evidence suggests that, at least for morphine and cocaine, rats avoid the taste cue because they are anticipating the rewarding properties of the drug. Thus, the suppressive effects of a rewarding sucrose solution and cocaine, but not those of the putatively aversive agent, LiCl, are exaggerated in drug sensitive Lewis rats. Likewise, the suppressive effects of sucrose and morphine, but not those of LiCl, are eliminated by bilateral lesions of the gustatory thalamus. Unlike morphine and cocaine, it is less clear whether rewarding or aversive drug properties are responsible for ethanol-induced suppression of intake of a taste cue. The present set of studies tests whether, like cocaine, ethanol-induced suppression of intake of a taste cue also is greater in the drug-sensitive Lewis rats and whether the suppressive effects of the drug are prevented by bilateral lesions of the taste thalamus.

Methods—In Experiment 1, fluid deprived Lewis and Fischer rats were given 5 min access to 0.15% saccharin and then injected with saline or a range of doses of ethanol (0.5, 0.75, 1.0, or 1.5 g/kg). There was a total of 6 such pairings. In Experiments 2 and 3, Sprague-Dawley rats received bilateral electrophysiologically-guided lesions of the gustatory thalamus. After recovery, suppression of intake of the saccharin cue was evaluated following repeated daily pairings with either a high (1.5 g/kg) or a low (0.75 g/kg) dose of ethanol.

Results—Ethanol-induced suppression of intake of the saccharin conditioned stimulus (CS) did not differ between the drug-sensitive Lewis rats relative to the less-sensitive Fischer rats. Lesions of the taste thalamus, however, prevented the suppressive effect of the 0.75 g/kg dose of the drug, but had no impact on the suppressive effect of the 1.5 g/kg dose of ethanol.

Conclusion—The results suggest that the suppressive effects of ethanol on CS intake are mediated by both rewarding and aversive consequences, varying as a function of dose.

Keywords

alcohol; gustatory thalamus; Lewis; Fischer; CTA; reward comparison

Introduction

Rats suppress intake of an otherwise palatable gustatory conditioned stimulus (CS) when paired with a putatively aversive or rewarding unconditioned stimulus (US). A conditioned taste aversion (CTA) occurs when rats suppress intake of a taste cue following pairings with a known aversive agent such as lithium chloride (LiCl) or x-radiation (Garcia et al., 1955; Nachman and Ashe, 1973). This phenomenon has been interpreted as a conditioned disgust

reaction established by the development of an association between the flavor and activation of the emetic system (Garcia et al., 1974). Rats also avoid intake of a similar taste cue (such as saccharin) when paired with a highly preferred sucrose US (Flaherty and Checke, 1982). This phenomenon is referred to as an anticipatory contrast effect (ACE) and is thought to occur because the perceived value of the saccharin CS pales in anticipation of the availability of the preferred sucrose US (Flaherty and Checke, 1982; Flaherty and Grigson, 1988; Flaherty and Rowan, 1985).

As with LiCl and sucrose, rats also avoid intake of a saccharin cue when paired with all drugs of abuse tested (Hunt and Amit, 1987; Le Magnen, 1969). For years, these effects have been assumed to be due to the development of an association between the taste cue and some aversive stimulus property of these drugs (Cappell and Le Blanc, 1971; Goudie, 1979; Hunt and Amit 1987; Nachman et al., 1970), Parker (1984; 1988; 1991; 1993; 1995; 2003), however, used the taste reactivity test (Grill and Norgren, 1978) and revealed that the taste avoidance produced by experimenter delivered drugs such as cocaine, amphetamine, methamphetamine, methylphenidate, morphine, nicotine and phencyclidine differs qualitatively from that produced by the emetic agent, LiCl. Recent data confirm and extend this interpretation by showing that rewarding, rather than aversive, drug properties are responsible for the reduction in CS intake. This account is referred to as the reward comparison hypothesis (Grigson, 1997). Supporting evidence is as follows: (1) As with abused substances, rats also markedly avoid intake of a saccharin cue when paired with a highly rewarding sucrose solution (Flaherty and Checke, 1982). (2) Rats exhibit aversive orofacial responses to gustatory stimuli when paired with the aversive agent, LiCl, but not when paired with experimenter delivered morphine, cocaine, or a range of abused substances (Grill and Norgren, 1978; Parker, 1984; 1988; 1991; 1993; 1995). (3) The suppressive effects of sucrose and cocaine, but not LiCl, are augmented in Sprague-Dawley rats with a history of chronic morphine treatment (Grigson et al., 2001). (4) The suppressive effects of cocaine and sucrose, but not LiCl, are greater in drug-sensitive Lewis rats than in the less sensitive Fischer rats (Grigson and Freet, 2000). (5) The suppressive effects of a rewarding sucrose solution and morphine, but not those of a matched dose of LiCl, are eliminated by bilateral lesions of the gustatory thalamus (Flynn et al., 1991; Grigson et al., 2000; Reilly and Pritchard, 1996; Reilly and Trifunovic, 1999; Scalera et al., 1997; Schroy et al., 2005).

In support of the hypothesis that these drugs are reinforcing, most drugs of abuse such as heroin, morphine, cocaine, and amphetamine also produce conditioned place preference in rats (Carr, et al 1989; Tzschentke 1998). This, however, is not the case for moderate to high doses (1.0-5.0 g/kg) of ethanol (EtOH) which consistently produce conditioned place aversion in rats. A few studies report conditioned place preference with low to moderate doses (0.5-2.0 g/kg) of EtOH under certain experimental conditions (Fidler et al 2004). For example, when drug-naïve subjects are used, most studies report no effect or a conditioned place aversion in rats (Broadbent et al., 2002; Froehlich et al., 1988; Risinger and Boyce, 2002; Risinger and Cunningham, 1998). Data of this nature suggest that ethanol-induced suppression of CS intake may be due to aversive, rather than rewarding, drug properties.

Lewis and Fischer rats differ in their behavioral response to EtOH. Relative to Fischer rats, Lewis rats orally self-administer more EtOH and show significantly higher response rates for EtOH (Suzuki et al., 1988a). A similar pattern is evidenced in response to other drugs of abuse where Lewis rats also show greater self-administration, place conditioning, and locomotor sensitization (Ambrosio et al., 1995; Camp et al., 1994; Guitart, et al., 1992; Kosten, et al., 1994; 1997; Martin, et al., 1999; Suzuki, et al., 1987, 1988a; 1988b; 1992), but see Kosten et al. (2007). Moreover, as referred to above, Lewis rats also exhibit greater suppression of intake of a saccharin cue than Fischer rats when the saccharin solution is

paired with cocaine or sucrose (Grigson and Freet, 2000). Hence, Lewis rats appear to be more responsive to the rewarding properties of drugs of abuse, and also show greater reward comparison effects. We hypothesized, then, that if EtOH-induced suppression of saccharin intake is mediated by rewarding drug properties, 'reward-sensitive' Lewis rats should show greater avoidance of the EtOH-associated taste cue than Fischer rats. If, on the other hand, the suppressive effects are mediated by aversive properties, then the magnitude of the suppressive effect of EtOH should be equal between the two strains as is the case with LiCl-induced CTA (Grigson and Freet, 2000). Experiment 1 used Lewis and Fischer rats to test these hypotheses.

Gustatory thalamus is the functional name for the parvocellular component of the nucleus ventralis posteromedialis (VPMpc) which receives the projection from the gustatory zone of the pontine parabrachial nucleus (PBN) and then projects to the anterior insular gustatory cortex (Norgren, 1984;1995). This structure is critically involved in the comparison of rewards over time. Bilateral lesions of the gustatory thalamus disrupt successive negative contrast effects (i.e., avoidance of a weaker concentration of sucrose when unexpectedly downshifted from a preferred higher concentration (Reilly, 1998). This lesion also disrupts anticipatory contrast — avoidance of a saccharin cue that predicts access to a preferred sucrose reward in the very near future (Reilly and Pritchard, 1996;Schroy, et al., 2005). Finally, bilateral lesions of the taste thalamus also fully eliminate avoidance of a palatable taste cue when paired with a 10 mg/kg dose of either cocaine- (Baldwin et al., 2005) or a 15 mg/kg dose of morphine (Grigson et al., 2000). LiCl-induced CTAs, on the other hand, remain intact across all doses tested (Flynn et al., 1991;Grigson et al., 2000;Reilly, 1998;Scalera et al., 1997). Taken together, these data suggest that bilateral lesions of the taste thalamus also can be used to identify the rewarding or aversive properties of drugs. Thus, if EtOH-induced suppression of CS intake is mediated by aversive drug properties, then the conditioned reduction in CS intake should not be altered by the thalamic lesion. If, on the other hand, the suppression of CS intake is mediated by rewarding drug properties, then EtOH-induced suppression should be disrupted in rats with bilateral lesions of the gustatory thalamus. Experiments 2 and 3 tested these hypotheses in Sprague-Dawley rats.

This conditioned reduction in CS intake is a classic paradigm used to identify aversive properties of EtOH and other drugs of abuse. Suppression of CS intake, however, can be induced by known aversive agents such as lithium chloride (LiCl) or x-radiation (Garcia et al., 1955; Nachman and Ashe, 1973) or by the availability of a highly preferred sucrose solution (Flaherty & Checke, 1982) or a drug of abuse (Cappell and Le Blanc, 1971; Le Magnen, 1969; Grigson, 1997; Goudie, 1979; Hunt and Amit 1987; Nachman et al., 1970). Given that a "CTA", alone, is inadequate to dissociate reward from aversion, the present set of studies employed additional manipulations (e.g., selectively bred strains and lesions of the gustatory thalamus) to determine when EtOH-induced avoidance of CS intake is mediated by rewarding consequences and when it is mediated by aversive consequences.

Experiment 1

As stated in the Introduction, Lewis rats generally exhibit greater conditioned place preferences for drugs of abuse, including EtOH, than do Fischer rats (Suzuki et al., 1988a). These data may suggest that EtOH is rewarding, particularly in Lewis rats. However, a study by Roma et al. (2006) found that Fischer rats exhibited greater EtOH-induced suppression of CS intake than did Lewis rats. This was true for a 1.25 g/kg and a 1.5 g/kg dose of EtOH. Strain differences were not found for a more moderate 1.0 g/kg dose of the drug. These results suggest that EtOH may suppress saccharin intake through its aversive properties at the doses of 1.25 and 1.5 g/kg, but not at the dose of 1.0 g/kg. The present study will revisit this question across a range of doses, including those below and above 1.0 g/kg. In addition, the Lewis and Fischer rats tested in this study will be examined using a less restrictive

water-deprivation regimen, potentially allowing for the expression of greater suppression of CS intake in both strains of rat.

Materials and Methods

Subjects: The subjects were 40 male Fischer rats (Harlan Laboratories, Indianapolis, IN) and 40 male Lewis rats (Harlan Laboratories, Indianapolis, IN). The Fischer rats weighed between 283 and 340 g and the Lewis rats between 288 and 396 g at the beginning of the experiment. All were experimentally naïve. The rats were housed individually in standard wire-mesh cages in a colony room with temperature, humidity, and ventilation automatically controlled. Except where noted otherwise, the rats received water and food *ad libitum*. They were maintained on a 12/12 h light/dark cycle, and all experimental manipulations began approximately 2 h into the light phase of the cycle.

Apparatus: All experiments were conducted on the home cage using inverted Nalgene graduated cylinders with silicone stoppers and stainless steel spouts. Fluid intake was recorded to the nearest 0.5 ml.

Procedure: The rats were placed on a water-deprivation schedule for 10 days before the start of the experiment in which they received access to dH₂O for 5 min in the morning and for 1 hr in the afternoon. During testing, the subjects received 5 min access to 0.15% saccharin, followed 5 min later by an i.p. injection of either 0.5, 0.75, 1.0, or 1.5 g/kg 15% (v/v) EtOH or an equal volume (1.0 g/kg EtOH) of saline (n=8/cell). There was one taste-drug pairing a day, occurring every other day, for a total of six trials. All subjects received 5 min access to dH₂O each morning between conditioning trials and 1 hr access each afternoon to rehydrate.

Drugs and solutions: Fifteen ml 200 proof EtOH was diluted to 100ml in 0.9% saline immediately before each experimental session. Saccharin sodium salt hydrate (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and was presented at room temperature.

Data analysis: The data were analyzed using a $2 \times 5 \times 6$ mixed factorial analysis of variance (ANOVA) varying Strain (Lewis vs. Fischer), Dose (0, 0.5, 0.75, 1.0, or 1.5 g/kg), and Trials (1-6). Post hoc tests were conducted, where appropriate, using Newman-Keuls tests, $p < 0.05$.

Results

Saccharin intake: Rats avoided intake of the saccharin cue following pairings with a range of doses of EtOH. The suppressive effects were dose dependent, but did not differ as a function of strain, see Figure 1.

Support for this conclusion is provided by a non-significant Strain $\times$ Dose interaction, $F(4,71) = 2.0$, $p < 0.1$, and Strain $\times$ Dose $\times$ Trials interaction, $F(20,355) = 1.22$, $p < 0.23$. There was, however, a significant main effect of dose, $F(4,71) = 106.3$, $p < 0.0001$, and post hoc analysis confirmed that all rats injected with the three higher doses of EtOH drank less of the saccharin CS than did rats injected with either saline or the 0.5 g/kg dose of EtOH, $ps < 0.05$. The Dose $\times$ Trials interaction also was significant, $F(20, 355) = 16.77$, $p < 0.0001$. Post hoc tests showed no significant difference between three higher doses of EtOH, $ps > 0.05$. In both Lewis and Fischer rats, the suppressive effects of three higher doses of EtOH were significant on trials 3 – 6, $ps < 0.05$, relative to intake by the saline treated controls. Finally, the main effect of strain attained statistical significance, $F(1,71) = 19.38$, $p < 0.0001$, indicating that Lewis rats drank more saccharin than the Fischer 344 rats overall, $p < 0.05$. This effect was primarily carried by differences in intake on Trial 1. In support,

post hoc analysis of a significant Strain $\times$ Trials interaction, $F(5,355) = 10.06$, $p < 0.0001$, confirmed that Lewis rats drank more saccharin, overall, than the Fischer rats on Trial 1, $p < 0.05$. No other group differences were significant, $ps > 0.05$.

Saccharin intake (% of trial 1): Because the Fischer rats drank less saccharin on trial one than did the Lewis rats (3.5 vs. 6.0 ml, respectively), the data were expressed as a percent of trial 1 intake and were re-analyzed using the same $2 \times 5 \times 5$ mixed factorial ANOVA. This conversion also allowed for ready comparison with the Roma et al. (2006) paper, which employed this conversion and reported greater EtOH-induced suppression of CS intake in Fischer vs. Lewis rats.

As is evident from Figure 2, the Fischer rats in the saline condition increased intake of the saccharin cue from Trial 1 to Trial 2. This was not the case for the similarly-treated Lewis rats.

The results of the analysis of these converted data revealed significant main effects of dose, $F(4, 71) = 12.16$, $p < 0.0001$, and trials, $F(4, 284) = 4.68$, $p < 0.01$, but a non significant main effect of strain, $F(1, 71) = 3.83$, $p > 0.05$. Post hoc tests on the dose data revealed that intake of the saccharin CS decreased as a function of dose, with the saline and 0.5 g/kg groups exhibiting less suppression of CS intake than the other groups, $ps < 0.05$. Finally, the Dose $\times$ Trials interaction was significant, $F(16, 284) = 1.87$, $p < 0.05$, but the Strain $\times$ Trials and the Strains $\times$ Dose $\times$ Trials interaction were not significant, $F(4, 284) = 0.16$, $p > 0.5$, $F(16, 284) = 1.29$, $p > 0.05$, respectively.

Experiment 2

As discussed, rats consume less 0.15% saccharin when it predicts subsequent access to a highly preferred sucrose solution (Flaherty and Checke, 1982; Flaherty and Grigson, 1988; Flaherty and Rowan, 1985) or a drug of abuse (Hunt and Amit, 1987) and bilateral lesions of the gustatory thalamus eliminate these effects (Grigson et al., 2000; Reilly and Trifunovic, 1999; Schroy et al., 2005). Repeated studies, however, have shown that this same lesion has no effect on the development of a LiCl-induced CTA (Flynn et al., 1991; Reilly, 1998; Grigson et al., 2000; Scalera et al., 1997). The results shown above, along with those reported by Roma et al. (2006), provide a dose range to work with and, while inconclusive, suggest at the very least that doses of EtOH greater than 1.0 g/kg may be aversive. The present experiment was designed to test whether the suppressive effect of a 1.5 g/kg dose of EtOH is mediated by aversive drug properties. If so, the suppressive effect of this dose of EtOH, like that of LiCl, should not be prevented by bilateral lesions of the gustatory thalamus.

Materials and Methods

Subjects and Apparatus: Twenty-nine male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing between 450 and 650 g at the start of the experiment were housed and maintained as described in Experiment 1. All rats previously served in an anticipatory contrast experiment and, as such, had experience with both saccharin and sucrose (Schroy et al., 2005). They were, however, naïve to Polycose, which served as the gustatory CS for this experiment. The apparatus was the same as that described in Experiment 1.

Surgery: Seventeen rats received bilateral, electrophysiologically-guided, ibotenic acid lesions of the gustatory thalamus (THLX); 6 rats received vehicle infusions (sodium phosphate buffer, PBS) as surgical controls using previously described methods (Grigson et al., 2000); and 6 rats served as non-surgical controls. Twenty minutes prior to anesthesia, all

rats were injected with atropine sulfate (0.25 mg/rat i.p.) and gentamicin (6 mg/rat, i.p.). They were then anesthetized with sodium pentobarbital (50.0 mg/kg b.w., i.p., supplemented as necessary throughout surgery) and mounted in a stereotaxic instrument using nontraumatic earbars. The skin was opened with a mid-line incision. Using a 4-mm trephine, a hole was drilled in the skull on either side of the midline, 3.0 mm caudal to bregma. Physiological saline was used to keep the skull and dura moist during surgery. Gustatory neurons in the VPMpc were identified by recording multiunit activity with a glass-insulated tungsten microelectrode ($Z=1.0\text{--}1.5\text{M}\Omega$ at 1 kHz) while stimulating the anterior part of the tongue with 0.3 M NaCl and rinsing with distilled water. The coordinates ranged between -3.5 to -4.0 mm posterior to bregma and ± 1.1 to ± 1.3 mm lateral to the midline; -5.7 to -7.0 mm below the dura. After the gustatory area was found, the searching electrode was replaced with a glass pipette containing 0.2 μl (20 $\mu\text{g}/\mu\text{l}$) of ibotenic acid. Once the taste area was relocated electrophysiologically, the solution was infused over 10 min. After the injection, the micropipette remained in place for an additional 10 min, before repeating the procedure on the opposite side. The PBS rats were treated identically, but 0.2 μl of PBS, rather than ibotenic acid, was infused into the gustatory thalamus. After removal of the micropipette, the wound was closed with wound clips. The animals' body weights returned to presurgical levels within a week.

Procedure: Once the rats recovered from surgery, they were handled for 4 days and then placed on the water-deprivation schedule described above (5 min am/1 h pm). After 5 days on this schedule, the rats were matched on the basis of 5 min water intake during the final two days baseline and on experimental history (e.g., whether having served in the saccharin-saccharin or the saccharin-sucrose group in Schroy et al., 2005) and divided into one of two US conditions: Polycose-saline or Polycose-EtOH. During testing, all rats were weighed and given 5 min access to a 0.03 M Polycose. After a 5 min interstimulus interval, they received i.p. injections of either 1.5 g/kg 15% (w/v) EtOH or an equal volume of saline. One such CS-US pairing occurred every other day for a total of seven trials, followed by one CS-only test. Rats were rehydrated daily for the duration of the study from 2:00-3:00 p.m., leaving them 18 hr fluid deprived for 9:00 a.m. testing. On the mornings between CS-US pairings, 5 min dH₂O intake also was measured.

Drugs and solutions: Nineteen ml 200 proof EtOH (Pharmco Products Inc, Brookfield, CT) was diluted to 100ml in 0.9% saline immediately before each experimental session. Polycose (Abbott laboratories, Columbus, OH) was dissolved in dH₂O and was presented at room temperature.

Histology: At the end of all behavioral tests, the rats were given an overdose of sodium pentobarbital (100mg/kg, IP). Once deeply anesthetized, they were perfused transcardially with physiological saline, immediately followed by 10% formalin. The brains were removed, stored in 10% formalin and then transferred to 30% sucrose + 10% formalin for 24 hr before the brains were sliced. They were then frozen and cut coronally in 50 μm sections. One series of alternate sections was stained for cell bodies with Cresyl Lecht violet, the other for fibers with the Weil procedure. The adequacy of the lesions was judged by comparing the acellular areas in the brains that had been injected with ibotenic acid with comparable areas in the brains of the PBS injected controls. The boundaries of the VPMpc were defined from neuroanatomical and electrophysiological data from this and other laboratories (Kosar, Grill, & Norgren, 1986; Norgren, 1984; for a review see Norgren, 1995; Oakley and Pfaffmann, 1962).

Data analysis: The data were analyzed using a $2 \times 2 \times 8$ mixed factorial ANOVA varying Lesion (PBS vs. THLX), Drug (saline vs. EtOH), and Trials (1-8). Post hoc tests were conducted, where appropriate, using Newman-Keuls tests with $p < 0.05$.

Results and Discussion

Histology: The data from 7 THLX rats were eliminated because of inadequate lesion placement. Thus, 10 rats served in the THLX group and 12 rats (6 PBS and 6 non-surgical controls) served in the SHAM group. The saline-injected brains showed no evidence of damage, aside from an occasional electrode track. All 10 rats in the THLX group that contributed data had complete bilateral damage to the VPMpc. See Schroy et al., 2005, page R968 and accompanying Figure 1 for Histology and a thorough description.

Behavior: A 2 (Lesion) $\times$ 2 (Drug) $\times$ 8 (Trial) mixed factorial ANOVA revealed a significant main effect of Drug, $F(1, 18)=87.50, p<0.0001$, showing that rats injected with the 1.5 g/kg dose of EtOH consumed less of the Polycose CS than did the saline injected rats overall. The Drug $\times$ Trials interaction also was significant, $F(7, 126)=20.96, p<0.0001$, and post hoc tests revealed that EtOH-induced suppression of Polycose intake was significant beginning with trial 3, $ps < 0.05$, see Fig 3.

Neither the main effect of lesion $F<1$, the Lesion $\times$ Drug interaction, $F<1$, nor the Lesion $\times$ Drug $\times$ Trials interaction, $F<1$, however, were statistically significant. Together, these data show that, at the high dose of EtOH, the gustatory thalamus lesion does not prevent EtOH-induced suppression of CS intake.

Experiment 3

The results of Experiment 1 suggest that 0.75 g/kg EtOH may be a more appropriate dose to use to test whether the bilateral, electrophysiologically-guided ibotenic acid lesions of the gustatory thalamus can eliminate EtOH-induced suppression of CS intake. That is, while the high dose may be aversive, this lower dose may be primarily rewarding. As such, the thalamic lesion may be effective in disrupting the suppressive effects of this lower dose of EtOH, as it is in disrupting the suppressive effects of a rewarding sucrose solution and matched or higher doses of morphine (Grigson et al., 2000). Finally, it is important to note that the same lesion is not effective in disrupting the suppressive effects of even a low, matched dose of LiCl (Grigson et al., 2000).

Materials and Methods

Subjects, surgery, apparatus and procedure: Thirty-four naïve male Sprague Dawley rats weighing between 397-570g at the beginning of the experiment were assigned into EtOH or saline groups. Each group (EtOH and saline) consisted of 9 rats with lesions of gustatory thalamus, 4 surgical controls, and 4 non-surgical controls. The rats were obtained as described in Experiment 2 and housed and maintained as described in Experiment 1. The apparatus, surgery, procedure, and histology were the same as described in Experiment 2 except that all rats were weighed and given 5 min access to 0.15% saccharin during training. After a 5 min interstimulus interval they were injected i.p. with either 0.75 g/kg 15% (v/v) EtOH or an equal volume of saline. There were a total of 7 pairings, occurring at 48 h intervals as described above, followed by one CS only test. Fifteen ml 200 proof EtOH was diluted to 100 ml in 0.9% saline immediately before each experimental session. Saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and was presented at room temperature.

Results and Discussion

Histology: The data from 8 THLX rats were eliminated because of inadequate lesion placement. Thus, 10 rats served in the THLX group (5 saline and 5 EtOH) and 16 rats (8 PBS and 8 non-surgical controls) served in the SHAM group (8 saline and 8 EtOH). Lesions were assessed at four levels (Paxinos and Watson, 1986). Level I is considered pre-taste and

occurs at the final point at which the medial lemniscus splits. Level II marks the point at which taste cells first spill into the thalamus. The fasciculus retroflexus (FR) is just evident. Level III is thought to fall right in the middle of the thalamic taste area. At this level the FR and the parafascicular nucleus (PF) are frankly evident. Level IV is best demarcated by the FR, which runs in long parallel lines dorsal to ventral and is thought to be the location of the most posterior taste cells in this nucleus.

All rats in the THLX group had complete bilateral damage at levels II, III, and IV of the VPMpc. As is typically the case with these ibotenic acid lesions (Schroy et al., 2005), most lesions extended beyond the boundaries of the VPMpc to include parts of the centromedial (CM), ventromedial, paracentral, centrolateral, VPM, posterior (Po), parafascicular (PF), subparafascicular (SPF), and mediodorsal thalamic nuclei. Damage to these structures was at most partial, with the exception of the CM and SPF which was extensive and bilateral in some rats. Even so, the VPMpc is the only structure that sustained complete bilateral damage in all lesioned subjects.

Behavior: A 2 (Lesion) $\times$ 2 (Drug) $\times$ 8 (Trial) mixed factorial ANOVA revealed a significant main effect of drug, $F(1, 22)=9.38, p<0.01$, a significant Drug $\times$ Trials interaction, $F(7, 154)=4.91, p<0.0001$, and a significant Lesion $\times$ Drug $\times$ Trials interaction, $F(7, 154)=2.25, p<0.05$, see Fig 5.

Post hoc Newman-Keuls tests of this 3-way interaction showed that EtOH-treated SHAM rats (left panel) suppressed intake of the saccharin cue relative to the saline controls on trials 3-8, $ps < 0.05$. Rats with bilateral lesions of the gustatory thalamus, on the other hand, failed to suppress intake of the saccharin cue despite repeated saccharin-EtOH pairings, $ps > 0.05$. Finally, SHAM rats treated with EtOH consumed less saccharin than did rats in the THLX EtOH group on trials 3-8, $ps < 0.05$. These results are consistent with the hypothesis that this low dose of EtOH may suppress CS intake via rewarding drug properties.

General discussion

Published data suggest that the rewarding and aversive effects of EtOH play an important role in determining whether people who drink will continue to consume alcohol (Cunningham et al., 2000). These factors, however, have proven difficult to dissociate. The present study used two manipulations, selectively bred strains and bilateral lesions of the gustatory thalamus, in an effort to identify the rewarding and/or aversive properties of EtOH. The results of Experiment 1 evidenced an orderly dose-response function, with EtOH-induced suppression of CS intake increasing with increasing doses of the drug in both the Lewis and the Fischer rats. This finding contrasts with that of Roma et al. (2006) where EtOH-induced suppression of CS intake was greater in male Fisher than male Lewis rats when using a 1.0 or 1.5 g/kg dose of the drug. We believe that the different findings between the two experiments relates to the use of differing fluid deprivation regimens. Specifically, while the rats had a 1 h rehydration period in the present study, the rats in the Roma et al. (2006) report were given just 20 min access to fluid daily. Thus, if the rats failed to consume during the 20 min test period, they were essentially deprived of fluid for a 48 h period. The Lewis rats, then, may have exhibited smaller EtOH-induced suppression of CS intake in the Roma et al. (2006) study because they were less willing to forego fluid than were the Fischer rats. In a subsequent study comparing EtOH-induced suppression of CS intake in female Lewis and Fischer rats, the same group (Roma et al., 2007) reported no strain differences.

Although it is interesting to consider the impact of various parametric manipulations on drug-induced suppression of CS intake, this was not the focus of the first study. The objective was to use these selectively bred strains in an attempt to dissociate the rewarding

and aversive properties of EtOH. To this end, the data were inconclusive. This finding is not completely surprising. That is, while Lewis rats exhibit greater avoidance of a taste cue that predicts access to a rewarding sucrose solution or an injection of cocaine (Grigson and Freet, 2000), Fischer rats exhibit greater avoidance of a taste cue that predicts an injection of morphine (Lancellotti et al., 2001) or nicotine (Pescatore et al., 2005). Regarding the suppressive effects of morphine, both strains exhibit equal suppression of CS intake when paired with the selective μ agonist, DAMGO (Liu and Grigson, 2005), but Fischer rats exhibit markedly greater avoidance of a taste cue paired with a kappa agonist (Wheeler et al., 2002). Together, these data suggest that Fischer rats may exhibit greater avoidance of a saccharin cue following saccharin-morphine pairings because they are more sensitive to the aversive kappa-mediated properties of the drug. We have no hints, as of yet, as to why the Fischer rats also are more sensitive to the suppressive effects of nicotine. Overall, however, the data indicate that conclusions need to be drawn with caution. Neither the reduction in CS intake, nor strain differences in the reduction in CS intake can be taken, out of hand, as evidence for either rewarding or aversive drug properties. The nature of suppression, whether mediated by rewarding and/or aversive drug properties, can differ as a function of drug, dose, and strain. Thus, additional studies are required to triangulate the argument.

The results of Experiments 2 and 3 were helpful in this regard. As is the case with LiCl-induced conditioned taste aversions (Grigson et al., 2000), bilateral lesions of the gustatory thalamus failed to reduce the suppressive effects of the 1.5 g/kg dose of EtOH on intake of the Polycose CS in Sprague-Dawley rats. The same lesion, however, blocked the suppressive effect of the lower 0.75 g/kg dose of EtOH, which was otherwise quite effective in the SHAM lesioned Sprague-Dawley rats. Taken together, these data suggest that the low dose may be primarily rewarding, and the high dose, aversive. As mentioned, the same lesion also eliminates avoidance of a saccharin cue when paired with a rewarding sucrose solution or morphine, but not when paired with a matched low dose of LiCl (Grigson et al., 2000).

This conclusion is consistent with previous studies showing that middle to high doses of EtOH induce conditioned place aversion, while low doses of EtOH support conditioned place preference learning (Fidler et al., 2004). Cunningham (1981) showed that IP injections of 1.0 and 2.0 g/kg of EtOH caused spatial aversion conditioning in male albino rats, while Ciccocioppo, et al. (1999) showed that a dose of 0.7 g/kg of EtOH delivered by intragastric (IG) administration to Marchigian Sardinian alcohol-preferring (msP) rats caused conditioned place preference. These dose effects, however, are not likely set in stone. Thus, it is also likely that the perception of 'reward' changes with experience, since tolerance is a common phenomenon in humans that chronically abuse alcohol. In support, Ciccocioppo et al. (1999) showed that EtOH experienced msP rats showed conditioned place preference after IG administration of not only 0.7 g/kg, but also 1.5 g/kg of EtOH as well. Finally, Froehlich et al. (1988) showed that alcohol-nonpreferring (NP) rats exhibited greater avoidance of a saccharin cue than alcohol-preferring (P) rats when paired with a 1.0 g/kg dose of EtOH administered IP. At the EtOH dose of 1.87 g/kg, saccharin intake was strongly suppressed in both P and NP rats. No suppression occurred at the EtOH doses of 0.5 and 0.25 g/kg. The finding that NP rats receiving 1.0 g/kg EtOH showed greater suppression of saccharin intake than P rats is consistent with the conclusion that the 1.0 g/kg dose of EtOH is more aversive to the NP than the P rats. This conclusion is consistent with our data suggesting that EtOH doses of 1.0 g/kg or higher are aversive.

In sum, the data suggest that, in drug naïve rats, EtOH demonstrates a particularly narrow range of rewarding doses: 0.75 g/kg is rewarding, while doses greater than 1.0 g/kg are aversive. This dose range, however, can be greatly expanded with experience. Future studies will be designed to track these changes in affective state and to determine how their

expression contributes to the transition from use to abuse of EtOH. In addition, having separated the rewarding and aversive properties of the drug with the thalamic lesion, it will now be important to identify other nuclei in the circuit that mediates the perception of the rewarding and aversive properties of EtOH.

Finally, it should be noted that 'aversion' as indexed here may be evidence of aversive drug properties and/or, alternatively, it may be evidence of the onset of a conditioned aversive state. With cocaine, we have recently determined that the greater the aversive state elicited by the drug-associated taste cue, the greater the drug-seeking and drug-taking behavior (Wheeler et al., 2008). Consequently, with EtOH, as with cocaine, it is critical that we understand if and how aversion to an EtOH-associated taste cue predicts subsequent drug-taking behavior.

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Fig 1. Mean ($\pm$ S.E.M.) intake (ml/5 min) of the 0.15% saccharin CS in Lewis and Fischer rats. Intake of the CS was paired with an i.p. injection of saline, 0.5/kg, 0.75/kg, 1.0/kg or a 1.5/kg dose of 15% (v/v) ethanol. There was one taste—drug pairing a day, occurring at 48-h intervals, for a total of six trials.

Fig 2. Mean ($\pm$ S.E.M.) percent of baseline (trial 1) intake of the 0.15% saccharin CS in Lewis (top panel) and Fischer (bottom panel) rats. Intake of the CS was paired with an i.p. injection of saline, 0.5/kg, 0.75/kg, 1.0/kg or a 1.5/kg dose of 15% (v/v) ethanol. There was one taste—drug pairing a day, occurring at 48-h intervals, for a total of six trials.

Fig 3.

Mean ($\pm$ S.E.M.) intake (ml/ 5 min) of the 0.03 M Polycose CS in sham lesioned rats (SHAM) and in rats with bilateral ibotenic acid lesions of the gustatory thalamus (THLX). Intake of the CS was paired with an i.p. injection of saline or a 1.5/kg dose of 15% (w/v) ethanol. There was one taste—drug pairing a day, occurring at 48-h intervals, for a total of eight trials.

Fig 4.

Panel (A) Coronal section of the gustatory thalamus in an intact rat brain ($\times 2$). (B) Shows the representative section of an ideal lesion in the gustatory thalamus produced by directly injecting ibotenic acid into the nucleus. VPMpc, ventroposteromedial nucleus of the thalamus pars compacta; VPM, ventral posteromedial nucleus; Po, posterior nucleus; PF, parafascicular nucleus; SPF, subparafascicular nucleus; MD, mediodorsal nucleus; PVP, paraventricular nucleus; fr, fasciculus retroflexus; 3V, third cerebral ventricle.

Fig 5.

Mean ($\pm$ S.E.M.) intake (ml/ 5 min) of the 0.15% saccharin CS in sham lesioned rats (SHAM) and in rats with bilateral ibotenic acid lesions of the gustatory thalamus (THLX). Intake of the CS was paired with an i.p. injection of saline or a 1.5/kg dose of 15% (v/v) ethanol. There was one taste—drug pairing a day, occurring at 48-h intervals, for a total of eight trials.