CONDITIONED TASTE AVERSION AND THE Myers’ HIGH-ETHANOL-PREFERRING RAT

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Abstract — Aims: Male and female Myers’ high-ethanol-preferring (mHEP) rats were compared to outbred controls in a taste aversion paradigm. Methods: Alcohol-naïve rats were adapted to a 2-h access to water. Each rat was given either 0.05% saccharin (w/v) or 7% ethanol (v/v) as a novel solution for 1 h, after which either 0.5 M LiCl, as the aversive stimulus, or NaCl, as the control, was injected intraperitoneally. Each rat was tested 48 h later by presentation of the same solution. Results: After LiCl injections, saccharin consumption declined 21.6% in female Sprague–Dawley, 9.5% in female mHEP, 33.3% in male Wistar, and 38.3% in male mHEP rats. Ethanol consumption in these groups declined by 88.5, 30, 45 and 52%, respectively. These mHEP rats were then screened for 24-h alcohol consumption on a 10-day 3–30% ethanol vs water ‘step-up’ procedure. During the step-up procedure, only the male mHEP rats trained with ethanol for taste aversion drank less ethanol at the 3–5% concentrations than did rats trained with saccharin. The female mHEP rats did not learn an aversion to either saccharin or ethanol. Conclusions: The female mHEP rat consumes copious amounts of ethanol, but the basis for this consumption may be different from that of the male mHEP rat.

INTRODUCTION

Taste and related orosensory cues are believed to be important for the selection of foods and fluids by rodents (Lush, 1991; McMillen and Williams, 1998). Rodents also appear to have varying degrees of conditionability in learning an aversion to a particular taste (Elkins, 1986; Orr et al., 1993). Several studies have suggested that a genetic difference in susceptibility to develop conditioned taste aversions (CTAs) may result in differences in ethanol preferences (Elkins et al., 1992; Orr et al., 1993, 1997). Furthermore, it was postulated that differences in CTA acquisition are responsible for the different propensities of some humans to ingest ethanol (Elkins, 1991). That is, the tendency to develop a taste aversion may protect taste aversion-prone individuals from consuming the high levels of ethanol that typically precede ethanol tolerance and dependence.

A relationship between taste aversion conditionability and ethanol acceptance was demonstrated within rats selectively bred for high and low acceptance of ethanol. Alcohol-non-preferring (NP) rats were more susceptible to developing a taste aversion to saccharin than alcohol-preferring (P) rats when ethanol was used as the aversive-conditioning agent (Froehlich et al., 1988). On the other hand, selective breeding for taste aversion conditionability produces rat lines that differ in ethanol preference, as seen when comparing TAP (taste aversion prone) and TAR (taste aversion resistant) line preferences with respect to ethanol free-choice consumption. Thus, rats genetically selected for high and low CTA acquisition, based on an emetic type of unconditioned stimulus and a saccharin solution condition stimulus, consume relatively low and high levels of ethanol, respectively, in a free-choice paradigm (Orr et al., 1997). The amount of ethanol consumed by the TAR rats was also comparable with that of the P rats, which were reported to drink regularly >5 g/kg body wt/day in a free-choice paradigm (Gatto et al., 1987; Orr et al., 1997). TAP rats had higher serotonin levels than TAR rats in whole brain tissue, which was in harmony with the relationship between the NP and P rats (Murphy et al., 1982, 1987; Orr et al., 1993). The behaviour of the TAP and TAR lines also resembles that of the NP and P rat lines in that both models display line differences in response to CTA paradigms in which ethanol is used as the aversive conditioning stimulus (Froehlich et al., 1988; Elkins et al., 1991, 1992). This behaviour has also been observed more recently in other high- and low-ethanol drinking inbred lines, like the HAD/LAD and the UChA/UChB rats (Badia-Elder et al., 2000; Quintanilla et al., 2001). Therefore, it is possible that genetically mediated substrates of individual differences in taste aversion conditionability contribute to individual differences in propensities for ethanol consumption.

The mHEP (Myers’ high-ethanol-preferring) rat was recently developed at East Carolina University by crossing female Sprague–Dawley rats selected for high consumption of ethanol after a 10-day step-up procedure with male P rats. mHEP rats consume larger than normal quantities of ethanol and prefer high concentrations of ethanol. Similar to the P rat, they maintain their preference for ethanol in the presence of other palatable drinks. They have also demonstrated increased levels of anxiety in behaviour tests, such as the elevated plus-maze (Myers et al., 1998).

The selectively bred P rat does not exhibit a taste aversion to ethanol at low to moderate concentrations, and TAR rats selectively bred for their inability to learn the taste aversion paradigm consume high quantities of ethanol, but where does the mHEP rat fall? Does the mHEP rat respond differently from an outbred rat in the conditioned taste aversion paradigm? Also, with the determination of the ability or inability of the mHEP rat to learn the taste aversion paradigm, the role of taste in high ethanol consumption may be further elucidated. In the CTA paradigm, solutions of ethanol and saccharin were used as the conditioned stimulus (CS) since they represented a novel taste to the animals. An aversive dose of LiCl for the animal was used as the unconditioned stimulus (US).

MATERIALS AND METHODS

Subjects

Male Wistar rats and female Sprague–Dawley rats were obtained from Harlan Sprague–Dawley for use as control...
subjects. Because the lineage for the mHEP rat comes from female Sprague–Dawley and male P line rats, which were selectively bred from NIH Wistar stock, female Sprague–Dawley and male Wistar rats were selected as the appropriate control strains. Male and female mHEP rats bred at East Carolina University were taken from the eighth generation (F8). The P1 generation consisted of three male P line rats from T.-K. Li of the Indiana University Alcohol Research Center and three female Sprague–Dawley rats purchased from Harlan Sprague–Dawley. The following generations were bred by crossing non-sibling male and female mHEP rats chosen based on g/kg ethanol consumption, proportion ratio, and preferred concentration calculated from a 10-day screening procedure at 40 days and 80 days of age (Myers et al., 1998). For this study, all animals were alcohol naïve and ~60 days of age (200–225 g for female rats and 250–275 g for male rats). Animals were housed individually in a suspended metal cage during the course of the experiment. The room was maintained on a 12 h light/12 h dark cycle and a temperature at 24°C. Care and handling of animals was in accordance with the ‘NIH Guide for the Care and Use of Laboratory Animals’. East Carolina University is accredited by AAALAC-International.

Taste aversion

Two days prior to the beginning of the experiment, animals were placed in suspended wire cages with unlimited access to water and food. Approximately 24 h before beginning training for limited access to fluids, all fluids were removed (McMillen and Williams, 1998). Animals were randomly assigned to one of three treatment groups. On days 1 and 2, animals were adapted to a 2 h presentation of water beginning in the early afternoon between 13:00 and 14:00. On day 3, the animals received either 0.05% (w/v) saccharin or 7% (v/v) ethanol for 1 h and the amount consumed was noted. At the end of 1 h, either 0.5 M NaCl or 0.5 M LiCl was injected intraperitoneally at a volume of 0.2 ml/100 g body weight. This dose is equivalent to 42 mg LiCl/kg. Novel solutions were removed immediately following each injection, so that each rat had no further access to solutions that day. The presentation of the novel solutions was limited to 1 h, as our experience with limited access has shown that most of the consumption occurs during the first hour and the injection of a noxious stimulus should be paired with consumption. On day 4, water was presented for 2 h. On the final day, the novel solutions were presented again for 1 h and consumption noted. The decrease in consumption was taken as a measurement of the strength of the aversion learned. Data were analysed using a three-way, repeated measures analysis of variance (ANOVA) and Newman Keuls’ post hoc analysis, using GB-STAT (Dynamic Microsystems, Inc., Silver Spring, MD, USA).

Table 1. Taste aversion training with 0.05% saccharin (w/v) as the novel taste in female and male rats from different strains: comparison of injections with 0.5 M NaCl or 0.5 M LiCl

<table>
<thead>
<tr>
<th></th>
<th>Female rats</th>
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<th>Male rats</th>
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<tbody>
<tr>
<td></td>
<td>Sprague–Dawley</td>
<td>mHEP</td>
<td>Wistar</td>
<td>mHEP</td>
</tr>
<tr>
<td></td>
<td>NaCl (n = 8)</td>
<td>LiCl (n = 8)</td>
<td>NaCl (n = 8)</td>
<td>LiCl (n = 8)</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>11.6 ± 0.7</td>
<td>12.1 ± 1.2</td>
<td>10.9 ± 0.8</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>13.5 ± .2</td>
<td>9.5 ± 1.0†</td>
<td>12.9 ± 1.9</td>
<td>9.5 ± 0.7</td>
</tr>
</tbody>
</table>

|               | Wistar                                                                       |                                                                  | Wistar                                                                    |                                                                  |
|               | NaCl (n = 8)                                                                  | LiCl (n = 8)                                                     | NaCl (n = 8)                                                              | LiCl (n = 8)                                                     |
| Pre-treatment | 15.9 ± 3.2                                                                    | 18.4 ± 1.7                                                      | 13.0 ± 1.5                                                                | 16.6 ± 1.1                                                      |
| Post-treatment | 23.1 ± 1.7†                                                                    | 12.3 ± 0.8†                                                    | 16.3 ± 1.4                                                                | 10.3 ± 1.8†                                                     |

n = number of rats in each group. Values are means ± SEM saccharin consumed in 60 min (ml).
*Different from 0.5 M NaCl-treated rats, P < 0.05.
†Different from pre-treatment, P < 0.05.

Screening for ethanol preference and consumption

Those male and female mHEP rats which received ethanol or saccharin as the novel solutions followed by injection of LiCl for CTA were later screened, using a 10-day step-up procedure (Myers et al., 1998). Animals were housed individually in suspended metal cages. Three calibrated drinking tubes were attached to each cage: one for ethanol, one for water, and a blank. Drinking tubes were rotated daily in a semi-random manner. Animals received 3% (v/v) ethanol, which was increased daily to 30% over the 10-day period. Body weight, fluid intake, and food intake were measured daily. The data at each concentration were analysed by a two-way repeated measures ANOVA and Newman–Keuls post hoc analysis.

RESULTS

Taste aversion conditioning

Analysis of data for consumption of saccharin with a three-way ANOVA for repeated measures showed a significant interaction (main effect) between strain, sex, and treatment received [$F(2,67) = 6.33, P < 0.001$]. The interaction of strain with treatment was not significant [$F(2,33) = 1.91, P > 0.05$], but there was a significant interaction of sex with treatment [$F(1,34) = 6.28, P < 0.05$]. When female subjects were presented with saccharin as the novel solution, a difference was noticed (Table 1) between the Sprague–Dawley and mHEP
rats. Both female Sprague–Dawley and female mHEP rats given NaCl injections slightly increased, although not significantly, the amount of saccharin consumed on the test day (Table 1). However, when presentation of saccharin was followed by injection of LiCl, the female Sprague–Dawley rats significantly decreased their subsequent consumption of saccharin by 21.6% compared to the first presentation of this solution, whereas the female mHEP rat showed only a slight decrease in consumption (9.5%), which was not significant (Table 1).

When male subjects were presented with saccharin as the novel solution followed by NaCl injection (Table 1), male Wistar rats dramatically increased their consumption of saccharin (47%) as did male mHEP rats although not by as much (25%). Both male Wistar and male mHEP rats significantly decreased their consumption of saccharin when treated with LiCl, 33 and 38.3% respectively (Table 1). The data in Table 1 also demonstrate that the conditioning effect was due to LiCl, as an equal concentration of NaCl did not result in decreased drinking.

Analysis of data for ethanol consumption with a repeated measures three-way ANOVA showed a significant interaction between strain, sex, and treatment received \([F(2,65) = 26.12, P < 0.001]\). The interaction of strain with sex was not significant \([F(2,32) = 1.16, P > 0.05]\), but there was a significant interaction of sex with treatment \([F(1,33) = 5.70, P < 0.05]\). Table 2 shows that when animals were exposed to ethanol as the novel solution followed by injection of 0.5 M NaCl, consumption of ethanol tended to increase with the second ethanol exposure. The increase was significant for the male Wistar rat (41%) and the male mHEP rat (69%), but not for female Sprague–Dawley or female mHEP rats. This result demonstrated that the handling and injection of solution is not aversive to any of these strains and sexes of rat. In contrast, injection of 0.5 M LiCl produced an aversive response with the female Sprague–Dawley rat exhibiting the most dramatic decrease (–88.5%). Male Wistar, male mHEP and female mHEP rats also significantly decreased their consumption, by 45, 52 and 30%, respectively.

### Ethanol consumption and preference

Male and female mHEP rats that had received saccharin or ethanol as the novel solution followed by LiCl injection then underwent a 10-day step-up procedure. A two-way repeated measures ANOVA showed a significant interaction between sex and ethanol consumption during the 10-day step-up procedure \([F(9,31) = 2.22, P < 0.05]\). For female mHEP rats (Fig. 1), no significant difference was noted between the saccharin-exposed group and the ethanol-exposed group when comparing both g/kg/day intake (lower panel) and preference scores (ml ethanol/ml total consumed) at all concentrations, except 5% ethanol as g/kg/day. On the other hand, male mHEP rats exhibited a significant difference between the saccharin-exposed group and the ethanol-exposed group at lower concentrations of ethanol (3–7% ethanol) in g/kg/day intake and/or preference scores (Fig. 2). This difference disappeared at concentrations of ≥9% ethanol.

### DISCUSSION

The present results demonstrate that there is a difference between the female mHEP rat and the male mHEP rat in their ability to learn or exhibit a taste aversion to either ethanol or saccharin as novel solutions. The female Sprague–Dawley, male Wistar, and male mHEP rats all exhibited a learned taste aversion when either saccharin or ethanol was used as a novel solution. The female mHEP rat did not exhibit a learned taste aversion when either saccharin or ethanol was used as novel solutions, thereby resembling Elkins' TAR rats. It is interesting to note that Elkins and co-workers used both male and female rats and reported no difference in CTA or consumption based on sex (Orr et al., 1997).

One explanation for the difference in the ability of the male mHEP rat to learn the taste aversion paradigm and the female mHEP rat not to learn the paradigm is that taste aversion is not related to alcohol drinking, but rather has been ‘passed along’ during the selective breeding of various lines. That is, as one

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**Table 2.** Taste aversion training with 7% ethanol (v/v) as the novel taste in female and male rats from different strains: comparison of injections with 0.5 M NaCl or 0.5 M LiCl

<table>
<thead>
<tr>
<th></th>
<th>Sprague–Dawley</th>
<th>mHEP</th>
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<tbody>
<tr>
<td></td>
<td>NaCl (n = 6)</td>
<td>LiCl (n = 7)</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>4.0 ± 0.6</td>
<td>5.0 ± 0.5†</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>6.0 ± 0.3</td>
<td>0.6 ± 0.3†</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>mHEP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl (n = 6)</td>
<td>LiCl (n = 8)</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>7.8 ± 0.7†</td>
<td>7.5 ± 0.8†</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>11.0 ± 1.4†</td>
<td>4.1 ± 0.6*†</td>
</tr>
</tbody>
</table>

*n = number of rats in each group. Values are means ± SEM saccharin consumed in 60 min (ml).

*Different from 0.5 M NaCl-treated rats, \(P < 0.05\).

†Different from pre-treatment, \(P < 0.05\).
behavioural phenotype is selected for during the breeding process, unselected behavioural differences between the selected lines may develop, and the possibility that the unselected line differences are due to the inadvertent fixation of trait-irrelevant genes cannot therefore be ruled out (Orr et al., 1997). Another more likely line of reasoning is that the male and female mHEP rats consume high quantities of ethanol for different reasons. Earlier generations of mHEP rats were already characterized as having biochemical and behavioural differences between the male and female mHEP rats (Myers et al., 1998). The female mHEP rats not only consume more ethanol when considering g/kg intake and proportion to total fluid intake, compared with their male counterparts, but they also prefer a higher concentration of ethanol. Perhaps most interesting are the differences in brain chemistry between mHEP male and female rats (Lucas and McMillen, 2002). Male mHEP rats have lower than normal brain concentrations of serotonin, similar to the P rat. Low serotonin concentrations have long been implicated as 'causes' of high alcohol consumption in humans (Olson et al., 1960; Thomson and McMillen, 1987), but males represent the majority of subjects in these studies.

The female mHEP rat with the exception of the nucleus accumbens, however, has normal concentrations of serotonin (Lucas and McMillen, 2002). It is possible that the male mHEP rat is disposed to consuming high amounts of ethanol partly because of a deficiency in brain serotonin, whereas something else related to taste reactivity disposes the female mHEP rat to consume high quantities of ethanol.

The present data also demonstrate that both male and female mHEP rats continue to consume high amounts of ethanol when screened with a 10-day step-up procedure, regardless of whether they received the taste aversion paradigm with ethanol or saccharin as the novel solution. With the female mHEP rat, there appears to be no significant difference in g/kg intake or preference between either treatment group throughout the step-up procedure. On the other hand, after learning a taste aversion to 7% (v/v) ethanol, the male mHEP rat will still consume high amounts of ethanol. At lower concentrations (3, 4, 5 and 7%), however, the amount consumed is significantly less in the group that received the ethanol/LiCl treatment in the taste aversion paradigm than the group that received the saccharin/LiCl treatment. This difference disappeared at ethanol.
concentrations >9%, when consumption by the two groups became approximately the same. This would suggest that, after several days of ethanol presentation in the step-up procedure, the male mHEP rats that had learned a taste aversion to ethanol are able to extinguish the aversion and begin to consume quantities of ethanol similar to male mHEP rats which did not receive a taste aversion to ethanol. Another possibility is that there is something different in how the ethanol taste is perceived, so that the taste of higher concentrations of ethanol is perceived differently from lower concentrations. Therefore, animals that received a taste aversion to 7% ethanol would consume less ethanol at concentrations of ≤7%, but at higher concentrations would consume more ethanol because it presents a different taste than the one an aversion was learned to. This is in agreement with human studies, which show that different concentrations of ethanol exert different orosensory responses (Scinska et al., 2000; Mattes and DiMeglio, 2001).

In summary, the male mHEP rat will learn and exhibit a CTA with either saccharin or ethanol as the novel solution. The learning from CTA with ethanol carries over into the ethanol preference step-up procedure, but only at the lower concentrations of ethanol. The female mHEP rat exhibits neither response. These data indicate that the basis for high ethanol consumption by the male mHEP rat is different from the female mHEP rat.

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