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Effects of the Combined Administration of the 5-HT_3 Antagonist MDL 72222 and Ethanol on Conditioning in the Periadolescent and Adult Rat

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RAJACHANDRAN, L., N. E. SPEAR AND L. P. SPEAR. *Effects of the combined administration of the 5-HT_3 antagonist MDL 72222 and ethanol on conditioning in the periadolescent and adult rat.* PHARMACOL BIOCHEM BEHAV 46(3) 535–542, 1993. – The effects of acute ethanol and treatment with the 5-HT_3 antagonist MDL 72222 on conditioning of a visual pattern discrimination and an olfactory context were examined in periadolescent (35–38-day-old) and adult (60–70-day-old) rats. In Experiment 1, the effects of acute ethanol exposure on conditioning of a visual pattern discrimination (horizontal vs. vertical black and white stripes) and an olfactory context were investigated. The results indicated that a moderate dose of ethanol, 2 g/kg, disrupted conditioning at both ages to the visual stimulus but not to the olfactory context in which conditioning occurred. This may reflect differential susceptibility of target and contextual learning to the effects of ethanol, or might instead confirm previous suggestions that the visual system is more susceptible than the olfactory system to the effects of acute ethanol exposure. The effects of 5.0 and 10.0 mg/kg MDL 72222 on the ethanol-induced impairment in pattern discrimination conditioning were examined in Experiment 2. Pretreatment with these doses of MDL 72222 did not reverse the cognitive impairments produced by acute ethanol exposure at either age. However, MDL 72222 pretreatment attenuated the hyperlocomotion evident in ethanol-treated male and female periadolescents and adult females, as indexed by the number of crossovers during the preference test. Thus, MDL 72222 does not appear to ameliorate the cognitive impairment induced by acute ethanol exposure, although the antagonist was observed to attenuate ethanol-induced hyperlocomotion in the same test situation.

Ethanol Pattern discrimination 5-HT_3 antagonist Hyperlocomotion

ACUTE ethanol exposure is known to stimulate in vivo dopamine (DA) release from the nucleus accumbens, with this release implicated in mediating some of the rewarding effects of ethanol (12). Pretreatment with antagonists of the 5-hydroxytryptamine$_3$ (5-HT_3) receptor subtype (ICS 205-930 and tropisetron) has been shown in vitro to attenuate increases in DA release in nucleus accumbens and striatum secondary to ethanol administration (12,32). Recent studies in humans have shown that the 5-HT_3 antagonist ondansetron can reduce alcohol craving and intake as well as prevent withdrawal associated with chronic alcohol exposure (12). It is not yet known whether 5-HT_3 antagonists can reduce other consequences of ethanol exposure, such as the cognitive impairment in conditioning that is often seen following acute ethanol exposure in both human and rodent models (1,7,16,22,30). However, 5-HT_3 antagonists have been implicated in the enhancement of normal cognitive function and in reversing cognitive deficits induced by scopolamine (3,9). Although the exact mechanism by which 5-HT_3 antagonists may facilitate cognitive performance is still a topic of much investigation and speculation, a serotonergic/cholinergic mechanism has been postulated (3,12).

Two experiments were conducted to assess the effects of the 5-HT_3 antagonist MDL 72222 on conditioning in the rat following acute ethanol exposure. The effects of acute ethanol exposure on conditioning of a pattern discrimination and an olfactory context were assessed in Experiment 1, whereas Experiment 2 examined the influence of pretreatment with MDL 72222 on ethanol-induced alterations in conditioning in the pattern discrimination task. Both periadolescent (35–38-day-old) and adult (60–70-day-old) rats were used in these experi-

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ments given that, although there is frequent consumption of alcohol among the teenage population (33), very little is known about the effects of ethanol in either periadolescent humans or laboratory animals.

METHOD

Subjects

Subjects used in Experiments 1 and 2 were 412 female and male Sprague–Dawley rats born and reared in two separate Binghamton University breeding colonies. Animals were tested at one of two ages: periadolescent (35–42 days postnatal) and adult (60–70 days postnatal), with each animal being tested at only one age. Half of the subjects were housed in a temperature-controlled colony room on a 14L : 10D (0800–2200 h) light schedule (colony room 1) while the other animals were housed in a similar colony room on a 12L : 12D (0700–1900 h) light schedule (colony room 2). Colony room of origin was used as a variable in data analysis. All subjects were handled for 5 min daily for 3–5 days prior to being used in these procedures.

Apparatus

Conditioning was conducted in a patterned chamber (35 × 13 × 13 cm for periadolescents; 35 × 15 × 15 cm for adults) with solid black Plexiglas chambers of the same dimensions being used to deliver foot shock for animals in the unpaired condition. The walls of one side of each patterned apparatus consisted of horizontal white/black stripes, while the other side consisted of vertical white/black stripes. The width of each stripe was approximately 1.9 cm. Chambers were fitted with stainless steel grid floors connected to a variable intensity shock generator and scrambler (Grason Stadler shock generator). A strip of cotton scented with 2 cc of orange odor (Spectrum Laboratories) was placed under the grid floor so that it covered the bottom area of both patterned chambers. Discrimination testing was conducted in the same patterned chambers used for conditioning at both ages, but with no partition between the two compartments. A similar strip of cotton scented with 2 cc of orange odor (Spectrum laboratories) was placed under the grid floor during testing. A clear Plexiglas test apparatus (60 × 13 × 25 cm) was employed for the odor preference test in Experiment 1 for adults as well as adolescents. This apparatus had a similar grid floor, with strips of cotton scented with 1 cc of lemon odor (Humco) being placed underneath the floor on one side and 1 cc of orange odor placed underneath the other side. Stainless steel feeding tubes (20 ga for periadolescents, 16 ga for adults) were used to administer the alcohol intragastrically.

Design

For Experiment 1, 10 to 12 animals at each age were randomly assigned to each of the eight treatment conditions defined by a two treatment (saline or 17% ETOH) × two conditioning procedure (unpaired vs. paired) × two type of test (pattern discrimination vs. odor preference test) factorial design. Each animal was tested only once, and animals were semirandomly assigned to the eight treatment conditions, with the constraint that the sex of animals be equated to the extent possible in each treatment condition. In Experiment 2, 9–10 animals at each of the two test ages were semirandomly assigned (equating sex within each treatment condition) to each of the 12 treatment conditions defined by a two treatment (saline vs. alcohol) × three drug (vehicle, 5 or 10 mg/kg/cc MDL 72222) × two condition (paired vs. unpaired) factorial design.

Drugs

Based on pilot testing conducted to determine a dose of ethanol that appeared to be effective in altering test performance at each age, an intragastric dose of 2 g/kg of 17% w/v ETOH prepared in distilled water was chosen for these experiments. Control animals received an equivalent volume of saline intragastrically. Doses of 5 and 10 mg/kg of MDL 72222 were chosen for use as these doses were observed in pilot testing to have minimal effects on baseline locomotor activity, in contrast to the marked hypoactivity induced by higher (15 and 20 mg/kg) doses of MDL 72222 (10). In both experiments, intubations of ethanol or saline were given 10 min prior to the onset of conditioning (or foot shock exposure for animals in the unpaired group). MDL 72222 was dissolved in one drop of glacial acetic acid, taken to volume with saline, and adjusted with NaOH to a final pH of 5.5.

In Experiment 2, MDL 72222 or vehicle was administered intraperitoneally (IP) 20 min prior to intubation with alcohol or saline.

Pattern Conditioning Procedure

At the onset of conditioning, paired animals were placed in the vertical white/black stripes compartment (CS−) of the apparatus for 20 s and then placed immediately in the horizontal white/black stripes compartment (CS+) for 20 s. Foot shocks of 3 s duration (1.0 mA) were delivered during seconds 8–10 and 18–20 of placement in the horizontal stripes compartment (CS+). The entire apparatus was scented with 2.0 ml of orange odor. Paired animals received six trials of conditioning separated by a 1-min intertrial interval, during which time they were placed in a holding cage. Unpaired animals were exposed to six trials of foot shock, each separated by a 1-min intertrial interval, in a black Plexiglas apparatus in an odor-free room. For these exposures, the animals were placed into one side of the foot shock exposure apparatus for 20 s and then immediately placed into the other side of the apparatus for 20 s where they received two foot shocks as described above. After completion of the last foot shock, each unpaired animal was returned to a holding cage for 10 min. The unpaired animals then received six unreinforced exposures (each separated by a 1-min interval in the holding cage) to both the vertical stripes compartment and the horizontal stripes compartment for 20 s each, with both sides of the apparatus being scented with orange odor.

Testing

In Experiment 1, 25 min after the delivery of the last foot shock, half of the animals in each group were examined for freezing behavior (see below) emitted in the CS+ as well as the CS− compartment followed 2 min later by a preference test between the CS+ and CS− chambers. The remaining animals were examined for freezing behavior emitted in the presence of the contextual odor (orange) and a novel odor (lemon) followed 2 min later by a contextual odor preference test. In Experiment 2, only the CS+/CS− test of freezing behavior followed by a CS+/CS− preference test was employed for all animals.

Assessment of freezing. For the examination of freezing behavior in the CS+ and CS−, animals were placed in one

side of the patterned compartment of the conditioning apparatus for 3 min and then placed into the other compartment for an additional 3 min; during these observation periods, orange odor was present in the chambers, as was the case during conditioning. Order of presentation of the different compartments was counterbalanced within this group. The frequency (number of bouts of freezing) and duration of freezing behavior emitted by the animals during exposure to the CS+ and CS− chambers were recorded continuously using a button keyboard connected to an Apple IIe computer. For the measurement of freezing behavior in the context odor or a novel odor, each animal was placed on one side of a divided clear Plexiglas chamber for 3 min followed by placement on the other side for an equivalent amount of time. One side of the chamber was scented with the contextual odor used during conditioning (orange) while the other half was scented with a novel odor (lemon). Order of odor exposure was counterbalanced. Again, frequency and duration of freezing behavior emitted on each side of the apparatus were recorded during a 3-min observation period as outlined above.

Preference testing. The CS+/CS− preference test was conducted in the conditioning apparatus, with the same contextual odor being placed under both sides of the apparatus as during conditioning. Each animal was placed in the center of the apparatus (randomly oriented either towards or away from the experimenter) and allowed to locomote freely between the two compartments. The criterion for being on either side of the apparatus was that the snout and two front paws were on that side of the apparatus. The amount of time spent on the horizontal striped side of the apparatus (the CS+ side) was cumulatively recorded each minute for a test duration of 5 min. For the contextual odor preference test, animals were given an odor preference test between orange odor (the contextual odor used during conditioning) and a novel odor (lemon). Each animal was placed on the midline axis of the clear Plexiglas apparatus and allowed to locomote freely over both sides of the apparatus. The amount of time spent over the orange-scented side of the apparatus was cumulatively recorded each minute during the 5-min test.

Activity testing. The number of whole body crosses from one side of the preference test apparatus to the other was recorded for each minute of the preference test in Experiment 2 as an index of locomotor activity.

Assessment of Blood Alcohol Levels

An additional 10 animals (five males, five females) at each age were sacrificed to determine blood alcohol concentrations 52 min following intragastric administration of a 2 g/kg dose of 17% ethanol. This time period was chosen to reflect approximate blood alcohol levels at the time of test. Experimental animals were sacrificed by decapitation and trunk blood was immediately collected. Blood from nonethanol-treated animals at each age was similarly collected and used in the preparation of standards. Blood alcohol levels (BALs) were measured by spectrophotometry, using an alcohol dehydrogenase-based assay procedure derived from Howerton and colleagues (14) and adapted for use in our laboratory [e.g., see (15)].

RESULTS

Experiment 1

The amount of time spent on the CS+ side (visual preference test) and on the contextual odor side (odor preference test) was separately analyzed at each age using a two (sex) × two (colony room) × two treatment (alcohol or saline) × two condition (paired vs. unpaired) × five Blocks of Time repeated measures analysis of variance (ANOVA) across time.

CS+/CS− Visual Preference Test

Analysis of these data revealed no significant effects of sex or colony room of origin. The ANOVAs revealed significant main effects of treatment, $F(1, 32) = 7.283$, $p < 0.05$, $F(1, 24) = 8.699$, $p < 0.05$, and condition, $F(1, 32) = 4.993$, $p < 0.05$, $F(1, 24) = 15.227$, $p < 0.05$, for periadolescents and adults, respectively (Fig. 1). In addition, at both ages there was also a significant treatment × condition interaction, $F(1, 32) = 6.786$, $p < 0.05$, $F(1, 24) = 15.239$, $p < 0.05$. Tukey's tests conducted comparing paired and unpaired animals within each treatment group at each age indicated that paired animals that received saline spent significantly less time on the CS+ side than unpaired saline animals, indicating conditioning. In contrast, as can be seen in Fig. 1, there were no significant differences at either age in the amount of time spent on the CS+ side between paired and unpaired animals treated with ethanol, indicating an ethanol-induced disruption of conditioning in these animals.

Contextual Odor Preference Test

These data revealed a significant main effect of condition, $F(1, 35) = 14.753$, $p < 0.05$, $F(1, 26) = 17.747$, $p < 0.05$, for periadolescent and adult animals, respectively. There was

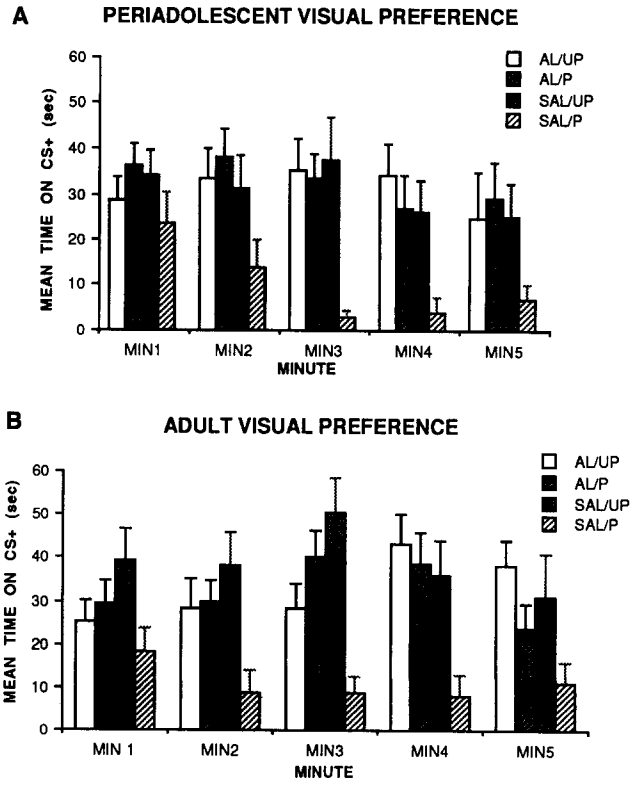

FIG. 1. Mean time (seconds ± SEM) per minute spent in the CS+ for periadolescent (A) and adult (B) unpaired (UP) and paired (P) animals following administration of alcohol (AL) or saline (SAL).

also a main effect of time, $F(4, 140) = 4.578$, $p < 0.01$, in the periadolescent data. Tukey's tests revealed that at both ages paired animals spent significantly less time in the presence of the contextual orange odor than unpaired animals, regardless of treatment with alcohol or saline (Fig. 2). The main effect of time in the periadolescent data reflects a decrease in the amount of time spent in orange (context) odor during minutes 4 and 5 relative to earlier minutes (Fig. 2). Thus, whereas ethanol disrupted visual conditioning or its expression in the visual preference test, no ethanol-related disruption in conditioning to the contextual odor was observed in periadolescents and adults pretreated with alcohol or saline.

Freezing Behavior Tests

The frequency and duration of the occurrence of freezing behavior during the two types of tests (CS+/CS− and contextual odor/novel odor) were initially analyzed for order effects. Preliminary analyses of these data revealed several order effects; therefore, analysis of the frequency and duration of freezing behavior was conducted only in the initial test situation by a two (treatment) × two (condition) × two (test situation) ANOVA. In these analyses, due to the reduced amount of data available for analysis, sex or colony room of origin were not included as factors. The analysis of the frequency of freezing behavior in adult animals revealed only a main effect of condition, $F(1, 24) = 6.833$, $p < 0.05$, in adult animals in the visual test of conditioned behaviors. Paired animals, regardless of treatment or test situation (placement in CS+ vs. CS−), exhibited more freezing bouts (8.0 ± 0.9) than their unpaired counterparts (4.9 ± 0.9).

Analysis of the frequency of freezing in periadolescents revealed only significant main effects of treatment, $F(1, 39) = 7.014$, $p < 0.05$, and test situation (contextual vs. novel odor), $F(1, 33) = 4.731$, $p < 0.05$, in the context vs. novel odor test. Tukey's tests revealed that ethanol-treated animals exhibited more bouts of freezing (4.1 ± 0.7) compared to saline-treated animals (2.1 ± 0.4) and animals placed in the novel lemon odor exhibited more freezing bouts (4.1 ± 0.7) relative to animals in the contextual orange odor (2.3 ± 0.5). There were no significant effects with regard to the duration of freezing behavior at either age in the visual or odor tests.

Experiment 2

Data from the visual preference test and the crossover measure were analyzed at each age using a five-way ANOVA: two colony room × two sex × two treatment (alcohol vs. saline) × three drug (0, 5, 10 mg/kg MDL 72222) × two condition (paired vs. unpaired). The frequency and duration of freezing behavior were analyzed at each age as outlined in Experiment 1 using a two condition × two treatment × three drug ANOVA. Post hoc analyses were conducted using Tukey's tests as discussed previously.

CS+/CS− Visual Preference Test

The ANOVAs of the preference data (Fig. 3) revealed significant main effects of condition, $F(1, 68) = 46.380$, $p < 0.001$, $F(1, 68) = 42.153$, $p < 0.001$, and treatment, $F(1, 68) = 15.784$, $p < 0.001$, $F(1, 68) = 44.957$, $p < 0.001$, for periadolescent and adult animals, respectively. In addition, at both ages there was also a significant treatment × condition interaction, $F(1, 68) = 41.61$, $p < 0.001$, $F(1, 68) = 46.438$, $p < 0.001$. Tukey's tests revealed that paired saline animals at both ages spent significantly less time in the CS+ (horizontal stripes compartment) than the unpaired saline animals, indicating conditioning. In contrast, as can be seen in Fig. 3, there were no significant differences between the paired and unpaired alcohol-treated animals in the amount of time spent on the CS+ side of the apparatus at either age, indicating a lack of conditioning following ethanol treatment in these animals. There were no effects of MDL 72222 pretreatment on performance in this test in either animals receiving saline or alcohol. Thus, administration of doses of 5 or 10 mg/kg of MDL 72222 prior to intubation with alcohol did not reverse the impairment in visual conditioning induced by alcohol in periadolescent and adult animals.

The ANOVA for adult animals also revealed a significant treatment × sex × colony room interaction, $F(1, 68) = 4.071$, $p < 0.05$, and treatment × drug × sex interaction, $F(2, 68) = 3.274$, $p < 0.05$. Tukey's tests were conducted on data collapsed across drug and condition to determine the locus of the treatment × sex × colony room interaction. These tests revealed that males from colony room 1 that received alcohol spent significantly more time in the CS+ (mean ± SEM in s: 41.7 ± 2.4) than males receiving alcohol from colony room 2 (32.3 ± 2.5). Analyses of the treatment × drug × sex interaction (on data collapsed across condition and colony room) revealed only that male animals that received vehicle and alcohol spent more time on the CS+ side (41.0 ± 3.4) than females receiving the same treatments (29.9 ± 2.4).

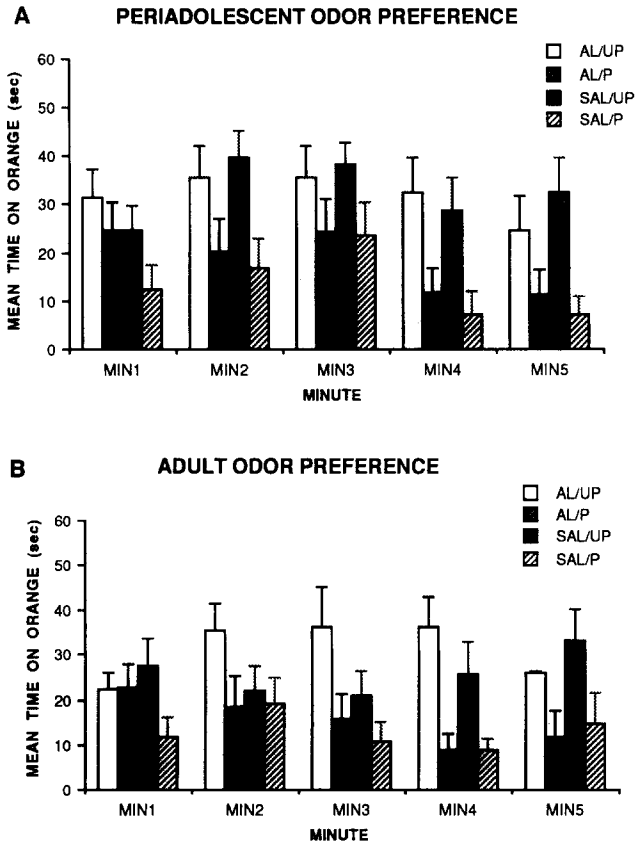

FIG. 2. Mean time (seconds ± SEM) per minute spent in the contextual orange odor for periadolescent (A) and adult (B) unpaired (UP) and paired (P) animals following administration of alcohol (AL) or saline (SAL).

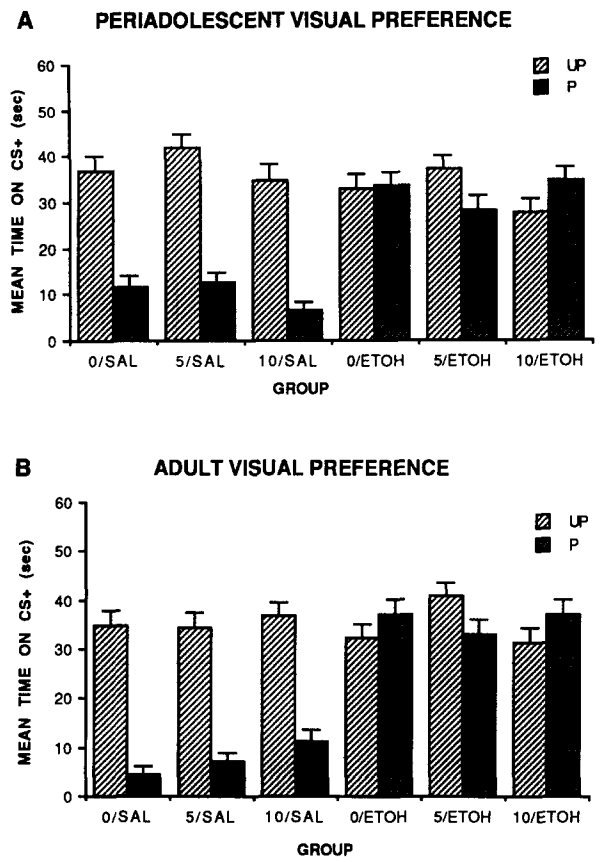

FIG. 3. Mean time (seconds ± SEM) per minute spent on the CS+ by periadolescent (A) and adult (B) animals during the CS+/CS− preference test. Animals were pretreated with either vehicle (0), 5, or 10 mg/kg MDL 72222 prior to intragastric administration of saline (SAL) or ethanol (ETOH).

Crossover Data

The number of whole body crosses measured during the preference test (Fig. 4) was analyzed at each age using a five-way ANOVA and post hoc Tukey's tests as outlined previously for the visual preference test data. The ANOVA of the periadolescent data revealed significant main effects of drug, $F(2, 68) = 6.946$, $p < 0.01$, treatment, $F(1, 68) = 32.792$, $p < 0.01$, and time, $F(4, 272) = 31.721$, $p < 0.001$, along with a significant condition × treatment interaction, $F(1, 68) = 4.300$, $p < 0.05$, and a significant drug × treatment interaction, $F(2, 68) = 3.103$, $p < 0.05$. There was also a significant condition × treatment × colony room interaction, $F(1, 68) = 4.116$, $p < 0.05$. The significant main effect of time reflects a significant decrease in the number of whole body crosses across all 5 min of the preference test (data not shown). Tukey's tests to determine the locus of the condition × treatment interaction indicated that paired periadolescent saline-treated animals exhibited fewer crossings than paired alcohol-treated animals, whereas there were no significant differences in the number of crosses made between unpaired animals given saline or alcohol. These findings reflect a tendency for paired saline-treated animals to exhibit fewer crossings than their unpaired counterparts, a trend that was not evident in ethanol-treated periadolescents (Fig. 4). Tukey's tests conducted to determine the locus of the condition × treatment × colony room interaction revealed only that paired alcohol animals from colony room 1 (1.7 ± 0.2) made more crosses than their counterparts from colony room 2 (1.0 ± 0.1). With regard to the drug × treatment interaction, animals receiving alcohol alone exhibited significantly more crosses than animals receiving saline. Tukey's tests failed to reveal any significant differences in the number of crosses exhibited between animals receiving saline and pretreated with the 0, 5, or 10 mg/kg doses of MDL 72222. However, animals in the alcohol/vehicle group exhibited significantly more crosses than animals receiving alcohol and pretreatment with either the 5 or 10 mg/kg dose of MDL 72222. Thus, as can be seen in Fig. 4, the ethanol-induced increase in crossovers was attenuated by both test doses of MDL 72222.

Analysis of the adult crossover data (Fig. 5) revealed significant main effects of treatment, $F(1, 64) = 11.344$, $p < 0.01$, condition, $F(1, 64) = 6.516$, $p < 0.001$, sex, $F(1, 64) = 4.757$, $p < 0.05$, and time, $F(4, 256) = 23.646$, $p < 0.05$, along with a significant four-way drug × sex × condition × treatment interaction, $F(2, 64) = 4.066$, $p < 0.05$. There was also a significant condition × sex × colony room interaction, $F(1, 64) = 5.518$, $p < 0.05$. Adult animals showed a significant decrease in the mean number of crosses across all 5 min of the preference test (data not shown). Generally, paired animals exhibited fewer crosses than unpaired animals, female animals made more crosses than males, and animals that received alcohol made more than saline animals. However, these main effects were tempered by the significant four-way interaction of drug × sex × condition × treatment. As can be seen in Fig. 5B, female unpaired animals given alcohol and vehicle were more active in terms of crossovers than females given saline and vehicle alone. This ethanol-induced increase in activity was not evident in adult unpaired males; indeed, as can be seen in Fig. 5A, unpaired adult males tended to exhibit a decrease in crossovers in response to ethanol alone, although this difference was not significant. The ethanol-induced increase in activity in adult unpaired females was attenuated by the low dose of MDL 72222. In contrast, unpaired males treated with alcohol and 5 mg/kg of MDL 72222 showed an increase in crossovers compared to unpaired males given vehicle and alcohol. Male unpaired animals receiving saline and the vehicle exhibited significantly more crosses than paired animals given the same treatments; a nonsignificant trend in

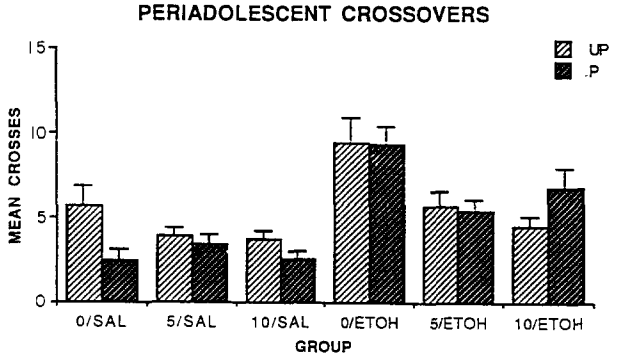

FIG. 4. Mean number of crosses (±SEM) exhibited by unpaired (UP) and paired (P) periadolescents during the 5-min CS+/CS− preference test. Animals were pretreated with either vehicle (0), 5, or 10 mg/kg MDL 72222 prior to intragastric administration of saline (SAL) or ethanol (ETOH).

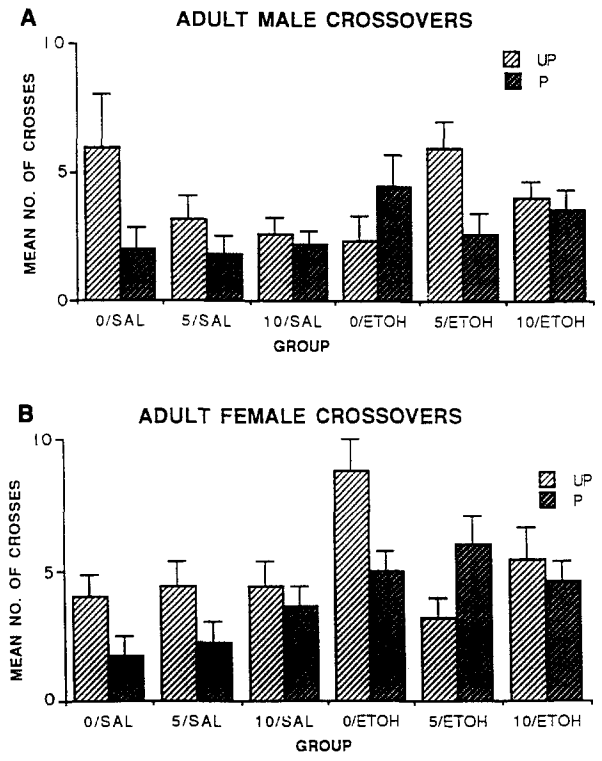

FIG. 5. Mean number of crosses (±SEM) for unpaired (UP) and paired (P) male (A) and female (B) adult animals during the 5-min CS+/CS− preference test. Animals were pretreated with either vehicle (0), 5, or 10 mg/kg MDL 72222 prior to intragastric administration of saline (SAL) or ethanol (ETOH).

this direction was also seen in comparably treated females. Tukey's tests conducted to determine the locus of the condition × sex × colony room interaction revealed only that male unpaired animals from colony room 2 (1.1 ± 0.2) made significantly more crosses than unpaired male animals from colony room 1 (0.6 ± 0.1).

CS+/CS− Freezing Behavior Test

Analysis of the frequency of freezing behavior in the presence of the CS+ and CS− in periadolescent animals revealed only a treatment × drug × condition interaction, $F(2, 86) = 3.72$, $p < 0.05$. Tukey's tests revealed that vehicle/saline-treated unpaired periadolescents exhibited more bouts of freezing (5.0 ± 1.0) than their vehicle/saline-treated paired counterparts (1.8 ± 0.6); this difference between paired and unpaired animals was not seen in vehicle/alcohol-treated periadolescents (paired: 3.0 ± 0.9, unpaired: 2.4 ± 0.9). The duration data for periadolescents revealed only a main effect of treatment, $F(1, 83) = 6.111$, $p < 0.05$. Alcohol-treated animals showed a greater duration of freezing behavior (64.7 ± 6.5) than saline-treated animals (43.4 ± 5.7), an effect that was seen in both the CS+ and CS− test. The frequency of freezing behavior in adult animals revealed a significant treatment × test situation interaction, $F(1, 92) = 6.22$, $p < 0.05$, although Tukey's tests revealed no significant differences between the groups. However, there was a trend in these data for saline-treated animals receiving the CS+ test to exhibit more freezing bouts (3.9 ± 0.3) than their counterparts in the CS− test situation (3.1 ± 0.5), whereas there was a trend for alcohol-treated animals in the CS+ test to exhibit fewer freezing bouts (3.1 ± 0.3) than their counterparts in the CS− test (4.1 ± 0.3). The analysis of the duration of freezing behavior in adults revealed no significant effects.

Blood Alcohol Concentration

A two (age) × two (sex) ANOVA on the blood alcohol level (BAL) data revealed a significant main effect of age, $F(1, 16) = 5.085$, $p < 0.05$, with periadolescents exhibiting slightly higher BALs (135.05 ± 11.68 mg/dl) than adults (98.59 ± 12.70 mg/dl) at a time equivalent to the onset of testing. There was no main effect or interaction of sex in this ANOVA.

DISCUSSION

In Experiment 1, acute alcohol exposure in adult and periadolescent rats was observed to interfere with conditioning to a visual CS+ but not an odor context. At both ages, animals pretreated with alcohol failed to learn a CS-US association with a visual CS+, conditioning that was evident in saline-treated periadolescents and adults. In contrast, ethanol had no effect on conditioned avoidance of the contextual odor in either periadolescents or adults. Taken together, the preference data from Experiment 1 provide evidence that acute alcohol administration differentially affects different aspects of a simple classical conditioning procedure.

There are several potential explanations of the differential effect of ethanol on the visual vs. odor preference tests. General context conditioning may be less susceptible to disruption by ethanol than the learning of a specific CS-US association, as evidenced by the visual preference test. Alternatively, it is possible that olfactory learning is more resistant to the detrimental effects of ethanol than is visual learning. There is some evidence to support this latter suggestion. For instance, acute alcohol administration to 21-day-old rats was not observed to impair conditioning to an olfactory stimulus, but did disrupt the acquisition of a conditioned aversion to a visual cue paired with a foot shock (20). This effect was observed both when the visual or odor cue was presented as a single element conditioned stimulus or as part of an odor/visual compound stimulus. (The latter might be seen as more analogous to the present study, where the learning of the visual discrimination occurred within the context of an odor.) According to the results of the present study, the relative resistance of olfactory conditioning to ethanol disruption appears to be maintained in periadolescence and adulthood, suggesting that the well-established sense of olfaction may be more resistant to the effects of ethanol throughout life. The validity of this suggestion will become clearer when amodal characteristics (e.g., intensity) of the alternative stimuli are equated.

Despite the slightly higher BALs of periadolescent animals at the time of test relative to adult animals, there were few differential effects of ethanol at the two test ages. Both periadolescents and adults exhibited an ethanol-related disruption of visual conditioning and a lack of effect of ethanol on conditioning to the olfactory context. These findings appear to be fairly generalizable, in that few effects of sex or colony room of origin were seen during analysis of these data. Thus, in terms of preference test performance, there was no evidence for any marked differential influence of ethanol in adolescence as compared to adulthood. There has been little prior investigation of the effects of ethanol on conditioning during

periadolescence, and hence additional work is needed to determine the generality of these findings.

The failure of MDL 72222 to restore conditioning in ethanol-treated periadolescent and adults observed in Experiment 2 suggests that blockade of 5-HT_3 receptors, a manipulation that presumably decreases dopamine release in the nucleus accumbens, is not sufficient to ameliorate ethanol's effects observed on learning in this paradigm. It is not the case that behaviorally ineffective doses of MDL 72222 were used, given that MDL 72222 was effective in attenuating the ethanol-induced hyperlocomotion that was seen in periadolescents as well as adult females during the preference test situation. The lack of protective effects of MDL 72222 on cognitive functioning in ethanol-treated animals was somewhat surprising given that 5-HT_3 antagonists have been previously observed to reverse scopolamine-induced cognitive impairments (3) and to attenuate the reinforcing effects of ethanol (27). Yet, it is possible that the locus of ethanol's effects on cognitive processes does not involve the mesolimbic DA areas in the brain. As mentioned previously, ethanol in low to moderate doses induces DA release from mesolimbic structures like the nucleus accumbens (5). This property of ethanol is thought to contribute to its reinforcing/rewarding effects and can be successfully blocked by administration of 5-HT_3 antagonists like MDL 72222 and ICS 205-930 (5). Since there was no reversal of the cognitive impairment in animals pretreated with MDL 72222 and intubated with alcohol, it appears more likely that ethanol's disruption of cognitive processes may be mediated by its effects on other brain structures (6).

Analysis of the crossover data revealed that periadolescent animals pretreated with vehicle and intubated with ethanol were more active during the preference test than vehicle animals intubated with saline. Similarly, adult female (but not male) unpaired animals treated with alcohol alone exhibited more crossovers than their counterparts treated with saline. These ethanol-related increases in activity during the preference test were somewhat surprising. Although low doses of ethanol frequently increase activity in mice [see (13) for review], suppressant effects of ethanol on locomotion typically have been observed in rats in open field or activity cage testing [e.g., (24,25,31), but see also (11,18,22) for exceptions]. In contrast, ethanol has been reported to increase intertrial crossings in rats acquiring or performing an active avoidance task (13) and to increase running speed during extinction of an escape task (26). Indeed, ethanol has been observed to prevent the decrease in open field activity normally seen in adult male rats following exposure to a mild stressor (2,28,29). Given that all animals in the present study had received foot shock prior to the preference test, it is possible that the increase in crossovers seen during preference testing in the ethanol-treated animals is a result of an ethanol-related blockade of the suppression in locomotion induced by prior exposure to foot shock.

It is not clear why this ethanol-induced increase in activity during the preference test was not evident in adult male rats. Although sex differences in locomotor activity following ethanol rarely have been reported [e.g., see (13)], there is one report of an ethanol-related increase in open field activity in females that was not evident in males, although this sex difference was evident only in Maudsley reactive, and not Sprague-Dawley, rats (11). Perhaps more to the point, studies to date examining the effects of prior stress on locomotor activity in ethanol-treated animals have not examined potential sex differences in this effect. The results of the present study tentatively suggest that adult female rats may be more responsive than males to an ethanol-related blockade of the stress-induced suppression in locomotion, with this sex difference emerging following puberty. In support of this possibility, there is recent neurochemical evidence that the dopamine system of adult female rats may be more sensitive than that of males to the effects of acute ethanol exposure (4). Ethanol induces increases in extracellular levels of dopamine in striatum and nucleus accumbens in female rats, whereas ethanol-induced increases in dopamine are seen only in striatum in males (4). Given that specific environmental factors such as shock and acute ethanol exposure appear to increase dopamine release (8,19), these regional sex differences in ethanol-induced dopamine release may be related to the increased susceptibility of adult females to the locomotor stimulant effects of ethanol following prior stress.

It is possible that the increase in crossovers observed during the preference test in periadolescents and adult females could have contributed to the ethanol-induced impairment in performance of these animals on the visual preference test. This possibility is unlikely, however, given that pretreatment with MDL 72222 attenuated this ethanol-induced increase in activity during the preference test without improving the performance of ethanol-treated animals on the preference test per se. Indeed, both doses of MDL 72222 used in this experiment attenuated the increase in crossovers seen following acute ethanol exposure in periadolescent animals and adult females. This attenuation is reminiscent of that reported previously by Reith where administration of the 5-HT_3 antagonists zacropride and ICS 205-930 was shown to significantly attenuate cocaine-induced locomotor activity in mice (23). Reith concluded that this effect was not due to the general sedating properties of these antagonists (23). In the present test situation, MDL 72222 did not suppress baseline levels of activity in periadolescents or adult females, and hence the attenuation of ethanol-induced activity in these animals likewise does not appear to be the result of a general sedative effect. Thus, in contrast to the lack of effects of MDL 72222 in reversing the cognitive impairing effects of ethanol, both doses of MDL 72222 attenuated the hyperactivity induced by ethanol in this testing situation.

The assessment of freezing behavior in these experiments did not provide a clear delineation of alcohol's effects on the conditioning process. Generally there were very few conditioning effects in these data, and the effects that were observed did not provide a valuable addition to the preference test measure. It is possible that the assessment of behavioral responses like freezing is dependent on the particular circumstances of the test situation. Perhaps if the CS was presented as a more discrete, punctate stimulus after the animal had been allowed to acclimate to the test situation, a better indication of conditioned suppression of activity (freezing) might have been obtained (17). In addition, it would probably be more advantageous to test for suppression of activity some time after the conditioning episode rather than immediately following it, eliminating the immediate effects of the US on the behavior of the animal [e.g., see (17)].

A major impetus behind much of the recent interest into the serotonergic 5-HT_3 receptor system has been the potential therapeutic efficacy of 5-HT_3 antagonist compounds. This interest is predicated on the fact that these substances exhibit antidopaminergic activity in mesolimbic areas (5). The present study suggests, however, that ethanol's cognitive effects are not ameliorated with 5-HT_3 antagonist administration. Although blockade of 5-HT_3 receptors may reverse ethanol's reinforcing effects (27), consequences of ethanol exposure, such

as cognitive impairment, appear to be relatively unaffected. Thus, there appears to be a clear delineation between the various consequences of acute ethanol exposure and their susceptibility to reversal by 5-HT_3 antagonists. Given that interactions between 5-HT_3 receptors and mesolimbic DA systems have been implicated in the ability of 5-HT_3 antagonists to reverse certain adverse consequences of ethanol and other drugs of abuse (8,12), the inability of MDL 72222 to reverse the ethanol-related cognitive impairment suggests the possibility that ethanol's effects on cognitive processes may not be modulated by mesolimbic dopamine structures. Future studies to develop pharmacological strategies to reverse the cognitive consequences of ethanol exposure may best be directed toward manipulations of other neural systems.

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