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Reduced 5-HT$_3$ receptor binding and lower baseline plus maze anxiety in the alcohol-preferring inbred fawn-hooded rat

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Abstract

The present investigation sought to explore the relationship between the 5-HT$_3$ receptor and anxiety-like behavior in fawn-hooded (FH/Wjd) rats, an inbred strain that exhibits a high intake and preference for ethanol, and the alcohol-nonpreferring ACI/N strain. Using quantitative autoradiography, we examined whether there were differences in central 5-HT$_3$ receptor binding in FH/Wjd versus ACI/N rats. Ten to 14 days prior to being used in the autoradiographic studies, rats were first confirmed to be representative of their strains by subjecting them to a two-bottle choice procedure for 2 weeks. The binding of [^{3}H]LY 278584 to 5-HT$_3$ receptors was significantly reduced in frontal cortex, CA1 region of hippocampus, and in the medial and lateral nuclei of the amygdala of FH/Wjd versus ACI/N rats. In the anterior cingulate cortex and in the dentate gyrus region of the hippocampus the reduction in [^{3}H]LY 278548 binding in the FH/Wjd versus ACI/N strain (40% and 41%, respectively) did not reach statistical significance. In a separate group of animals, the effects of the 5-HT$_3$ receptor antagonist MDL 72222 (3 mg/kg ip) on anxiety-related behaviors were assessed in the elevated plus maze. In vehicle-treated rats, the FH/Wjd strain exhibited significantly greater percent of time spent on the open arms and percent open arm entries, an indication of less anxiety. Pretreatment with MDL 72222 did not alter these behaviors in the FH/Wjd rats, but had an anxiolytic-like effect in the ACI/N strain, significantly increasing the percent of time spent on the open arms and percent open arm entries. Further research into 5-HT$_3$ receptor function in the alcohol-preferring FH/Wjd rats is needed to elucidate the relationship among 5-HT$_3$ receptors, alcohol drinking, and anxiety.

Keywords: Fawn-hooded rats; 5-HT$_3$ receptor; Elevated plus maze; ACI rats; MDL 72222

1. Introduction

Abnormalities in serotonergic neurotransmission in the brain have been implicated in the pathogenesis and maintenance of alcoholism (e.g., Heinz et al., 2001) and in alcohol-drinking behavior of rats (e.g., Chen and Lawrence, 2000; Murphy et al., 1987; Zhou et al., 1991) and mice (Hensler et al., 2003). However, drugs that interact with serotonergic neurotransmission have had limited success in treating alcoholism (e.g., LeMarquand et al., 1994; Sellers et al., 1992). More recently, the 5-HT$_3$ receptor antagonist ondansetron has been found effective in reducing drinking and in prolonging abstinence in a subpopulation of alcoholics with an early age of onset of alcoholism (Johnson et al., 2000). This subpopulation of alcoholics develops problem drinking during youth, experiences severe behavioral problems, and has a high familial or biological predisposition towards alcoholism (Johnson et al., 2000).

Studies with rodents suggest that there might be a genetic relationship between the 5-HT$_3$ receptor and alcohol sensitivity and intake. Indeed, recent studies have demonstrated that 5-HT$_3$ receptor binding is decreased in the alcohol-preferring, selectively bred P rat (Ciccocioppo et al., 1998). Moreover, transgenic mice overexpressing the 5-HT$_3$ receptor voluntarily drink less alcohol than wild type mice (Engel et al., 1998) and are more sensitive to the effects of alcohol (Engel and Allan, 1999).

A substantial amount of literature has linked 5-HT$_3$ receptor function with anxiety-like behavior. Preclinical studies indicate that 5-HT$_3$ receptor antagonists induce anxiolytic-like effects in a variety of animal models (Jones et al., 1988; Costall and Naylor, 1991, 1992; Barnes et al.,

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1992; Cutler et al., 1997; Barnes and Sharp, 1999; Olivier et al., 2000); although this has not been a consistent observation (File and Johnston, 1989; Rodgers et al., 1997). The direct application of a 5-HT_3 receptor agonist into the amygdala or dorsal raphe nucleus induces an anxiogenic-like response (Costall et al., 1989). Mice having a deletion of the 5-HT_{3A} subunit exhibit an anxiolytic profile in several tests of anxiety-like behavior (Kelley et al., 2003). Moreover, several clinical studies have demonstrated the efficacy of 5-HT_3 receptor antagonists in the treatment of generalized anxiety disorder (Freeman et al., 1997) and panic disorder (Schneier et al., 1996). Therefore, a reduction in 5-HT_3 receptor function appears to be associated with lower anxiety.

The fawn-hooded (FH/Wjd) rat is an inbred strain that exhibits central serotonin (5-HT) dysfunction and voluntarily consumes substantially more ethanol than the inbred ACI/N strain (Overstreet et al., 1999; Rezvani et al., 2002). The present investigation sought to explore the relationship between the 5-HT_3 receptor and anxiety-like behavior in FH/Wjd and ACI/N rats. In the current study, we did not examine the link between alcohol preference and the 5-HT_3 receptor or test the impact of alcohol on these receptors. Using quantitative autoradiography, we have measured in these two rat strains 5-HT_3 receptor binding sites in cortical and limbic structures, specifically the hippocampus and amygdala. We have also assessed in these two strains anxiety-related behaviors in the elevated plus maze and the effect of the 5-HT_3 receptor antagonist MDL 72222 on these behaviors. Our data indicate that in the FH/Wjd rat, 5-HT_3 receptor binding in cortical and limbic structures is reduced and that these rats exhibit lower anxiety when compared with the ACI/N rat. Further research into 5-HT_3 receptor function in the alcohol-preferring FH/Wjd rats is needed to elucidate the relationship among 5-HT_3 receptors, alcohol drinking, and anxiety.

2. Methods

2.1. Animals

Male FH/Wjd and ACI/N rats, approximately 70 days of age, were selected from the breeding colonies maintained in the UNC Center for Alcohol Studies. Unless otherwise indicated, animals were group housed and maintained on a 12:12-h day/night cycle with constant access to food and water. Rats used in the autoradiographic study were confirmed to be representative of their strains by subjecting them to two-bottle choice procedure for 2 weeks. Rats used in the elevated plus maze test were alcohol naïve. These experiments were performed according to the Guide for the Care and Use of Laboratory Animals (2002) as adopted and promulgated by the National Institutes of Health and were approved by the UNC Institutional Animal Care and Use Committee.

2.2. Drugs and radioisotopes

[^{3}H]LY 278548 (84 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). [^{3}H]BRL 43694 (82 Ci/mmol) was purchased from PerkinElmer Life Sciences/NEN (Boston, MA). ICS 205,930 and MDL 72222 were purchased from Sigma/RBI (St. Louis, MO).

2.3. Two-bottle choice procedure

Rats were individually housed and given free access to water in a graduated Richter tube for 2 days. Food was available ad lib. Rats were then given free access to food and a solution of 10% (v/v) ethanol as the sole source of fluid for 3 days. The rats were then given free access to food, water, and a 10% (v/v) solution of ethanol for 10 days. The positions of the two drinking tubes were randomly changed each day to prevent position preference. Water and ethanol intake were recorded every day at 9:00 a.m.; food and body weights were recorded three times per week (Rezvani et al., 1999). Ethanol intake was calculated as g/kg body weight/day. Rats were then returned to normal group housing conditions in which they received food and water ad lib for 10–14 days before being sacrificed for quantitative autoradiography.

2.4. Quantitative autoradiography

2.4.1. Tissue preparation

Rat brains were rapidly removed and frozen on powdered dry ice. Brains were stored at −80 °C until sectioning. Coronal sections of 20 μm thickness were cut at −17 °C in a cryostat microtome at the level of the frontal and anterior cingulate cortex (Plate 8, bregma 3.20 mm) and dorsal hippocampus (Plate 30, bregma −3.14 mm) according to the atlas of the rat brain of Paxinos and Watson (1986). Sections were thaw mounted onto gelatin-coated glass slides, desiccated at 4 °C for 18 h under vacuum, and then stored at −80 °C until use.

2.4.2. [^{3}H]LY 278548 autoradiography

Autoradiography of the binding of [^{3}H]LY 278548 to 5-HT_3 receptors in brain sections was performed as described (Gehlert et al., 1991). Briefly, slide-mounted sections were thawed and desiccated at 4 °C for 2 h. Sections were preincubated at room temperature in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl for 30 min. Sections were then incubated in the same buffer containing 4 mM $CaCl_2$ and 2 nM [^{3}H] LY 278548 (specific activity = 84 Ci/mmol) for 30 min at room temperature. Nonspecific binding was defined by incubating adjacent sections in the presence of 1 μM ICS 205,930. Incubation was terminated by two washes for 10 min each in ice-cold incubation buffer (pH 7.6), followed by a dip in ice-cold deionized water. Sections were dried on a slide warmer and exposed

to Kodak Biomax MR Film for a period of 16 weeks to generate autoradiograms.

2.4.3. Image analysis

Analysis of the digitized autoradiograms was performed using the image analysis program NIH Image, version 1.47 (NIH, Bethesda, MD). For each brain region, autoradiograms of two tissue sections were analyzed per animal. Tissue sections were stained with thionin and the brain areas identified using the atlas of the rat brain of Paxinos and Watson (1986). Autoradiograms of [^{3}H]LY 278548 binding were quantified by the use of simultaneously exposed [^{3}H] standards (ART-123, American Radiochemicals, St. Louis, MO), which had been calibrated using brain-mash sections according to the method of Geary et al. (Geary and Wooten, 1983; Geary et al., 1985). The amount of ligand bound was determined by converting optical density measurements to femtomoles per milligram of protein. Specific binding was calculated by subtracting nonspecific binding from total binding on adjacent sections.

2.5. Homogenate radioligand binding studies

The binding of [^{3}H]BRL 43694 (specific activity = 82 Ci/mmol) to 5-HT$_3$ receptors in membranes prepared from rat hippocampus and cortex was performed as described (Nelson and Thomas, 1989). Tissue was homogenized with a Teflon-glass homogenizer in 10 volumes of ice-cold HEPES buffer (50 mM, pH 7.5). The homogenate was washed three times by centrifugation (50,000 × g for 10 min) and the final pellet suspended in the same buffer. Saturation binding experiments were performed using 12 concentrations of [^{3}H]BRL 43694 (0.1–4 nM). In preliminary saturation binding experiments using Sprague–Dawley rats, [^{3}H]BRL 43694 bound to 5-HT$_3$ receptors in cortical homogenates with a B_{max} of 13 ± 1.4 fmol/mg protein and a K_d of 0.4 ± 0.14 nM (n = 3 animals) in close agreement with Nelson and Thomas (1989). For single-point binding, a concentration of 0.8 nM [^{3}H]BRL 43694 was used. Nonspecific binding was defined in the presence of 1 μM ICS 205,930. Binding was initiated by the addition of homogenate (250 μg protein per tube). Assay tubes were incubated for 30 min at 23 °C. Using a Brandel cell harvester (Gaithersburg, MD), binding reactions were terminated by the addition of 5 ml of ice-cold buffer (50 mM HEPES, pH 7.4), and membranes were collected on glass fiber filters (Schleicher and Schuell) that had been presoaked in 0.3% polyethyleneimine. Filters were washed three times with ice-cold buffer. Protein concentration was determined by the method of Bradford.

2.6. Elevated plus maze

Behavioral testing in the elevated plus maze was conducted as previously described by Gonzalez et al. (1998) and Kampov-Polevoy et al. (2000). The apparatus was made of black perspex and consisted of two closed arms and two open arms opposite each other, with a central arena of 10 × 10 cm. The maze was elevated 50 cm off of the floor and lit by bright light. Rats were placed in the elevated plus maze 30 min after injection with either isotonic saline vehicle or the 5-HT$_3$ receptor antagonist, MDL 72222 (3 mg/kg ip). This dose of MDL 72222 was selected for the current studies on the basis of the findings of Fadda et al. (1991); it is the lowest dose to suppress alcohol intake in Sardinian ethanol-preferring (SP) rats (Fadda et al., 1991). The rat was placed in the apparatus with the head in the central arena and the following measures were recorded during a 5-min session: number of entries into the closed arms by the whole rat, number of entries into the open arms by the whole rat, and time spent in the open arms by at least two forepaws. Observers were blind to treatment. The percent of time spent on the open arms of the maze (time spent on open arms divided by the time spent on open arms plus time spent in closed arms) provides the measure of anxiety, and the number of closed-arm entries provides the measure of locomotor activity.

2.7. Statistical analyses

2.7.1. Receptor binding

Saturation binding data were fitted by nonlinear regression using Kaleidagraph (version 3.08, Synergy Software) to the model $B=[B_{max}/1+(K_d/[D])^{m1})]+(m^2*[D])$, where B is the amount of radioligand bound at the radioligand concentration D, B_{max} is the maximal concentration of bound ligand, K_d is the equilibrium dissociation constant, m_1 is the slope of the total binding curve, and m_2 is the slope of the nonspecific binding curve. Statistical comparisons of binding data were made by ANOVA. F values reaching significance ($P < .05$) were evaluated further by post hoc analysis using Fisher's protected least significant difference test. Statistical tests were performed using Statistica software (version 4.1, Statsoft, Tulsa, OK).

2.7.2. Behavioral data

Analyses of the behavioral data were facilitated by use of the GBstat statistical package (version 5.0, Dynamic Microsystems, Silver Spring, MD). Initially, statistical comparisons were made by two-way ANOVA, with strain and treatment as the two main factors. F values reaching significance ($P < .05$) were evaluated further using Tukey's protected t tests.

3. Results

3.1. Receptor binding

To determine whether there were differences between the ACI/N and FH/Wjd strains in central 5-HT$_3$ receptor sites,

quantitative autoradiography of the binding of the antagonist radioligand [^{3}H]LY 278548 was performed. Rats used in the autoradiographic study were confirmed to be representative of their strains by subjecting them to two-bottle choice procedure for 2 weeks. Ethanol intake (g/kg body weight/day) was 0.49 ± 0.28 for ACI/N rats and 6.13 ± 1.32 for FH/Wjd rats (n = 5 animals per group). Rats were returned to normal housing conditions for 10–14 days before being used for the autoradiographic studies. Autoradiograms of the binding of [^{3}H]LY 278548 to sections of rat brain taken at the level of the hippocampus are shown in Fig. 1. The binding of [^{3}H]LY 278548 to 5-HT$_3$ receptor sites was reduced in the cortical regions, hippocampus, and amygdaloid nuclei of FH/Wjd rats when compared with ACI/N rats (Fig. 2). This reduction in [^{3}H]LY 278548 binding reached statistical significance in frontal cortex [$F(1,8) = 18.23$, $P = .002$], CA1 region of hippocampus [$F(1,8) = 17.32$, $P = .003$], and in the medial [$F(1,8) = 45.57$, $P < .001$] and lateral [$F(1,8) = 28.26$, $P < .001$] nuclei of the amygdala. In the anterior cingulate cortex and in the dentate gyrus region of the hippocampus, the reduction in [^{3}H]LY 278548 binding in the FH/Wjd versus ACI/N strain (40% and 41%, respectively) did not reach statistical significance [anterior cingulate cortex, $F(1,8) = 3.548$, $P = .102$; dentate gyrus, $F(1,8) = 4.093$, $P = .077$]. These data suggest that 5-HT$_3$ receptor sites are reduced in frontal cortex and several limbic brain structures in FH/Wjd versus ACI/N rat.

Ethanol exposure has been reported to up-regulate 5-HT$_3$ receptor function (Yoshimoto et al., 1996), although this has not been a consistent observation (Chen and Lawrence, 2000). It is therefore unlikely that in the current study, the greater ethanol intake of the FH/Wjd rats during the two-bottle choice procedure contributed to the reductions in 5-HT$_3$ receptor binding observed in the autoradiographic studies. To address this question, radioligand binding studies were conducted in homogenates prepared from the cortex and hippocampus of alcohol-naïve FH/Wjd and ACI/N rats. In the cortex and hippocampus of FH/Wjd rats, the binding of a single concentration of [^{3}H]BRL 43694 (0.8 nM) was reduced in comparison with tissue from ACI/N rats (fmol/mg protein) {cortex: ACI/N = 8.5 ± 1.4, FH/Wjd, = 6.05 ± 0.07 [$F(1,4) = 2.93$, $P = .162$]; hippocampus: ACI/N = 6.5 ± 0.9, FH/Wjd, = 4.2 ± 0.47 [$F(1,4) = 4.99$, $P = .089$]} (n = 3 animals per group). These data indicate that the reductions in central 5-HT$_3$ receptor binding observed for FH/Wjd rats in the autoradiographic studies were not due to ethanol exposure.

3.2. Plus maze

Anxiety-related behaviors in the elevated plus maze and the effect of the 5-HT$_3$ receptor antagonist MDL 72222 on these behaviors are shown for the two strains in Fig. 3. Two-way ANOVA of the percent of time spent on the open arms (Fig. 3A) revealed a significant strain effect [$F(1,26) = 6.09$, $P = .02$], a nonsignificant effect of the drug MDL 72222 [$F(1,26) = 0.63$, $P > .25$], and a significant interaction effect [$F(1,26) = 8.28$, $P = .008$]. As shown in Fig. 3A, there was a marked difference between strains in the vehicle-treated rats, with the FH/Wjd rats spending a greater percent of time on

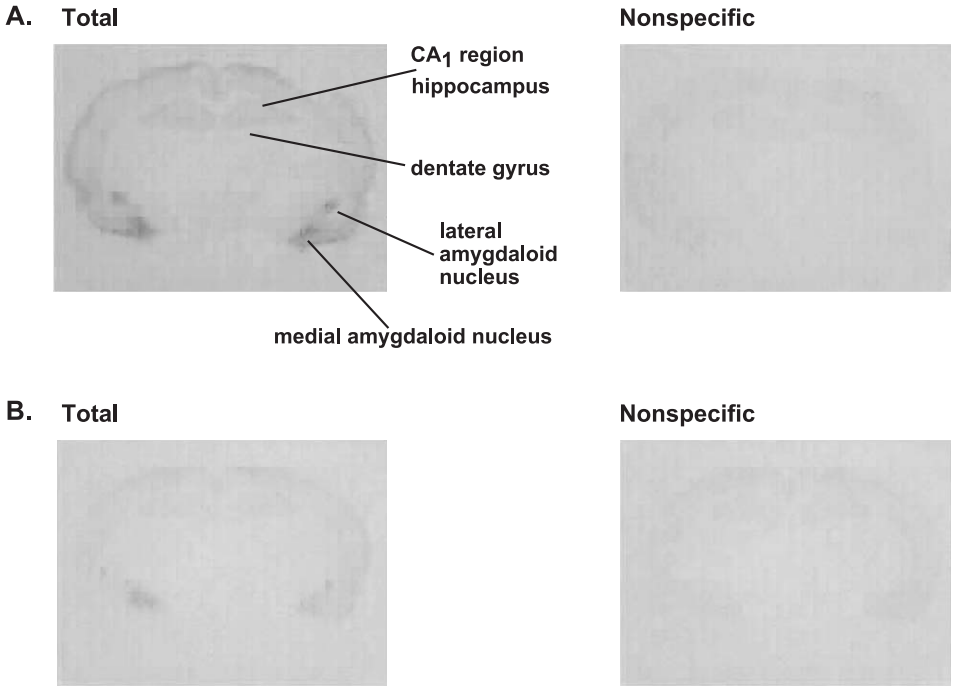

Fig. 1. Autoradiograms of the binding of [^{3}H]LY 278548 to sections of the brain from (A) ACI/N or (B) FH/Wjd rats. Coronal sections at the level of hippocampus (Plate 30) (Paxinos and Watson, 1986) were incubated with [^{3}H]LY 278548 (2 nM). Nonspecific binding was defined in the presence of 1 μM ICS 205,930.

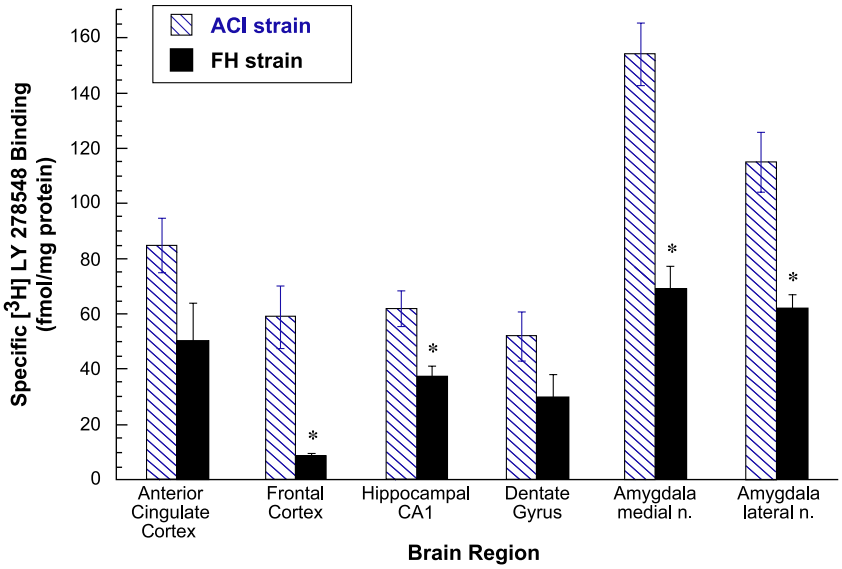

Fig. 2. Strain differences in the binding of [^{3}H]LY 278548 to 5-HT$_3$ receptors. Coronal sections of rat brain at the level of anterior cingulate cortex (Plate 8) and hippocampus (Plate 30) (Paxinos and Watson, 1986) were incubated with [^{3}H]LY 278548 (2 nM). Nonspecific binding was defined in the presence of 1 μM ICS 205,930. Specific binding is expressed as fmol/mg protein. Shown are the mean ± S.E.M. $n=5$ animals per experimental group; * $P<.05$.

the open arms than the ACI/N rats. This baseline difference in open arm exploration can be interpreted as a sign of less anxiety in the FH/Wjd rats. Pretreatment with the 5-HT$_3$ receptor antagonist MDL 72222 did not significantly alter the percent time spent on the open arms for the FH/Wjd rats but had an anxiolytic-like effect in the ACI/N strain, significantly increasing the percent time spent on the open arms (Fig. 3A). The percent of time spent on the open arms for the vehicle-treated FH strain, the MDL 72222-treated FH strain, and the MDL 72222-treated ACI strain were not statistically different from each other (Fig. 3A).

Open arm entries, expressed as a percentage, are shown in Fig. 3B. Two-way ANOVA revealed a highly significant strain effect [$F(1,26)=26.88$, $P<.001$], a nonsignificant effect of the drug MDL 72222 [$F(1,26)=2.01$, $P>.1$], and a significant interaction effect [$F(1,26)=12.15$, $P=.002$]. As shown in Fig. 3B, there was a marked difference between strains in the vehicle-treated rats, with a greater

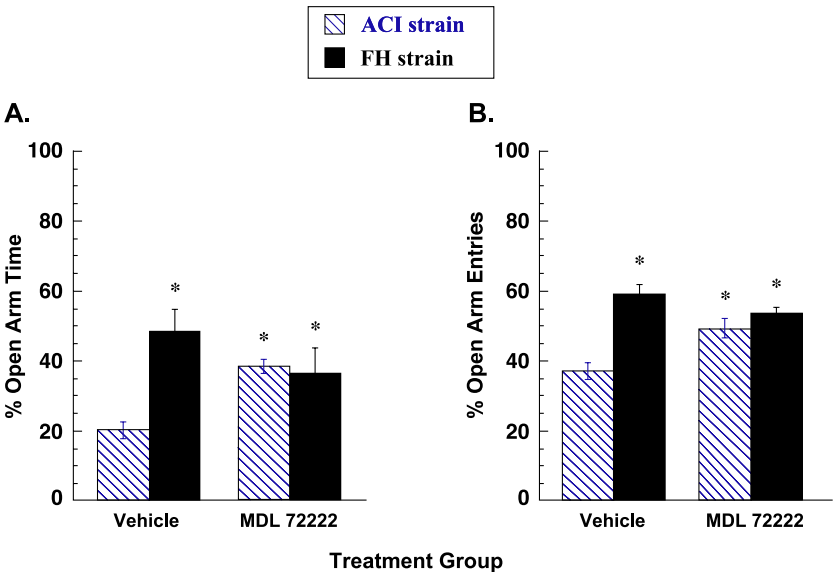

Fig. 3. Strain differences in the effect of a 5-HT$_3$ antagonist on the (A) % open arm time and (B) % open arm entries in the elevated plus maze. Rats were injected with either isotonic saline (1 ml/kg ip) or the 5-HT$_3$ receptor antagonist MDL 72222 (3 mg/kg ip) 30 min before being placed at the midpoint of the plus maze. % Open arm time is the time spent on the open arms divided by the time spent on open arms plus time spent in the closed arms. % Open arm entries are the number of entries into the open arms divided by the entries into the open arms plus entries into the closed arms. $n=8$ animals per experimental group; * $P<.05$ when compared with vehicle-treated ACI/N group.

percent of entries onto the open arms for FH/Wjd rats than for ACI/N rats. This baseline difference in open arm entries can be interpreted as a sign of less anxiety in the FH/Wjd rats and is consistent with the data shown in Fig. 3A. Pretreatment with the 5-HT_3 receptor antagonist MDL 72222 did not significantly alter the percent open arm entries for the FH/Wjd rats but had an anxiolytic-like effect in the ACI/N strain, significantly increasing the percent open arms entries (Fig. 3B). The percent open arm entries for the vehicle-treated FH strain, the MDL 72222-treated FH strain, and the MDL 72222-treated ACI strain were not statistically different from each other (Fig. 3B).

As increased time spent on the open arms or entries into the open arms of the elevated plus maze alone could be due to an increase in locomotion or exploration, the number of closed arm entries was used as a measure of locomotor activity. Two-way ANOVA revealed a significant strain effect [$F(1,26)=6.25$, $P=.02$], a nonsignificant effect of the drug MDL 72222 [$F(1,26)=2.01$, $P>.1$], and a nonsignificant interaction effect [$F(1,26)=0.11$, $P>.25$]. As shown in Fig. 4, there was a significant difference between strains in the number of entries into closed arms in the vehicle-treated rats, with FH/Wjd rats entering into the closed arms fewer times than ACI/N rats. Pretreatment with the 5-HT_3 receptor antagonist MDL 72222 did not affect the closed arm entries in either strain. These data suggest that the anxiolytic-like effect of MDL 72222 on anxiety-related behaviors observed for the ACI/N strain was not due to an effect on locomotor activity.

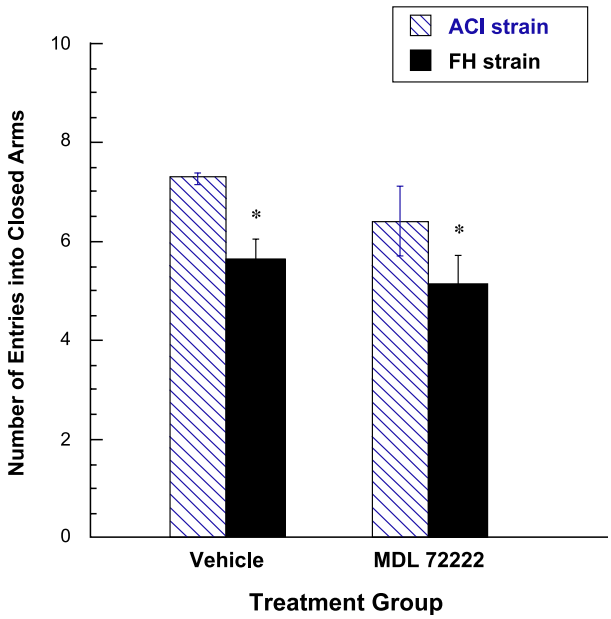

Fig. 4. Strain differences in the number of entries into the closed arms of the elevated plus maze. Rats were injected with either isotonic saline (1 ml/kg ip) or the 5-HT_3 receptor antagonist MDL 72222 (3 mg/kg ip) 30 min before being placed at the midpoint of the plus maze. $n=8$ animals per experimental group; $*P<.05$ when compared with vehicle-treated ACI/N group.

4. Discussion

The results of the present study show that 5-HT_3 receptor binding is reduced in the cortex, hippocampus, and amygdala of alcohol-preferring FH/Wjd rats when compared with their alcohol-nonpreferring counterparts, the ACI/N strain. Our data are in contrast to those of Chen and Lawrence (2000), who report no difference in 5-HT_3 receptor binding in the cortex of FH versus Wistar–Kyoto rats. This difference between the current study and that of Chen and Lawrence (2000) may be due to the use of different control strains. Chen and Lawrence (2000) did not examine 5-HT_3 receptor binding in amygdala or hippocampus. Our data indicating marked decreases in 5-HT_3 receptor binding in the amygdala of FH/Wjd rats are consistent with the decrease in 5-HT_3 receptor binding sites observed in the amygdala of alcohol-preferring P rats (Ciccocioppo et al., 1998). The results of the current study are consistent with the report of Ciccocioppo et al. (1998) in suggesting an inverse relationship between the number of 5-HT_3 receptors in the amygdala and alcohol intake.

We observed the greatest percent reductions in 5-HT_3 receptor sites in the frontal cortex (82%) and medial (54%) and lateral (47%) nuclei of the amygdala of FH/Wjd rats when compared with ACI/N rats. The medial and lateral nuclei of the amygdala are structures that are key components of a basal forebrain macrostructure termed the extended amygdala, the brain reward system implicated in the development of alcoholism (see Koob and Le Moal, 2001). Dysregulation of multiple neurotransmitter systems, including serotonin, in the extended amygdala occurs during the development of allostasis (the ability to achieve stability through change) and alcohol dependence (see Koob and Le Moal, 2001).

The amygdala is also a brain region that has been implicated in anxiety-like behavior (Davis, 1998), so it is quite feasible that reductions of 5-HT_3 receptors in this brain region in the FH/Wjd rats may contribute to lower anxiety in this strain. Indeed, we observed in the elevated plus maze test greater percent time spent on the open arms and greater percent open arm entries for FH/Wjd rats when compared with the ACI/N rats. The number of closed arm entries, an indication of locomotor activity, was greater for the ACI/N rats. Thus, the higher proportion of time in open arm exploration observed for the FH/Wjd rats was not due to increase locomotion but is indicative of lower anxiety in this strain.

Consistent with our findings indicative of lower anxiety in FH/Wjd rats are the observations of Knapp et al. (1997). These authors, in measuring ultrasonic vocalizations in response to aversive air puff stimulus, found that FH/Wjd rats vocalize less than the ACI strain (Knapp et al., 1997). However, the reduced anxiety-like behavior in the FH/Wjd strain compared to the ACI/N strain (Knapp et al., 1997; current report) appears at odds with some of the literature on FH/Wjd rats. For example, Kantor et al. (2000) found that

all social behaviors in the social interaction test are markedly diminished in Fawn-Hooded rats, suggesting higher anxiety in these animals. However, it is not clear whether the Fawn-Hooded rats used in this study are of the FH/Wjd substrain (Kantor et al., 2000). The FH/Har substrain has been shown to exhibit increased anxiety-like behavior in the open-field test (Hall et al., 2000). In a direct comparison of the FH/Wjd with the FH/Har substrain (from a colony established by Harrington and maintained at the National Cancer Institute), Overstreet and Rezvani (1996) found that FH/Har rats exhibit more anxiety-like behaviors than the FH/Wjd substrain. Overstreet and Rezvani (1996) found at least two remarkable differences between these strains: The FH/Wjd strain is more immobile in the swim test and drinks significantly more alcohol voluntarily. In contrast, the FH/Har strain spends very little time in the open arms of the elevated plus maze, suggesting increased anxiety in these animals. Recently, Lodge and Lawrence (2003) showed that FH/Wjd rats, isolated from weaning, exhibit more anxiety-like behavior than group-housed FH/Wjd rats, indicating that housing conditions can contribute to the behavioral profile of these rats. Thus, whether Fawn-Hooded rats exhibit anxiety-like behavior depends on the substrain of Fawn-Hooded rats, the strain used for comparison, and the housing conditions. All the rats used in the current study were group-housed under the same conditions.

A factor analysis study involving 18 measures in nine alcohol-preferring and -nonpreferring rat strains revealed that measures of anxiety, such as defecation in the open field and ultrasonic vocalizations after a tactile stimulus, were negatively correlated with alcohol intake and preference (Overstreet et al., 1997). Thus, alcohol-preferring rats appear less anxious than their alcohol-nonpreferring counterparts (see Overstreet et al., 1997). Reports on anxiety-related behaviors of the alcohol-preferring P rats in the elevated plus maze, however, are mixed. Alcohol-preferring P rats were reported to exhibit greater anxiety-like behavior in the elevated plus maze relative to the alcohol-nonpreferring NP rats (Stewart et al., 1993), but differences between the P and NP rats in anxiety-related behaviors in the elevated plus maze were not confirmed (Viglinskaya et al., 1995). P rats, as well as FH/Wjd and other alcohol-preferring rat strains, have been shown to exhibit fewer ultrasonic vocalizations after a tactile stimulus than their alcohol-nonpreferring counterparts, suggesting lower anxiety in these strains (Knapp et al., 1997). The less anxious behavioral profile shown by the alcohol-preferring FH/Wjd rats in the elevated plus maze relative to the alcohol-nonpreferring ACI/N rats confirms therefore the relationship between alcohol preference and anxiety-like behavior. The present findings also suggest that the reduction in 5-HT_3 receptors might be a contributing factor to both of these behavioral measures, although further study is needed.

The anxiolytic-like effects of selective 5-HT_3 receptor antagonists in animal models are inconsistent (see Olivier et al., 2000). Strain differences in effects of 5-HT_3 antagonists may be one contributing factor to the lack of consistency in the literature. Indeed in the current study, we observed a strain-dependent effect of the 5-HT_3 receptor antagonist MDL 72222 on anxiety-like behavior in the elevated plus maze. The FH/Wjd strain exhibited significantly greater percent of time spent on the open arms and percent open arm entries than the ACI/N strain, an indication of less anxiety. This difference between these two strains in baseline anxiety-like behavior may have contributed to the difference between these two strains in the effect of the 5-HT_3 receptor antagonist on behavior in the elevated plus maze. Pretreatment with the 5-HT_3 receptor antagonist MDL 72222 did not alter these behaviors in the FH/Wjd rats but had an anxiolytic-like effect in the ACI/N strain, significantly increasing the percent of time spent on the open arms and percent open arm entries. Strain differences to anxiolytic or anxiogenic agents other than 5-HT_3 receptor antagonists have also been reported in strains with baseline differences in anxiety-like behavior (Gonzalez et al., 1998; Keck et al., 2001). Thus, drugs that modify anxiety-like behavior have differential effects that may be associated with strain differences in baseline anxiety-like behavior. In the present study, the strain differences in baseline anxiety-like behavior between FH/Wjd and ACI/N rats may be related to differences in central 5-HT_3 receptor binding.

The differential anxiolytic response of the ACI/N and FH/Wjd rats to the 5-HT_3 receptor antagonist, MDL 72222, suggests that clinical studies on the anxiolytic properties of 5-HT_3 antagonists might produce mixed results. Indeed, although there are isolated reports of beneficial effects of 5-HT_3 receptor antagonists in treating anxiety disorders (e.g., Freeman et al., 1997; Schneier et al., 1996), there have also been several negative studies (see Olivier et al., 2000).

In sum, FH/Wjd rats have fewer 5-HT_3 receptors in frontal cortex and several limbic structures of the brain and exhibit an anxiolytic profile in the elevated plus maze compared to the ACI/N rats. These findings are consistent with other literature on 5-HT_3 receptor function and anxiety-like behavior. However, the relationship between central 5-HT_3 receptor binding or the elevated plus maze response and the alcohol drinking phenotype inherent to these rats is not clear. Indeed, further studies are needed to elucidate the relationship among 5-HT_3 receptors, alcohol drinking, and anxiety.

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