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Differences in Ethanol-Induced Behaviors in Normal and Acatalasemic Mice: Systematic Examination Using a Biobehavioral Approach

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ARAGON, C. M. G. AND Z. AMIT. *Differences in ethanol-induced behaviors in normal and acatalasemic mice: Systematic examination using a biobehavioral approach.* PHARMACOL BIOCHEM BEHAV 44(3) 547–554, 1993. – In studies designed to further examine the previously reported involvement of catalase in ethanol-induced effects, we attempted to confirm earlier observations by using normal (C3H-N) and acatalasemic (C3H-A) mice. These mice are identical in every respect and differ only in their catalase activity. Data suggested that the application of 3-amino-1,2,4-triazole (AT), a catalase inhibitor, to both substrains of mice resulted in a proportional decrease in motor activity, thus supporting our earlier observations. We also showed that this effect was specific to ethanol because AT did not have any effect on cocaine-induced motor activity in both substrains. Contrary to the effects of ethanol, these substrains did not differ in motor activity in response to cocaine. In an additional study, we observed that acatalasemic mice differed from the normals in their pattern of voluntary ethanol consumption. Acatalasemic mice consumed more ethanol but only when it was presented in the range of concentrations between 12 and 18%. Finally, we also obtained data suggesting that acatalasemic mice have longer duration of sleep time following ethanol administration compared to normals. Catalase activity was measured in both substrains. Results, once again, confirmed earlier data that the substrains differ in this activity and that AT further decreases brain catalase activity in both mice. Finally, when brain homogenates derived from both substrains were incubated with ethanol significant differences in the amount of generated acetaldehyde were found between the two mice strains. Together, these results provide strong support for the involvement of brain catalase in a variety of ethanol-induced behavioral effects.

Ethanol Acetaldehyde Catalase Acatalasemic mice Locomotor activity Narcosis Voluntary ethanol consumption Ethanol oxidation

ETHANOL, through its effects in the CNS, induces a variety of behavioral and physiological effects on mice. These include, among others, locomotor excitation and depression (2, 12,16), narcosis (12), conditioned taste aversion (20), and positive reinforcement (17). Acetaldehyde, the first metabolite of ethanol, has been implicated in the possible mediation of some of the central actions of ethanol (25). Thus, positive reinforcement as measured in the self-administration paradigm (9) and in the place preference paradigm (24), conditioned taste aversion (4,26), locomotor activity (5,27), and narcosis (6,29) were all shown to be mediated, in part, by acetaldehyde.

More recently, reports from several laboratories also suggested a role for brain catalase activity in the mediation of some of the psychopharmacological effects of ethanol (1,2,4–7,29). It has been suggested that the enzyme catalase, in conjunction with hydrogen peroxide, may metabolize ethanol directly in the brain (8,10). It has been shown that ethanol protected brain catalase from inhibition by 3-amino-1,2,4-triazole (AT) (10) as well as cyanamide (8), both catalase inhibitors. This prevention of the inhibition of catalase has been taken as indirect supportive evidence for the oxidation of ethanol to acetaldehyde in the CNS (8,10). Evidence suggesting a biologic significance to this central metabolic process or interaction of ethanol with catalase was reported in several studies. Pretreatment with AT, and the consequent loss of brain catalase activity, blocked or attenuated such different ethanol-induced behaviors as conditioned taste aversion (4), locomotor activity (5), narcosis (6,29), lethality (6,29), and voluntary ethanol drinking (1). Together, these studies suggested an involvement of brain catalase in ethanol's behavioral effects and supported the notion that this putative ability to synthesize acetaldehyde in brain may be an important regulator of ethanol's psychopharmacological effects.

Given these findings, it seemed logical to further extend these studies on the role of the enzyme catalase in the mediation of the central effects of ethanol. The present studies were

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designed in an attempt to rule out the possible artifactual confounds emanating from the use of inhibitors (19,23) as tools to manipulate catalase activities. Specifically, the role of brain catalase in mediating ethanol's behavioral effects was assessed by comparing ethanol's effects on locomotor activity, voluntary ethanol consumption, and narcosis in normal and acatalasemic mice (13-15). Genetically acatalasemic mice were originally produced by X-ray irradiation from normal mice (strain C3H) (14,15). This autosomally inherited acatalasemia resulted in the loss of catalase in the liver, blood, and brain (13-15). To confirm these reports on the differences between the two substrains, brain catalase activities of these mice was investigated. Finally, the possible differential capacity of brain homogenates extracted from both strains of mice to oxidize ethanol was also tested. It was hoped that the use of animals genetically deficient in catalase activity would exclude the possible confounding effects of the use of the somewhat toxic catalase inhibitors.

METHOD

Subjects

Wild-type mice C3H-N (normal) and a corresponding colony of C3H-A (acatalasemic) mice produced by X-ray irradiation were supplied by Oak Ridge Laboratory (Oak Ridge, TN). This colony was originally established by Dr. Feinstein at Argonne National Laboratory (14,15). Male mice of about 27 g were used in the present experiment.

Experiment 1: Locomotor Activity and Ethanol

This experiment constitutes a further examination as well as an extension and elaboration of data reported elsewhere (2).

Apparatus. Locomotor activities were measured in an open-field apparatus that consisted of a glass cylinder (30 cm in diameter) divided into four quadrants by black markings on the floor of the cylinder. A single locomotor activity count was considered each time the mouse crossed over from one quadrant to another with all four paws.

Procedure. Five hours prior to open-field testing, subjects received IP injections of either AT (0.5 g/kg) or saline (S). This dose of AT was chosen on the basis of previous studies demonstrating a reduction of about 95% of brain catalase activity following such treatment (2,3). Immediately before testing, mice received an IP injection of one of these doses of ethanol: 0.0, 0.8, 1.2, 1.6, 2.0, or 3.2 g/kg. Following injections, mice were placed in the open-field apparatus, where locomotor activity was recorded for a total of 10 consecutive min. For each treatment and mouse strain, $n = 8$.

Experiment 1a: Locomotor Activity and Cocaine

This experiment was designed to examine the presumed specificity of the interaction between AT and ethanol.

Procedure. Five hours prior to open-field testing, subjects received IP injections of either AT (0.5 g/kg) or S. Immediately before testing, mice received an IP injection of one of two doses of cocaine (2.0 or 4.0 mg/kg) or saline. Following injections, mice were placed in the open-field apparatus, where locomotor activity was recorded for a total of 10 consecutive min. For each treatment and mouse strain, $n = 8$.

Experiment 2: Voluntary Ethanol Consumption

Procedure. Twenty-one male mice of each strain were housed, three to a cage, during testing in a room regulated for constant temperature and humidity on a 12 L : 12 D cycle. Drinking fluids were presented in test tubes fitted with stainless steel ball-bearing spouts inserted through the wire mesh in the top of the cage. Animals were offered a free choice between water and increasing concentrations of ethanol (2-22%). Ethanol solutions were prepared by mixing 95% ethanol with tapwater. The concentrations of ethanol solutions offered to animals were raised by increments of 1%. To avoid a position bias, the location of the fluid containing tubes was shifted systematically. Each ethanol concentration was presented for 4 consecutive days. To reduce the confounding effects of variability, the data was then analyzed in 2-day blocks. In view of the difficulties in obtaining accurate fluid intake and weight measurement from individual mice, daily fluid consumption and body weights of all three animals residing in a cage were measured together. Ethanol consumption was, thus, calculated as g/kg body weight/cage. Daily ethanol preference ratio scores were obtained by dividing the consumption of ethanol (ml) derived from the ethanol bottle alone by the total fluid consumption (ml) derived from both the ethanol and water bottles.

Experiment 3: Duration of Narcosis

Procedure. Immediately before sleep-time testing, male mice of both substrains received an IP injection of ethanol, 3.0 or 4.0 g/kg (20%). The duration of narcosis or sleep time was defined as the time elapsed from loss of righting reflex to the time the righting reflex was regained. Recovery of the righting reflex was determined when subjects could right themselves three times within 60 s after being placed on their backs.

Experiment 4: Catalase Activity Determination

Procedure. Five hours after IP administration of AT (0.5 g/kg) or saline, normal and acatalasemic mice were placed in an ether inhalation chamber 1 min prior to being sacrificed by decapitation. Brains were removed and prepared for catalase activity determinations. Brain catalase activity was determined using a Yellow Springs oxygen monitor (Yellow Springs Instruments, Yellow Springs, OH) equipped with a Clark style oxygen electrode according to a procedure described by DeMaster et al. (11). Catalase activity is expressed in units of nM of O_2 formed per minute per μg protein.

Experiment 5: Ethanol Oxidation in Brain Homogenates

Procedure. Brains of C3H-N and C3H-A male mice were excised and 10% homogenates were prepared with 0.1% Triton X-100 in 0.1 mM potassium phosphate buffer (pH 7.6) at 4°C. All homogenates were stored at 0°C and assayed the same day. Aliquots of these homogenates equivalent to 25 mg wet tissue were incubated at 37°C for 1 h in sealed, clear 6-ml Hypo-vials with 90 mM potassium phosphate buffer (pH 7.6), 10 mM glucose, and ethanol (12.5, 25, 50, 100, and 200 mM) in the presence or absence of sodium azide (5 mM), a total inhibitor of catalase activity used as control for nonenzymatic formation of acetaldehyde. The acetaldehyde content of the gaseous phase of each vial was measured by a head-space gas chromatography procedure (28). The flasks were incubated at 65°C for 25 min and 2 ml of the head space was injected into a Varian Model 1400 gas chromatograph (Varian, Walnut Creek, CA) with flame ionization detectors. A 180 cm × 2-mm column of Chromosorb 101 mesh 80/100 was used with inlet and detector temperatures of 140 and 180°C, respectively, as well as a nitrogen flow rate of about 20 ml/min. Under these conditions, the retention time was 1.8 min for acetaldehyde and 3.4 min for ethanol. Relative peak heights

were determined by comparison with standards prepared by the addition of known amounts of acetaldehyde to "zero-time" controls. Blanks with boiled homogenates used as totally non-active tissue controls were employed in each experiment. For each strain and ethanol concentration, $n = 6$.

Statistical Analysis

Results where appropriate were expressed as group mean ± SEM. The appropriate analysis of variance (ANOVA) was performed on these means. Posthoc comparisons using Tukey tests or *t*-tests were carried out on each individual data point. p values of < 0.05 were accepted as significant.

RESULTS

Experiment 1: Locomotor Activity and Ethanol

Mean activity counts in 10-min blocks at each of the six treatment doses of ethanol for both substrains of mice are presented in Fig. 1. A two-way ANOVA yielded a significant two-way interaction, $F(5, 84) = 21.35$, $p < 0.001$. It also yielded a significant strain effect, $F(1, 84) = 441.38$, $p < 0.001$, as well as a significant dose effect, $F(5, 84) = 302.99$, $p < 0.001$. Ethanol induced biphasic effects on locomotor activity in both substrains of mice ($p < 0.01$). Moderate doses of ethanol (0.8, 1.2, 1.6, and 2.0 g/kg) produced excitation ($p < 0.01$), while higher doses of ethanol produced depression (3.2 g/kg; $p < 0.01$). These findings of a biphasic effect of ethanol on locomotor activity confirmed previous studies reported by this laboratory (2). Significant differences in locomotion between the two substrains of mice were found following administration of 0.8-, 1.2-, 1.6-, and 2.0-g/kg ($p < 0.01$) ethanol doses. Locomotor activity following all excitatory ethanol doses was significantly different from locomotor activity following the depressive dose (3.2 g/kg) in normal mice. It is worth noting that locomotor activity following the depressive dose of 3.2 g/kg was not significantly different from activity following the saline injection in those mice ($p > 0.05$). Acatalasemic mice also revealed significant differences in locomotion following injections of the excitatory doses of 1.6 and 2.0 g/kg ethanol as compared to the saline injection as well as the highest and depressive, 3.2-g/kg injection of ethanol ($p < 0.01$).

Both strains of mice were pretreated 5 h prior to ethanol injection with saline or AT (0.5g/kg). AT produced a significant decrease in ethanol-induced locomotor activity in mice from the normal substrain (C3H-N) (Fig. 2A). A two-way ANOVA yielded a significant interaction, $F(5, 84) = 37.08$, $p < 0.001$. It also yielded a significant AT treatment and ethanol dose effects, $F(1, 84) = 585.57$, $p < 0.001$; $F(5, 84) = 152.27$, $p < 0.001$, respectively. AT also produced a significant decrease in ethanol-induced locomotor activity in acatalasemic mice (Fig. 2B) following all ethanol doses tested with the exception of the highest dose of 3.2 g/kg. A two-way ANOVA yielded a significant interaction as well as a significant AT treatment and ethanol dose effect, $F(5, 84) = 63.87$, $p < 0.001$; $F(1, 84) = 482.45$, $p < 0.001$; $F(5, 84) = 184.79$, $p < 0.001$, respectively. Administration of AT did not affect the spontaneous activity of mice from the two substrains. Also, baseline (saline) activity scores of the two substrains were identical.

Experiment 1a: Locomotor Activity and Cocaine

The results depicted in Table 1 summarize the effects of saline or AT on cocaine-induced locomotor activity in both substrains of mice. The results revealed a significant effect of cocaine but not of strain or pretreatment. A three-way ANOVA yielded a significant cocaine dose effect, $F(2, 86) = 222.51$, $p < 0.001$. No significant interaction and no significant strain and AT treatment effects were found. The higher dose of cocaine (4 mg/kg) did produce locomotor excitation in all groups tested.

Experiment 2: Voluntary Ethanol Consumption

An examination of the ethanol drinking data of the two substrains following a wide range of ethanol concentrations offered to these animals (2–22%) revealed the following pic-

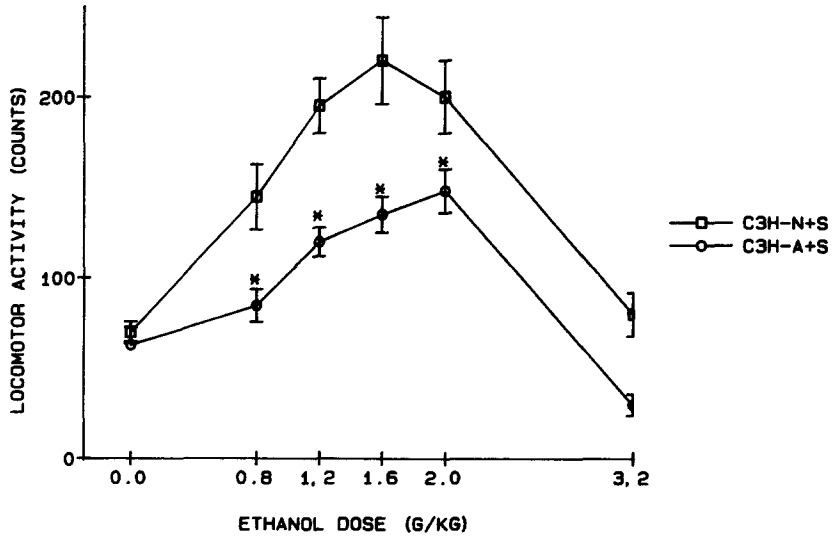

FIG. 1. Locomotor activity (counts/10 min) for normal (C3H-N) and acatalasemic (C3H-A) mice as a function of ethanol dose (g/kg). Data represent mean ± SEM of $n = 8$ (*$p < 0.05$).

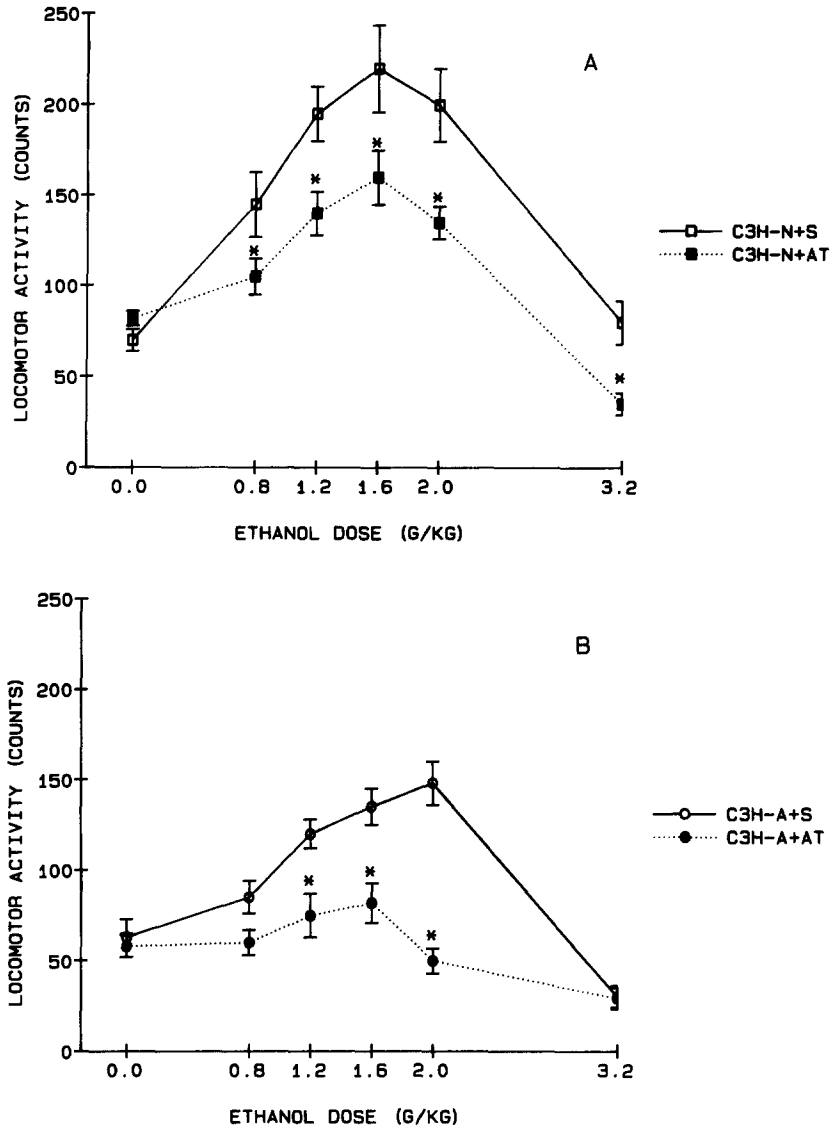

FIG. 2. Locomotor activity (counts/10 min) for normal (C3H-N) and acatalasemic (C3H-A) mice pretreated with saline (S) or 3-amino-1,2,4-triazole (AT) (0.5g/kg) as a function of ethanol dose (g/kg). (A). Normal mice. (B). Acatalasemic mice. Data represent mean ± SEM of $n = 8$ ($^*p < 0.05$).

TABLE 1

MEAN LOCOMOTOR ACTIVITY (COUNTS/10 min; ±SEM) FOR NORMAL (C3H-N) AND ACATALASEMIC (C3H-A) MICE PRETREATED WITH AT (0.5 g/kg) OR SALINE AS A FUNCTION OF COCAINE DOSE (mg/kg)

	Strain			
	C3H-N		C3H-A	
Treatment	S	AT	S	AT
Cocaine Dose (mg/kg)				
0.0	65.0 ± 5	55.0 ± 8	58.0 ± 9	62.0 ± 6
2.0	71.0 ± 16	73.0 ± 10	70.0 ± 18	68.0 ± 16
4.0	125.0 ± 16	132.0 ± 14	118.0 ± 15	123.0 ± 18

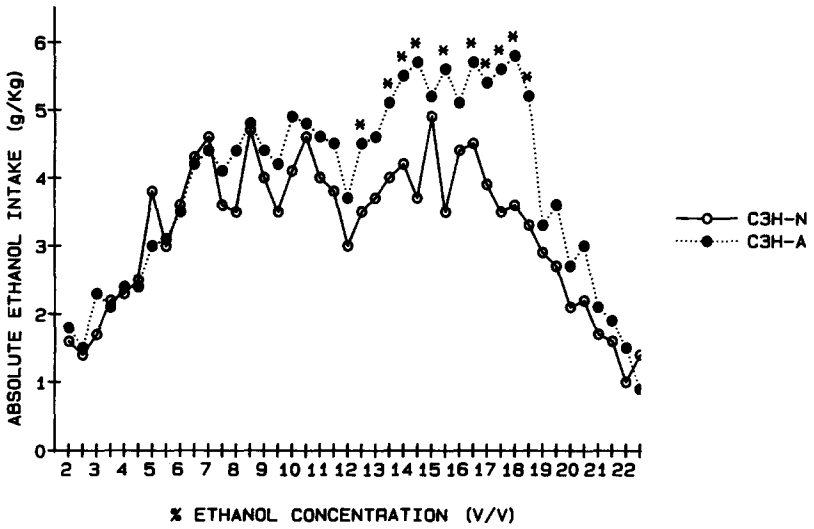

FIG. 3. Voluntary ethanol intake calculated in g ethanol/kg body weight/cage as a function of ethanol concentration for normal (C3H-N) and acatalasemic (C3H-A) mice. Each point represents mean of 2 days of exposure to one ethanol concentration (*$p < 0.05$).

ture, represented in Figs. 3 and 4. The intake of absolute ethanol in normal mice stabilized at 3.9 g/kg body weight/cage/day once animals reached the 5–18% ethanol concentration range. Acatalasemic mice consumed an average of 4.4 g/kg body weight/cage/day when ethanol solutions were presented in a range of concentrations around 6–12%. Ethanol intake rose further to 5.5 g/kg body weight/cage/day when ethanol was presented in the 14–18% concentration range to the same mice. A two-way ANOVA with repeated measures yielded a significant interaction, as well as a significant strain and days effect, $F(41, 492) = 283.07$, $p < 0.05$; $F(1, 492) = 3{,}651.09$, $p < 0.05$; $F(41, 492) = 3{,}419.21$, $p < 0.05$, respectively. Posthoc Tukey tests applied to the data revealed a significant effect between strains on 10 different 2-day blocks (see Fig. 3). No differences were found between the two substrains in total fluid intake.

A differential pattern of preference for ethanol was observed between the two substrains of mice (Fig. 4). A two-way ANOVA with repeated measures reveled a significant interaction, as well as a significant strain and days effect, $F(41, 492) = 5.825$, $p < 0.05$; $F(1, 492) = 138.93$, $p < 0.05$; $F(41, 492) = 60.95$, $p < 0.05$, respectively. Normal (C3H-N) mice

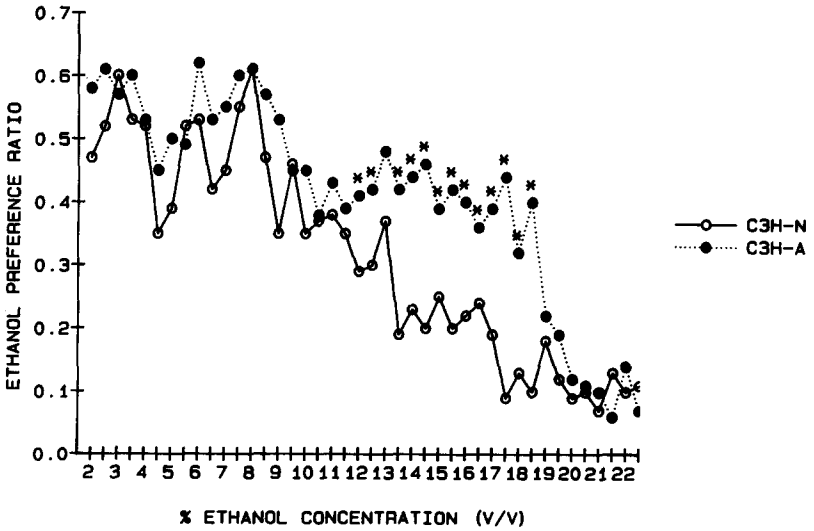

FIG. 4. Ethanol preference ratio calculated by dividing the consumption of ethanol (ml) by the total fluid consumption (ml) derived from both the ethanol and water bottles. Each dot point represents mean of 2 days of exposure to one ethanol concentration (*$p < 0.05$).

TABLE 2

DURATION OF NARCOSIS TIME INDUCED BY DIFFERENT DOSES OF ETHANOL IN NORMAL (C3H-N) AND ACATALASEMIC MICE (C3H-A) (n = 8 PER GROUP AND DOSE)

	Ethanol Dose	
Strain	3.0 g/kg IP	4.0 g/kg IP
C3H-N	0	32 ± 10*
C3H-A	16 ± 6*	76 ± 22*

Data represents mean ± SEM in minutes.
*$p < 0.01$.

maintained a relatively stable level of ethanol preference when the concentrations of ethanol solutions offered to them were between 2 and 13%. Preference for more concentrated solutions rapidly declined in these mice. Acatalasemic (C3H-A) mice, on the other hand, maintained the same level of ethanol preference (40–60% of total fluid intake) until the concentrations of ethanol solutions offered to them reached 19%. Post-hoc Tukey tests applied to the data revealed significant differences between strains when the ethanol concentrations were in the range of 12–18% (see Figure 4).

Experiment 3: Duration of Narcosis

Duration of ethanol-induced narcosis in both substrains is summarized in Table 2. Significant differences in duration of narcosis were observed between mice of the two substrains following administration of the two ethanol doses. A two-way ANOVA yielded a significant interaction, as well as a significant strain and ethanol dose effect, $F(1, 28) = 16.89$, $p < 0.001$; $F(1, 28) = 212.12$, $p < 0.001$; $F(4, 28) = 81.32$, $p < 0.001$, respectively. Acatalasemic mice revealed a significantly higher sensitivity to the narcotic effects of ethanol following both ethanol doses tested ($p < 0.01$).

Experiment 4: Catalase Activity Determinations

Mean brain catalase activity in both substrains of mice treated with either saline or AT are shown in Table 3. A two-way ANOVA revealed a significant interaction, as well as a significant strain and AT treatment effect, $F(1, 28) = 78.51$, $p < 0.001$; $F(1, 28) = 81.32$, $p < 0.001$; $F(1, 28) = 212.12$, $p < 0.001$, respectively. As can be seen in this table, differences in brain catalase activity were observed between the two mice substrains. It can also be seen that AT induced a further

TABLE 3

MEAN CATALATIC ACTIVITY OF BRAIN CATALASE OF NORMAL AND ACATALASEMIC MICE 5 h FOLLOWING ADMINISTRATION OF SALINE OR 3-AMINO-1,2,4-TRIAZOLE (n = 6)

Mouse Strain	nM O_2 Formed/min/μg protein in Brain
C3H-N + S	1.067 ± 0.050
C3H-N + AT	0.429 ± 0.029*
C3H-A + S	0.469 ± 0.017*
C3H-A + AT	0.056 ± 0.028*

*$p < 0.001$.

TABLE 4

ACETALDEHYDE RECOVERED AFTER INCUBATION OF MICE BRAIN HOMOGENATES FROM NORMAL AND ACATALASEMIC MICE WITH ETHANOL (n = 6)

	nM Acetaldehyde/mg Protein/h	
	Strain	
ETOH concentration (mM)	C3H-N	C3H-A
12.5	5.27 ± 0.11	0.22 ± 0.01
25.0	6.99 ± 0.16	0.27 ± 0.01
50.0	9.03 ± 0.16	0.34 ± 0.01
100.0	10.35 ± 0.12	0.36 ± 0.02
200.0	12.87 ± 0.17	0.55 ± 0.02

significant decrease in brain catalase activity in both substrains.

Experiment 5: Ethanol Oxidation in Brain Homogenates

Table 4 summarizes the results of acetaldehyde generated after incubation (60 min) of brain homogenates with ethanol (12.5, 25.0, 50.0, 100.0, and 200.0 mM). As can be seen from this table, pharmacologically meaningful amounts of acetaldehyde were generated after incubation of the brain homogenates with ethanol in both substrains of mice. A two-way ANOVA revealed a significant interaction and also a significant strain and ethanol concentration effect, $F(4, 50) = 128.65$, $p < 0.001$; $F(1, 50) = 5{,}853.89$, $p < 0.001$; $F(4, 50) = 151.43$, $p < 0.001$, respectively. Significant differences between the two substrains were found following incubation of tissue homogenates with all ethanol concentrations used in this experiment ($p < 0.01$). A dose–response relationship between ethanol doses and the acetaldehyde generated was observed only in the C3H-N (normals) substrain ($p < 0.01$). Negligible increases in acetaldehyde production following the increases in ethanol dose were observed in C3H-A mice ($p > 0.05$). The influence of different incubation periods on the production of acetaldehyde is presented in Table 5. A two-way ANOVA yielded a significant interaction and also a significant strain and time of incubation effect, $F(2, 30) = 202.87$, $p < 0.001$; $F(1, 30) = 1{,}316.89$, $p < 0.001$; $F(2, 30) = 207.03$, $p < 0.001$, respectively. Maximum generation of acetaldehyde was obtained after 1 h of incubation with 50 mM etha-

TABLE 5

TIME COURSE FOR THE OXIDATION OF ETHANOL (50 mM) BY BRAIN HOMOGENATES (25 mg WET TISSUE) OF NORMAL AND ACATALASEMIC MICE

	nM Acetaldehyde/mg Protein	
	Strain	
Incubation Time (min)	C3H-N	C3H-A
15	2.20 ± 0.53	0.35 ± 0.05
30	4.84 ± 0.17	0.11 ± 0.01
60	9.03 ± 0.16	0.34 ± 0.01

Reactions were carried out as described in the Method section and were initiated by the addition of ethanol (50 mM final concentration) (n = 6).

nol. However, it is important to note that shorter incubation periods yielded measurable levels of acetaldehyde in brain homogenates of normal mice (C3H-N) (see Table 5).

DISCUSSION

The results of the present series of experiments reveal marked differences between the two substrains of mice in terms of their behavioral responses following administration of ethanol. These mice also differed in terms of their catalase activity, and finally the data showed that the differences in catalase activity are probably related to the observed differences in ethanol-induced behaviors.

More specifically, the results of Experiment 1 revealed a significant difference in ethanol-induced motor activity between the two substrains. These results directly confirm earlier data reported by this laboratory (2). Normal (C3H-N) mice were found to be more sensitive to the effects of ethanol injections that resulted in higher activity scores for these animals. This difference between the two substrains was in particular obvious following administration of ethanol doses resulting in motor excitation (see Fig. 1). When catalase activity was blocked in both substrains following administration of AT, ethanol-induced motor activity was attenuated in both substrains. These observations tended to provide further support for the notion that it was the difference in catalase activity between the two substrains that underlied the observed differences in motor activity in those mice (2).

The results of Experiment 1a support the notion that the involvement of catalase activity in the mediation of behavior is specific to its interaction with ethanol. No differences in motor activity between the two substrains were observed following treatment with cocaine. These findings are in line with other reports on the lack of relationship between AT treatment and behavioral indices induced by such drugs as morphine and LiCl (4). This lack of difference in motor activity was maintained when animals were treated with both saline and AT. Both the initial lack of difference and the lack of effect of AT treatment tend to confirm the suggestion that the differences in ethanol-induced motor activity between the two substrains and its putative mediation by catalase were specific to ethanol.

A differential pattern of preference for ethanol was also observed between the two strains of mice. While normal mice rejected ethanol concentrations higher than 13%, acatalasemic mice maintained the same or almost the same level of ethanol preference until the ethanol concentrations reached the range of 19% (see Figs. 3 and 4). These results provide support for the notion that brain catalase activity exerts an influence on both the intake of and preference for ethanol. However, the direction of the effect seems to be in the opposite direction to those observed in rats with reduced catalase activity (1,7). While rats with lowered catalase activity displayed a lowered pattern of ethanol intake, the opposite was observed with these mice. Acatalasemic mice deficient in brain catalase activity revealed a higher tendency to drink ethanol. These species-specific differences between mice and rats seem to be similar to the observed differences between rats and mice in ethanol-induced motor activity (2,5,16).

Significant differences between normal and acatalasemic mice were also observed on the duration of narcosis or sleep time induced by ethanol (see Table 2). In similar fashion to the data on ethanol intake, we found that while rats with reduced levels of brain catalase activity due to treatment with AT displayed a reduction in ethanol-induced narcosis (6,29) acatalasemic mice in the present study displayed the opposite; an increase in ethanol-induced narcosis.

Given the behavioral data described above and our contention (1,2,4-7) that these behavioral responses are a function of changes in brain catalase activity, one must predict that there would be differences in brain catalase activity between the two mice substrains. Indeed, the results of Experiment 4 demonstrated differential levels of brain catalase in the two substrains. Such differences in brain catalase activity have also been reported elsewhere (2,13).

AT treatment further decreased catalase activity in both substrains. Because the inhibition of catalase by AT is H_2O_2 dependent (21,22), the present data supports the notion of the presence of H_2O_2 in the brain of this mice and the possibility of ethanol metabolism in vivo by catalase.

Experiment 5 was conducted in support of the notion that ethanol may be differentially metabolized in brains of mice from the two substrains via the peroxidatic activity of the enzyme catalase. Acetaldehyde was obtained after incubation of brain homogenates with low concentrations of ethanol similar to those found in rat brains to produce meaningful behavioral effects (18). Significant differences in the amount of generated acetaldehyde were found between the two mice strains after incubation of brain homogenates with ethanol. These data support similar data obtained in previous studies with the same mice (2). In these studies, ethanol incubation of brain homogenates of normal and acatalasemic mice treated with AT prior to sacrifice produced lower levels of acetaldehyde than those produced by normal and acatalasemic controls. A difference between both strains was also reported in the same study (2).

In conclusion, the results obtained in the present series of experiments tend to support the postulation that the putative role of the enzyme catalase in ethanol's effects may be through its ability to oxidize ethanol in the brain and that this capacity exerts at least some influence on ethanol-induced behaviors. We have shown in an in vitro experiment (Experiment 5) the capacity of catalase to oxidize ethanol in brain tissue. We further demonstrated in vivo that two substrains of mice differing in their behavioral response to ethanol also differed in their brain levels of catalase activity and, finally we reported that when brain catalase of both substrains is reduced by AT a corresponding change in ethanol-induced behavior occurred in both substrains.

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