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Sensitivity and tolerance to the motor impairing effects of moderate doses of ethanol

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Abstract

The present study assessed sensitivity and the development of tolerance to the motor impairing effects of moderate doses of ethanol, using an oscillating bar task. Adult male Wistar rats were trained for 5 consecutive days to stay on the oscillating bar for 120 s to avoid a 0.5-mA foot shock. On the 5 consecutive test days, animals were injected once a day with ethanol (ip: 1.0, 1.25, or 1.5 g/kg) and tested at 15 min intervals until recovery to the 120 s criterion. On test day 1, rats in the 1.5 g/kg group took significantly longer to recover (81 ± 9 min; mean ± S.E.M.) than did animals in the 1.25 (49 ± 9 min) and 1.0 (29 ± 5 min) g/kg groups. Tolerance developed to all doses by test day 3, with the 1.5, 1.25, and 1.0 g/kg groups reaching criterion in significantly shorter times (42 ± 8, 31 ± 5, and 18 ± 2 min, respectively), as compared to test day 1. BACs associated with recovery time on test day 3, for the 1.5 g/kg group, were significantly higher than the BACs associated with recovery time on test day 1. The data suggest that the oscillating bar task can be used to measure the acute ataxic effects of ethanol, across a narrow range of moderate ethanol doses, and, as well, the development of tolerance to the motor impairing effects of these ethanol doses.

Keywords: Alcohol; Ataxia; Sensitivity; Oscillating bar; Tolerance

The oscillating bar task was developed by Lê and Israel [6] to assess the acute effects of low dose ethanol on motor impairment. They found that, with minimal training, the motor impairing effects of a 0.5 g/kg dose of ethanol could be differentiated from that seen with a 1.0 or 1.5 g/kg dose of ethanol. In the past, relatively high doses of ethanol have been used to assess the acute and chronic effects of ethanol (c.f., Ref. [11]). For example, in rats, tolerance to hypnotic, with 5.0–8.0 g/kg pre-treatments and a 3.5 g/kg test treatment [5]; motor impairing, with 4.0 or 5.0 g/kg pre-treatments and a 3.0 g/kg test treatment [8]; and hypothermic, with 2.0–6.0 g/kg pre-treatments and a 3.0 g/kg test treatment [8] effects of ethanol have been reported.

An association between alcohol preference and the development of tolerance to alcohol's effects has been proposed [2,10,18,20]. However, the doses used in the past to examine ethanol tolerance have been relatively high, which has made it difficult to discuss the results in terms of physiological relevance. The oscillating bar task is sensitive in detecting the acute effects of low dose ethanol, to the extent that it detects the motor impairing effects of a 0.5-g/kg dose of ethanol [6]. Therefore, we hypothesized this task could not only be used to assess sensitivity to the motor impairing effects of moderate doses of ethanol, but it could also be used to assess the development of tolerance to the motor impairing effects of these moderate doses of ethanol.

1. Method

1.1. Subjects

Adult male Wistar rats (Harlan, Indianapolis, IN) weighing between 215 and 260 g at the beginning of training were used. Animals were double-housed and had free access to food and water, except during the training and testing

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sessions. The vivarium was temperature-controlled and on a 12/12 h dark/light cycle (0800–2000: light). Animals used in this experiment were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee, in accordance with the guidelines of the *Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH*, and the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996).

1.2. Test apparatus

The oscillating bar test apparatus has been described in detail elsewhere [6]. Briefly, an oscillating rectangular wooden bar (3.8 cm wide, 1.9 cm thick, and 89 cm long) was connected to a variable speed electric motor via a swing arm that allowed the bar to move in a 120° arc. The task had a passive avoidance component, in that a shock grid (0.5 mA scrambled shock) was located 42 cm below the oscillating bar. The bar and grid were inside a Plexiglas enclosure.

1.3. Procedures

Animals were handled intermittently and habituated to the test room for 3 days the week before training. The animals were then trained to remain on the oscillating bar for 120 s. Training was conducted for 5 days, with the oscillation rate being 20, 30, 40, 40, and 40 oscillation/min for days 1 through 5, respectively. Once an animal achieved the 120-s criterion, they were returned to the vivarium. Animals were allowed a maximum of six training trials each day. After training, rats were tested (40 oscillation/min) for ethanol motor impairment once daily for 5 consecutive days. Three ethanol doses were used (1.0: $n=14$, 1.25: $n=11$, and 1.5 g/kg: $n=10$) and injected ip. Ethanol (95%) was mixed with sterile saline for a 15% (v/v) concentration. On each test day, animals were allowed two baseline trials. After baselines were obtained, the animals rested for 20 min. Animals were then injected with the appropriate dose of ethanol and tested at 15-min intervals until they reached criterion (120 s).

1.4. Blood alcohol concentrations (BACs) analysis

Originally, tail bloods were taken at time of recovery on each test day. However, similar to the findings of Lumeng et al. [14], the BACs for the respective dose groups did not change appreciably across test days. For example, BACs for the 1.5 g/kg group on days 1 and 3 were (mean ± S.E.M.) 73 ± 7 mg% and 81 ± 7 mg%, respectively. Our data, and that of Lumeng et al. [14], suggest that BACs from tail blood plateau across the recovery times found in the present study (20 to 80 min). Therefore, BACs were determined by trunk blood samples in a separate group of adult male Wistar rats (270–325 g). Six animals were given a 1.5 g/kg ip injection of ethanol. Eighty-one minutes after injection (the average recovery time for the 1.5 g/kg group on the first test day), the animals were decapitated and two samples of trunk blood were taken from each animal. A second group of six rats received 1.5 g/kg ip injections of ethanol for 3 consecutive days. On the third day, the rats were decapitated 42 min after injection (the average recovery time for the 1.5 g/kg group on the third test day) and two samples of trunk blood were taken from each animal. Blood samples were collected in heparinized tubes and centrifuged in a Microfuge (Model B, Beckman: Palo Alto, CA) for 45 s. The supernatant fractions were used for determining BAC. Ethanol was measured by gas chromatography as previously described [14].

1.5. Statistical analyses

A 3×5 mixed ANOVA, with dose of ethanol as the between-subjects factor and test day as the within-subjects factor, was performed on the oscillating bar performance data. Appropriate interaction contrasts and simple effects were performed after significant interactions. A priori analyses to assess sensitivity to ethanol-induced motor impairment (simple main effects of dose for each test day) were planned during the design of the experiment. To prevent inflated alpha error for the a priori analyses, alpha was set at .01 for the simple main effects, followed by Tukey HSD post hoc comparisons. A one-way ANOVA was performed on the BAC data (one sample from the third test day group was discarded because of contamination).

2. Results

2.1. Oscillating bar performance data

The ethanol Dose × Test Day interaction was significant: $F(8,128)=4.31$, $P<.001$, with an effect size of $\eta^2=.21$ and

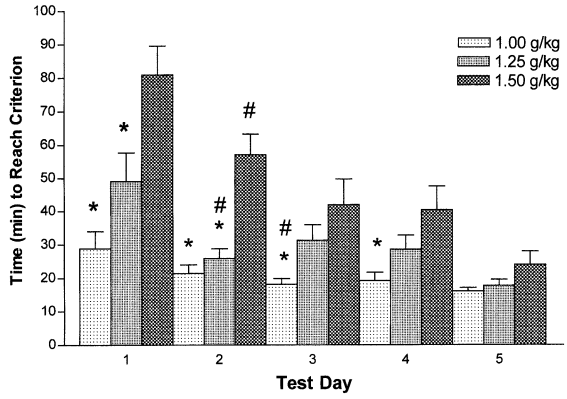

Fig. 1. Effects of ethanol dose and test day on the mean (± S.E.M.) time (min) to recover from the motor impairing effects of ethanol. * Significant ($P<.05$) differences from the 1.50 g/kg group for that test day. # Significant ($P<.05$) differences in performance between test day 1 and the respective test days. See text for statistical analyses.

a power of 0.99. As seen in Fig. 1, there is a robust dose-dependent effect on the first test day that diminishes across test days. The simple main effects of days (assessments of tolerance) for the 1.5 g/kg: $F(4,36)=17.60$, $P<.001$; 1.25 g/kg: $F(4,40)=5.03$, $P=.002$; and 1.0 g/kg: $F(4,52)=3.10$, $P=.023$ dose groups were significant. As seen in Fig. 1, there were significant decreases in recovery time on test day 2, as compared to test day 1, in both the 1.5 g/kg: $F(1,9)=18.58$, $P=.002$ and 1.25 g/kg: $F(1,10)=5.62$, $P=.039$ dose groups. The 1.0 g/kg group did not show a significant decrease in recovery time until test day 3, as compared to test day 1: $F(1,13)=5.51$, $P=.035$.

A priori analyses of sensitivity (simple main effects of ethanol dose for each test day) were performed. There were significant differences between the recovery times for the doses on test day 1: $F(2,32)=12.86$, $P<.001$, with the 1.0 g/kg group taking significantly less time to recover ($P<.05$) than both the 1.25 g/kg and the 1.5 g/kg dose groups. The 1.25 g/kg group also recovered significantly ($P<.05$) quicker than the 1.5 g/kg group. There were significant differences between the recovery times for the doses on test day 2: $F(2,32)=23.10$, $P<.001$, with both the 1.0 and 1.25 g/kg groups recovering in a significantly ($P<.05$) shorter time than the 1.5 g/kg group. There were also significant differences between the recovery times for the doses on test days 3: $F(2,32)=6.52$, $P=.004$ and 4: $F(2,32)=5.52$, $P=.009$, with only the 1.0 g/kg group recovering significantly ($P<.05$) faster than the 1.5 g/kg group on both of these test days. On test day 5, there were no significant differences between any of the treatment groups.

2.2. BAC data

Blood alcohol levels associated with the recovery time on test day 3 (161 ± 10 mg%; mean ± S.E.M.) for the 1.5 g/kg group were significantly ($F(1,21)=20.14$, $P<.001$) higher than the blood alcohol levels associated with the recovery time on test day 1 (109 ± 6 mg%), see Fig. 2. The BACs from tail blood for the 1.5 g/kg group, at time of recovery, on test days 1 and 3 were (mean ± S.E.M.) 73 ± 7 and 81 ± 7 mg%, respectively, and were not significantly different.

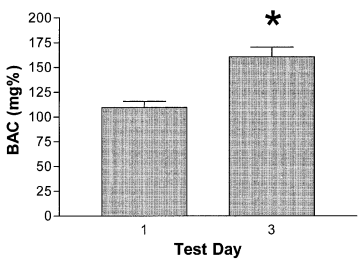

Fig. 2. Differences in trunk blood alcohol levels (mean ± S.E.M., mg%), yoked to the average recovery time, for the 1.5 g/kg dose group between test days 1 and 3. * Significant ($P<.05$) difference in levels between test days 1 and 3. See text for statistical analyses.

3. Discussion

The data indicate that the oscillating bar task is very sensitive in detecting ethanol-induced motor impairment. The task detects dose-dependent differences in ethanol's effects, even at small increments (0.25 g/kg), across a range of moderate doses of ethanol (1.0 to 1.5 g/kg). It is also noteworthy that the present paradigm can differentiate dose-dependent effects across repeated test days (i.e., in the presence of tolerance). In the original work with the oscillating bar task, Lê and Israel [6] were able to differentiate the effects of a 0.5 g/kg dose from that seen with a 1.0 or 1.5 g/kg dose. Pilot work, with the present experimental procedures, showed that initial test times earlier than 15 min did not reveal consistent ethanol-induced motor impairment. This, coupled with the fact that our experimental procedures involve more extensive training, up to 30 trials vs. 9 trials [6], may limit the lower range of ethanol doses that can be assessed with this technique. Nevertheless, the present results parallel that of Lê and Israel [6], in that the oscillating bar task can detect motor impairment induced by moderate doses of ethanol, and that dose-dependent effects are detected with both single and repeated injections.

In the present study, tolerance was demonstrated first by significantly improved performance across the test days, and secondly by the higher trunk BACs taken at the average recovery time of the 1.5 g/kg group on test day 3, compared to recovery time on test day 1. Rapid (test day 1 to test day 2) tolerance was demonstrated to the motor impairing effects of both 1.25 and 1.5 g/kg doses of ethanol. Tolerance was also shown by the 1.0 g/kg group on test day 3, compared to test day 1. The development of rapid tolerance demonstrated by the 1.25 and 1.5 g/kg dose groups, but not the 1.0 g/kg group, was probably due to the greater motor impairment seen with the 1.25 and 1.5 g/kg doses, compared to the 1.0 g/kg dose. In support of this conclusion, a dose–response relationship between the magnitude of ethanol dose on test day 1 and the amount of rapid tolerance shown on test day 2 has been reported [3].

In the past, relatively high doses of ethanol (2.0–5.0 g/kg) have been used to assess ethanol's effects in rats. With regard to motor impairment, some examples include the rotorod task [1], the tilting plane task [4,17], and the moving belt test [7,9]. In rats, rapid tolerance to the motor impairing effects of ethanol, as measured by the tilt-plane test (4.0 g/kg treatment on day 1 and 2.3 g/kg treatment on day 2), and the hypothermic effects of ethanol (4 g/kg treatment on day 1 and 2 g/kg treatment on day 2) have been reported [3]. The present paradigm detected tolerance, both chronic and rapid, following moderate doses of ethanol (1.0 to 1.5 g/kg), as opposed to the relatively large doses used in the past. Thus, the oscillating bar task can assess the development of tolerance to ethanol-induced motor impairment using more physiologically relevant ethanol doses.

Tolerance to ethanol-induced effects involves both associative and nonassociative mechanisms [11,19]. Associative tolerance is readily demonstrated by administering repeated treatments by the same method (ip injections in the present study) [19]. Associative tolerance is attributed, in part, to environmental cues that are present during both initial treatment and on subsequent test days. These cues may elicit compensatory mechanisms that decrease the treatments subsequent efficacy [16,17]. The oscillating bar task has a learning component, and the procedures are the same across the test days. Given this, an associative component is probably involved in the expression of rapid tolerance. For example, rapid tolerance to both ethanol-induced motor impairment (tilt-plane) and hypothermia has been demonstrated after either testing or sham testing (handling) on the first day, but not when testing or handling was absent on the first day [3]. Intoxicated practice also plays a role in the development of tolerance, when repeated sessions are used to test tolerance, such that the rate of tolerance development increases with increased intoxicated practice [12]. This is particularly true for tolerance to ethanol-induced motor impairment [7,13]. Therefore, it cannot be determined at this time the relative importance of associative and nonassociative factors nor the role of intoxicated practice in the development of tolerance seen in the present study.

The results indicate that the oscillating bar task is sensitive in detecting dose-dependent motor impairing effects of ethanol across a narrow range of moderate doses (1.0 to 1.5 g/kg). The ability to differentiate the level of impairment induced by different doses is retained even in the presence of tolerance. The task is also sensitive in detecting the development of tolerance to ethanol-induced motor impairment at these moderate doses. This is a potentially important advantage, in that an association between alcohol preference and the development of alcohol tolerance has been proposed [2,10,18,20]. Since rodent lines (e.g., the "P" alcohol-preferring rat) have been developed that self-administer physiologically relevant levels of ethanol [15], this task is well-suited for differentiating the expression of tolerance seen between these rats and their alcohol-avoiding (e.g., the "NP" alcohol-nonpreferring rat) counterparts at more physiologically relevant doses than previously tested.

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