

Influence of task parameters on rotarod performance and sensitivity to ethanol in mice

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Abstract:

Motor performance in mice can be assessed with multiple apparatus and protocols. Use of the rotarod (a.k.a. rotorod, rota-rod, roto-rod, or accelerod) is very common, and it is often used with the apparent assumption by the experimenters that it is a straightforward and simple assay of coordination. The rotarod is sensitive to drugs that affect motor coordination, including ethanol. However, there are few systematic data assessing the range of “normal” performance in mice. There are also few data exploring optimal task parameters (e.g. the influence of different speeds of rotation). In these experiments, we show that both accelerating and fixed-speed rotarod (FSRR) performance vary under different test protocols and conditions, and that moderate to high doses of ethanol disrupt performance. Under certain conditions, low doses of ethanol were found to enhance performance on the accelerating rotarod (ARR). Therefore, it is not possible to characterize individual differences fully using a single set of test parameters. For example, because of the biphasic effect of ethanol on performance, at least two doses of the drug are necessary to explore individual sensitivity differences. We offer recommendations of parameters we believe to be generally suitable for exploring the performance of new genotypes using the rotarod. We suggest that other putative tests of “ataxia” are similarly complex, and that characterizing the contribution of genetic differences will require similar attention to the details of task apparatus and protocols. These data also underscore the need to employ multiple behavioral assays in order to model a complex domain such as “ataxia” or “coordination.”

Keywords: Rotarod; Ethanol; Ataxia; Mice

Article:

1. Introduction

The loss of motor coordination is a common characteristic of many neurological disorders and is one of the most readily observable effects of drug intoxication. Although often associated with cerebellar dysfunction, ataxia can also be caused by impairment of motor cortex, striatum, or the spinal cord [17]; impairment of these areas can result in abnormal motor behavior. Motor coordination is a complex behavioral domain, and can reflect balance, muscle strength, and patterned gait, as well as sensory competence. Difficulties in motor performance can confound behavioral assays of learning and memory, exploration, and motivation. Therefore, ataxia is of interest to a wide range of researchers, from clinicians to experimental biologists. Many tests measure coordination, but studies of ethanol effects demonstrate that all motor tests cannot be measuring the same trait [1,3].

One of the most commonly used tests of motor incoordination is the rotarod [2]. This task was popularized by Dunham and Miya [9] to test neurological deficits in rats and mice. Their fixed-speed rotarod (FSRR) consisted of a 3 cm diameter horizontal wooden rod that rotated at a constant rate of 5 rpm. A thorough examination of important parameters on the FSRR was performed by Watzman et al. [25], who showed the importance of rod diameter and rate of rotation in studies of central nervous system depressants. Jones and Roberts [13] and Watzman and Barry III [24] developed another version of the task in which the rotation rate of the rod was accelerated over the course of the test session. According to Jones and Roberts, the development of the accelerating rotarod (ARR) eliminated the need for extensive training or the introduction of a maximal time

limit for performance. Their apparatus provided a model that started at a slow enough speed so that all mice could stay on, yet became difficult enough that all mice would eventually fall off. This apparatus also allowed for the measurement of drug-induced increases in performance, which would be difficult to observe if a low rotation rate was used in the fixed-speed model.

Today, the rotarod is still widely used in biomedical research, yet there is little consensus on which version of the task to use, or the ideal parameters and test schedules to produce optimal results. Many researchers design studies using apparatus that are unfamiliar to them, and simply adopt testing strategies used by others in the past. While often gaining useful information from these studies, few researchers know whether or not the design they adopted was appropriate or ideal for the questions that were asked. We have found different studies with mice that have used rod diameters from 3–8 cm [15,22], fixed-speed rates of 3–31 rpm [6,26], and acceleration rates of 3.5–60rpm/min [16,27]. Only a few of these studies [15,27] varied any parameters of the rotarod assay (e.g. acceleration rate, drug dose). Recently, the rotarod has been used to assess differences between wild-type and null mutant genotypes. Typically, such comparisons also employ only a single set of apparatus and test parameters [10,19,23]. It is unknown whether the conclusions about genotypic differences would generalize to other test conditions. The purpose of the present set of studies was to perform a systematic series of experiments on both the accelerating and FSRRs using an outbred stock of mice in order to determine a set of rotarod characteristics and testing protocols that would yield meaningful results that could be generalized to other experiments.

2. Materials and methods

2.1. Animals and husbandry

Male and female Withdrawal Seizure-Control (WSC-2) mice were used to test the different parameters of both the ARR and FSRR. WSC-2 mice are one replicate of a genetically segregating stock maintained in Portland, OR [5], and were originally derived from a genetically heterogeneous stock (HS/Ibg) maintained at the Institute for Behavioral Genetics (Boulder, CO). The HS/Ibg stock was itself derived by systematically intercrossing eight inbred mouse strains [18]. Mice were tested between 55–120 days of age. Approximately equal numbers of males and females were assigned to each treatment group, except where specified. All mice were bred, reared, and housed at the Veterinary Medical Unit, Department of Veterans Affairs Medical Center (Portland, OR). Same-sex groups of mice were housed 1–5 per cage on corncob bedding. The colony room was kept on a 12 h light:dark cycle with lights on at 6 a.m. Mice had access to food and water ad libitum except during experimental procedures. Testing was performed between 8 a.m. and 4 p.m. Mice were moved from the colony room to the experiment room and weighed. They were allowed to sit undisturbed for at least 30 min prior to the beginning of testing. For ARR ethanol studies, mice were tested 30 min post-injection. For these studies, mice were injected and immediately placed into individual holding cages for the 30 min wait. They were then tested on the ARR and returned to their home cage. All FSRR testing was done immediately after an injection, eliminating the need for a holding cage. Colony and experiment room temperatures were maintained at 20 ± 2 °C. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with NIH guidelines.

2.2. Drugs

Twenty percent (v/v) ethanol solutions were made by diluting 200 proof ethanol (Pharmco) with 0.9% saline. All solutions were made fresh on the day of testing. Injections were given intraperitoneally (i.p.) with volume adjusted according to weight. Control groups were given equivalent injections of 0.9% saline.

2.3. Rotarod

The AccuRotor Rota Rod (Accuscan Instruments, Columbus, OH) was used for both the ARR and FSRR tests. The apparatus was modified to have a 63 cm fall height to be consistent with our previous apparatus. Accuscan Instruments provided a 6.3 cm diameter dowel. A set of dowels with different diameters (5.1 and 7.6 cm) were obtained from Flair Plastics (Portland, OR). After receipt, dowel surfaces were covered with 320 grit wet/dry sandpaper to provide a uniform surface and to reduce slipping. Sandpaper was glued to the dowel as carefully as possible to minimize the seam, as mice can use any seam to grasp the rod and drastically increase their latency

to fall (Wahlsten et al., in press). For the ARR, four animals were placed on the stationary rod, one each in the four lanes created by Plexiglas rounds. Once all animals were on the rod, the motor was turned on and the rod rotation was continuously accelerated at a rate of 15–60 rpm/min, depending on the experiment (see each experiment separately). The maximum speed the ARR could reach was 99.9 rpm, but no mouse achieved this speed. The FSRR rotated at 3–20 rpm, depending on the experiment. Mice were placed on the rod while it was moving, except at 15 and 20 rpm. At these higher speeds, mice were placed on the static rod, and then the rod was accelerated at 40 rpm/min from zero to either 15 or 20 rpm and held constant at that rate.

2.4. Statistics

Systat (Chicago, IL) version 10 was used for all statistical analyses. Data were analyzed using ANOVA. Between-groups or between-groups with repeated measures comparisons were used where appropriate. When significant interactions were present, follow-up analyses were performed by separate one- or two-way ANOVAs between variables. Differences were considered significant at $P < 0.05$.

2.5. ARR

2.5.1. Experiment 1: ARR, training and saline injection

WSC mice were tested on a 6.3 cm diameter rod to determine the amount of training necessary to achieve stable performance on the ARR, to see whether saline injection would alter performance, and to see whether three or five trials on a second test day were sufficient to reestablish peak performance. Male and female mice were divided into three groups ($N = 12$ – 15 /group). Each mouse in a set of four was given 10 consecutive trials on the rotarod accelerating at 20 rpm/min, with 30 s rest between trials after the last mouse fell. The following day, mice received either 10 more consecutive trials (Group 1), five trials followed by a saline injection, 30 min delay, and five more trials (Group 2), or three trials followed by a saline injection, 30min delay, and five more trials (Group 3). Previous work in our lab had used three pre-injection trials to establish baseline performance. Group 3 was included to compare results from our previous method with those from the other groups.

2.5.2. Experiment 2: ARR, repeated testing

WSC mice were tested on the 6.3 cm diameter rod accelerating at 20 rpm/min to determine the stability of performance after repeated testing with saline and 2.5 g/kg EtOH. This study allowed assessment of test–retest reliability of performance. We hoped that it would indicate that expensive inbred strains could be tested repeatedly without carryover effects if a sufficiently long test–retest interval was used. Separate groups of mice ($N = 12$ /group) were trained with 10 consecutive trials with 30 s rest between trials. The following day, both groups were given three baseline trials followed by an injection of saline. Thirty min later, mice were tested three more times. Mice were tested in an identical manner before and after a saline injection two more times at either 72- (Group 1) or 96 (Group 2)-h intervals. Two weeks later, the same mice were tested for performance before and after 2.5 g/kg EtOH. Mice were given five baseline trials followed by an injection of EtOH. Thirty minutes later, mice were given three more tests. Mice were tested two more times before and after EtOH at either 48- (Group 1) or 72 (Group 2)-h intervals.

2.5.3. Experiment 3: ARR, dowel size and acceleration rate

The WSC mice tested in Experiment 2 were retested to examine the effects of different dowel sizes and acceleration rate on post-EtOH performance. Mice were re-assigned to one of three acceleration rate groups ($N = 8$ /group). Groups received either 15, 20, or 25 rpm/min during testing. All mice were tested on a 5.1, 6.3, and 7.6 cm diameter dowel consecutively, with 72 h between tests. For each test, all mice were given five baseline trials before an i.p. injection of 2.5 g/kg EtOH. Thirty minutes later, all mice were given three more tests. A more thorough examination of acceleration rate was performed 48 h after the last test. For this, mice were re-assigned to dose (1.5 or 2.5 g/kg) and dowel size groups (6.3 or 7.6 cm diameter). At 2.5 g/kg, separate groups of mice were tested on each dowel size. At 1.5 g/kg, mice were only tested on the 6.3 cm diameter dowel. All mice ($N = 8$ /group) were given five baseline trials, one each at 20, 30, 40, 50, and 60 rpm/min consecutively. After the last trial, each mouse was injected with EtOH. Thirty min later, all mice were tested once at 20, 30, 40, 50, and 60 rpm/min consecutively.

2.6. FSRR

2.6.1. Experiment 4: FSRR, criterion test length

The FSRR differs from the ARR in that not all mice necessarily fail the task. This requires that a criterion latency to fall be established, beyond which it is assumed that the mouse could continue to perform indefinitely. To use the FSRR for comparing genotypes or treatment groups, it would be necessary to be sure that the criterion adopted was well within the capability of all mice of all strains when undrugged. All tests on the FSRR were done using the 6.3 cm diameter dowel. Naive male and female WSC mice were divided into four groups ($N = 10/\text{group}$) to investigate the effect of length of criterion test on post-EtOH FSRR performance. All mice were given three practice trials at 3 rpm with a maximum time on the rod of 30 s. If mice stayed on for 30 s, they were taken off and placed in the holding area under the rod for a 30 s ITI. Following the practice trials, mice were given criterion tests at either 3 or 10 rpm (depending on the group). Mice in each rpm group were given criterion test lengths of 3 or 10 min and were said to have passed a criterion test if they walked on the rod without falling for the length of the criterion test. Once a mouse passed three consecutive criterion tests (or was given a maximum of six tests), it was injected with 2 g/kg EtOH and immediately placed on the rod rotating at its assigned speed (3 or 10 rpm). Latency to fall from the rod was recorded.

2.6.2. Experiment 5: FSRR, effect of rpm

The effect of rotation speed was tested in four groups of male and female WSC mice ($N = 12\text{--}13/\text{group}$). Mice were given three practice trials at 3 rpm with a 30 s ITI. Following the practice trials, mice were given criterion tests at 3 (Group 1), 5 (Group 2), or 10 (Group 3) rpm (Group 4 was not tested on this day). Mice were considered to have passed a criterion test if they were able to stay on the rod for 3 min. As soon as mice had passed two consecutive criterion tests (or had been given a maximum of nine tests) they were injected with 2 g/kg EtOH and immediately placed back on the rod rotating at the speed they were given on the criterion tests. Latency to fall from the rod was recorded. Seventy-two hours later, all four groups were given three practice trials (30s each, 3 rpm) and tested at either 10, 15, or 20 rpm. Group 1 was tested at 15 rpm, Group 2 at 20 rpm, and Group 3 again at 10 rpm. Group 4 was tested at 10 rpm to control for the repeated testing in Group 3. As soon as mice passed two consecutive criterion tests (or a maximum of nine tests), mice were injected with 2 g/kg and immediately tested at the assigned rpm. Latency to fall from the rod was recorded.

2.6.3. Experiment 6: FSRR, ethanol dose

WSC mice were divided into six groups ($N = 8/\text{group}$) to study the effects of ethanol dose on FSRR performance. Due to a lack of male mice, only female mice were tested. Mice were given three practice trials at 3 rpm (30 s maximum, 30 s ITI) before being given criterion tests at 3 or 10 rpm. Mice were tested until they were able to stay on for 3 min, with a maximum of six tests. Immediately after passing the single criterion test, mice were injected with either 2.0, 2.5, or 3.0 g/kg EtOH and placed back on the rod rotating at 3 or 10 rpm. Latency to fall from the rod was recorded.

3. Results

3.1. Experiment 1: ARR, training and saline injection

Two-way ANOVA (group \times trial) on Day 1 latency to fall showed a significant effect of trial ($F(9, 306) = 56.59, P < 0.001$), suggesting a strong learning component to ARR performance (Fig. 1). The groups did not differ in acquisition on Day 1, and there was no significant group \times trial interaction ($F_s < 1.22, \text{ns}$). We performed a three-way ANOVA (group \times trial \times day) comparing the last five trials on Days 1 and 2. This analysis revealed no effect of group, but significant effects of day ($F(1, 36) = 20.51, P < 0.001$) and trial ($F(4, 144) = 3.02, P < 0.03$). Overall, mice were performing better on Day 2 compared to Day 1 and were improving over trials. There was no effect of group or any significant interactions ($F_s < 1.28, \text{ns}$). To examine the effects of training and injection on ARR performance among groups, we used two-way ANOVA (group \times trial) to analyze the last five trials on Day 2. This analysis revealed no significant effect of group or group \times trial interaction ($F_s < 0.82, \text{ns}$), suggesting there was no effect of saline injection on the last five trials on the rotarod, or any differences across baseline treatments. Finding no effect of trial ($F < 1.43, \text{ns}$) shows that mice were not improving their performance significantly over these last trials, suggesting stable performance.

Because the initial training schedule of 10 massed trials on Day 1 produced stable performance on Day 2, it was used to train mice in all other ARR experiments.

Averaging Trials 2 and 3 on Day 2 showed that all three groups demonstrated significant savings in rotarod performance. Dividing the average of Trials 2 and 3 on Day 2 by the average of Trials 8–10 on Day 1 for each mouse showed that, on average mice had 99.8% savings of performance of Day 1. This also suggested that using three baseline trials for drug testing 24h after training would allow drug responses to be referred to performance similar to that at the end of training. For the group that received 10 consecutive trials on both days, test–retest reliability was assessed by Pearson correlation. This was done by using the average of Trials 8–10 on both days of testing. This analysis demonstrated a significant test–retest reliability of $r = 0.61$ ($P < 0.03$).

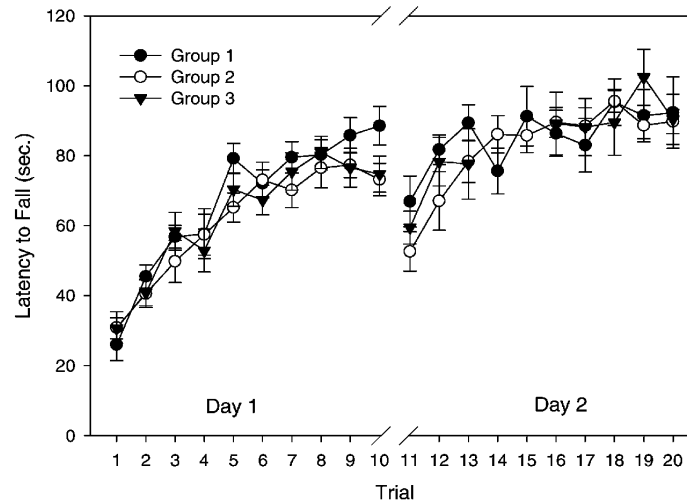


Fig. 1. Acquisition and retention of accelerating rotarod (ARR) performance. All mice received 10 consecutive trials on a 6.3 cm diameter dowel on Day 1. On Day 2, treatment was as follows: Group (1) 10 consecutive trials, Group (2) five trials, saline injection, 30 min delay, and five more trials, and Group (3) three trials, saline injection, 30 min delay, and five more trials. Values represent means \pm S.E.M. for $N = 12$ –15/group.

3.2. Experiment 2: ARR, repeated testing

Acquisition performance (10 massed trials) did not differ among groups ($F < 1.8$, ns, data not shown). Baseline performance was calculated by averaging the last two pre-injection values for each animal. Post-saline performance was calculated by taking the average of all three post-saline trials. A three-way ANOVA (group \times day \times treatment) was performed on mice repeatedly tested with saline. This analysis revealed only a significant main effect of treatment ($F(1, 23) = 56.52$, $P < 0.001$), showing that the saline injection significantly increased performance in both groups across all 3 days of testing (Fig. 2A). No effect of day ($F < 2.01$, ns) suggested that mice tested repeatedly with saline every 72 or 96 h showed stable baseline and post-saline performance across days. To illustrate the improved performance after the saline injection more clearly, Fig. 2B expresses the data as a change from baseline performance. Two-way ANOVA (group \times day) of these data again found no significant effect of group, day, or their interaction ($F < 0.98$, ns) suggesting that mice can be tested repeatedly.

A three-way ANOVA (group \times day \times treatment) was performed to look for differences between groups in baseline and post-EtOH performance across days. For this part of the study, mice were given five baseline trials to make sure baseline performance was not underestimated, as could be suggested by the increase in performance after saline in Fig. 2. This analysis revealed a main effect of day ($F(2, 44) = 7.04$, $P < 0.01$), showing that mice improved with repeated tests, and a main effect of treatment ($F(1, 22) = 73.78$, $P < 0.001$), demonstrating that mice performed significantly worse after EtOH compared to baseline (Fig. 3A). Groups treated 48 or 72h apart did not differ in performance before or after EtOH, or across tests ($F_s < 2.31$, ns). The increase in performance across days might suggest the development of tolerance to this dose of EtOH. However, a parallel increase in baseline performance was also seen, evidenced by the lack of a day \times treatment interaction. When the data were analyzed as a change from baseline score, there was no effect of group, day, or their interaction ($F_s < 1.13$, ns, Fig. 3B). This argues against development of any tolerance to EtOH in either group tested 48 or 72h apart.

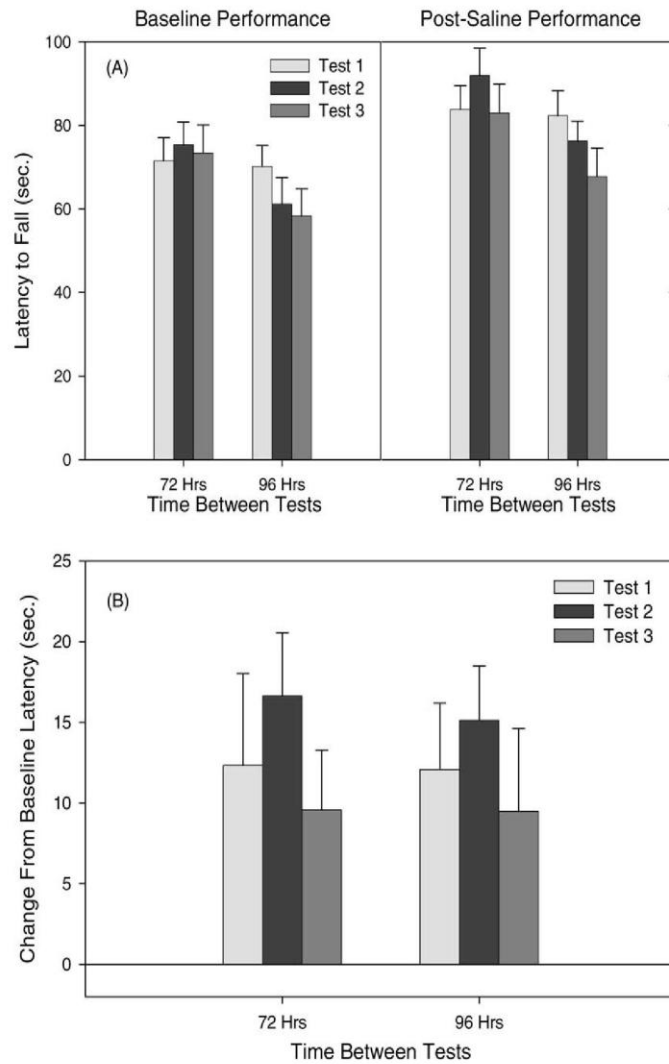


Fig. 2. Repeated testing after saline on the accelerating rotarod (ARR). (A) Latency to fall at baseline and post-saline over 3 days of testing. Test days were separated by 72 or 96 h depending on the group. (B) Improvement over baseline performance after saline on the 3 days of testing. Values represent means \pm S.E.M. for $N = 12/\text{group}$.

3.3. Experiment 3: ARR, dowel size and acceleration rate

Three-way ANOVA (rpm \times dowel size \times treatment) on latency to fall revealed a main effect of rpm ($F(2, 21) = 4.89, P < 0.02$) with faster acceleration rates leading to shorter latency to fall (Fig. 4A). There was also a main effect of treatment ($F(1, 21) = 138.59, P < 0.001$) showing that EtOH significantly decreased the latency to fall compared to baseline performance, and a rpm \times treatment interaction ($F(2, 21) = 4.91, P < 0.02$). The interaction suggested that treatment with 2.5 g/kg EtOH suppressed the effect of rpm on latency to fall. That is, after EtOH, mice fell off at the same time regardless of the acceleration rate. Further, there was a significant effect of dowel size ($F(2, 42) = 18.14, P < 0.001$) and a dowel size \times treatment interaction ($F(2, 42) = 13.29, P < 0.001$). There was no significant rpm \times dowel size or rpm \times dowel size \times treatment interaction ($F_s < 1.27, \text{ns}$). Examining the effect of dowel size at baseline and post-EtOH showed that after 2.5 g/kg EtOH, there was no effect of dowel size. Mice had the same latency to fall regardless of the diameter of the dowel. At baseline, however, individual one-way ANOVAs showed that mice tested on the 5.1 cm diameter dowel had significantly higher latency to fall than mice tested on either the 6.3 or 7.6 cm diameter dowel regardless of the acceleration rate at which they were tested ($F_s > 22.88, P_s < 0.001$). Mice tested on the 6.3 and 7.6 cm diameter dowel did not differ in their latency to fall. This increased latency to fall on the smallest diameter dowel tested is likely due to the fact that most of the mice tested at this size were able to cling to the dowel and rotate all the way around the rod at least one time. Eighteen of the 24 mice tested on the 5.1 cm diameter rod demonstrated this behavior. Of the 120 total baseline trials (24 mice \times 5 trials each) 31 (26%) resulted in mice passively rotating

on the rod. At the other sizes this behavior occurred on no more than 3% of the trials. After EtOH, no mouse exhibited this passive rotational behavior.

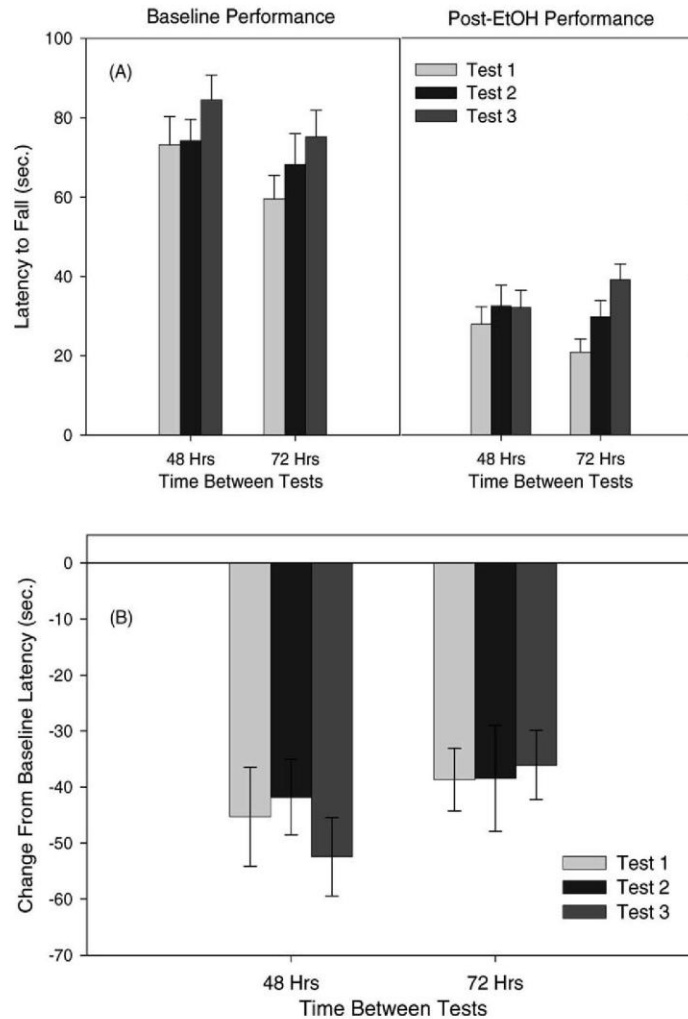


Fig. 3. Repeated testing after 2.5 g/kg EtOH on the accelerating rotarod (ARR). (A) Latency to fall at baseline and post-EtOH over 3 days of testing. Mice in the 72 and 96 h group in Fig. 2 were retested for their post-EtOH performance at 48 and 72 h intervals, respectively. (B) Change from baseline performance 30 min after 2.5 g/kg EtOH over the 3 days of testing. Values represent means \pm S.E.M. for $N = 12$ /group.

To adjust for the differences in baseline ability between the groups caused by effects of dowel size and rpm, the data were also analyzed as a change from baseline latency to fall after EtOH (Fig. 4B). Two-way ANOVA (rpm \times dowel size) revealed main effects of both rpm ($F(2, 21) = 4.91$, $P < 0.02$) and dowel size ($F(2, 42) = 13.29$, $P < 0.001$), showing a bigger decrease from baseline with slower acceleration rate and smaller dowel size.

To examine the role of rpm more thoroughly, we tested 20–60 rpm/min acceleration rates (Fig. 5). Mice that were tested on the 6.3 cm diameter dowel received either 1.5 or 2.5 g/kg EtOH after baseline testing. A three-way ANOVA (dose \times rpm \times treatment) for latency to fall revealed a main effect of rpm ($F(4, 48) = 16.95$, $P < 0.001$), with mice falling off sooner at higher rpm. There were also significant dose \times treatment ($F(1, 12) = 23.54$, $P < 0.001$) and dose \times rpm \times treatment ($F(4, 48) = 5.53$, $P < 0.01$) interactions.

The three-way interaction was examined further by two-way ANOVA (dose \times rpm) on baseline performance. This analysis found only a main effect of rpm ($F(4, 56) = 8.87$, $P < 0.001$), with mice falling off sooner with increasing acceleration rates (Fig. 5A). When the post-EtOH data were expressed as a change from baseline, it showed that the 1.5 g/kg group actually had enhanced performance that was most pronounced at the lowest acceleration rate (Fig. 5B). ANOVA (dose \times rpm) of the change from baseline data revealed a main effect of dose ($F(1, 12) = 23.16$, $P < 0.001$) and a dose \times rpm interaction ($F(4, 48) = 5.53$, $P < 0.01$), showing that the differences in performance were greatest at the lower acceleration rates, but were suppressed at higher rates.

Individual one-way ANOVAs revealed significant effects of dose at each rate; however, effect sizes were greater at 20 and 30rpm/min ($F_s > 14.6$, $P_s < 0.01$) than at 40, 50, or 60 rpm/min ($F_s > 5.26$, $P_s < 0.04$).

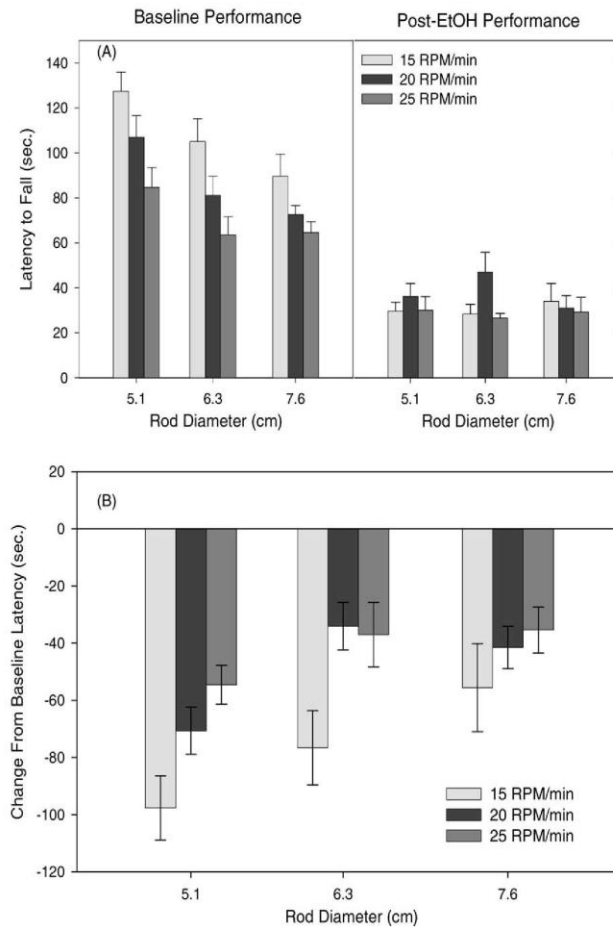


Fig. 4. Effect of rod diameter and acceleration rate on accelerating rotarod (ARR) performance. (A) Baseline and post-EtOH latency to fall at each acceleration rate and rod size. At baseline, 26% of mice tested on the 5.1 cm diameter rod were able to cling and passively rotate on the rod (Section 3.3). (B) Change from baseline latency to fall at each acceleration rate and rod size. Mice in Figs. 2 and 3 were re-randomized to acceleration rate group and retested for this experiment ($N = 8/\text{group}$). Values represent means \pm S.E.M.

A separate ANOVA (rpm \times dowel size \times treatment) was done comparing mice given 2.5 g/kg and tested on the 6.3 or 7.6 cm diameter dowel. This showed only a main effect of rpm ($F(4,56) = 8.28$, $P < 0.001$) and treatment ($F(1, 14) = 15.00$, $P < 0.001$), again with mice falling off sooner at the higher acceleration rates and after EtOH. There was no effect of dowel size or any interactions with dowel size ($F_s < 3.51$, ns), suggesting that mice tested on 6.3 or 7.6 cm diameter dowels performed similarly before and after EtOH. When expressed as a change from baseline score, there were also no significant differences between the groups. These results suggest that testing with a 6.3 cm diameter dowel allows detection of effects of both acceleration rate and EtOH dose. Importantly, it is also large enough to prevent mice from passively rotating on the rod. These experiments also illustrate the importance of using multiple doses of EtOH in order to gain a better understanding of the potential biphasic effects of EtOH on this test.

Although mice were repeatedly tested in Experiments 2 and 3, there were no differences in body weight among groups or across experiments (Experiment 2: 25.4 ± 0.83 g, Experiment 3: 25.7 ± 0.89 g). Further, there were no significant correlations of performance either before or after EtOH with body weight (data not shown).

3.4. Experiment 4: FSRR, criterion test length

The latency to fall immediately after a 2.0 g/kg EtOH injection is shown in Fig. 6. Two-way ANOVA (rpm \times criterion time) revealed only a main effect of rpm on latency to fall ($F(1, 34) = 9.27$, $P < 0.01$). Mice that were tested at 3 rpm had significantly longer latencies to fall than mice tested at 10 rpm. The length of the criterion test had no effect. That is, mice that were given a 3 min criterion test performed as well after EtOH as those

given a 10 min criterion test. This suggested to us that a 3 min criterion test on the FSRR is sufficient training to allow detection of an effect of EtOH.

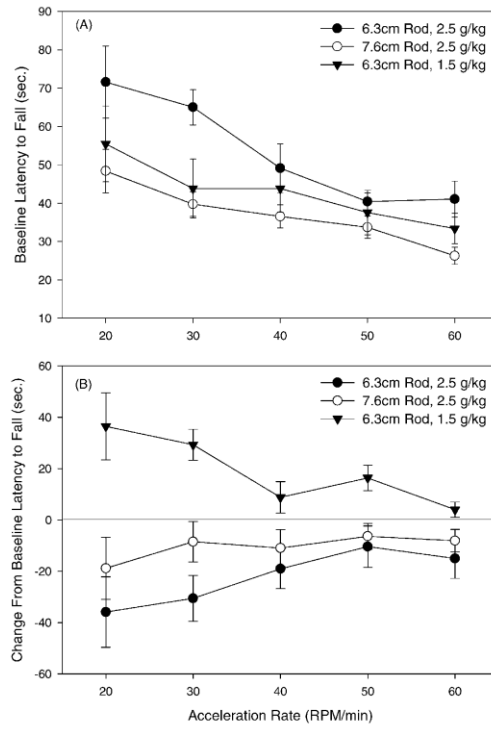


Fig. 5. Effect of acceleration rate and EtOH dose on accelerating rotarod (ARR) performance. (A) Baseline latency to fall for mice repeatedly tested at 20, 30, 40, 50, and 60 rpm/min. Mice from Figs. 2-4 were re-randomized to dose and dowel size groups ($N = 8/\text{group}$), and were retested for this experiment. (B) Change from baseline latency to fall 30 min after 1.5 or 2.5 g/kg EtOH. Values represent means \pm S.E.M.

3.5. Experiment 5: FSRR, effect of rpm

The effect of rpm on Day 1 performance showed decreasing latency to fall with increasing rpm ($F(2, 23) = 5.20$, $P < 0.02$) (Fig. 7A). Bonferroni post hoc analysis showed that mice tested at 10 rpm (Group 3) had a shorter latency to fall than did mice tested at 3 rpm ($P < 0.02$). Seventy-two hours later, higher rpms were tested. Analysis of latency to fall data showed no effect group on this day (Fig. 7B), suggesting that 10 rpm is the fastest rotation speed one needs to test to examine effects of rpm. None of our future studies used rpm > 10 . This analysis also showed that prior testing at the same rpm 3 days earlier (Group 3) failed to increase performance compared to the group that was tested for the first time at that rpm (Group 4), suggesting that repeated testing at the same speed is possible.

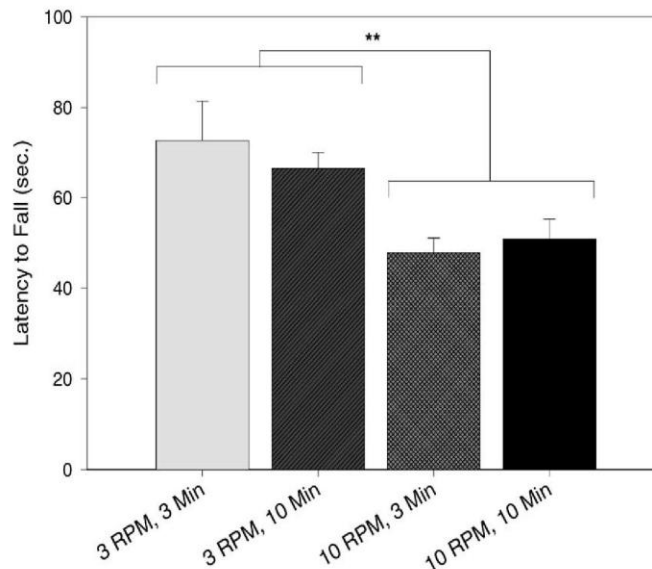


Fig. 6. Effect of criterion test length on FSRR performance after EtOH. Mice were given criterion test lengths of 3 or 10 min at either 3 or 10 rpm. Criterion test length had no effect on post-EtOH performance. Mice tested at 3 rpm fell at significantly higher latencies immediately after 2.0 g/kg than mice tested at 10 rpm. Values represent means \pm S.E.M. for $N = 10/\text{group}$. $**P < 0.01$.

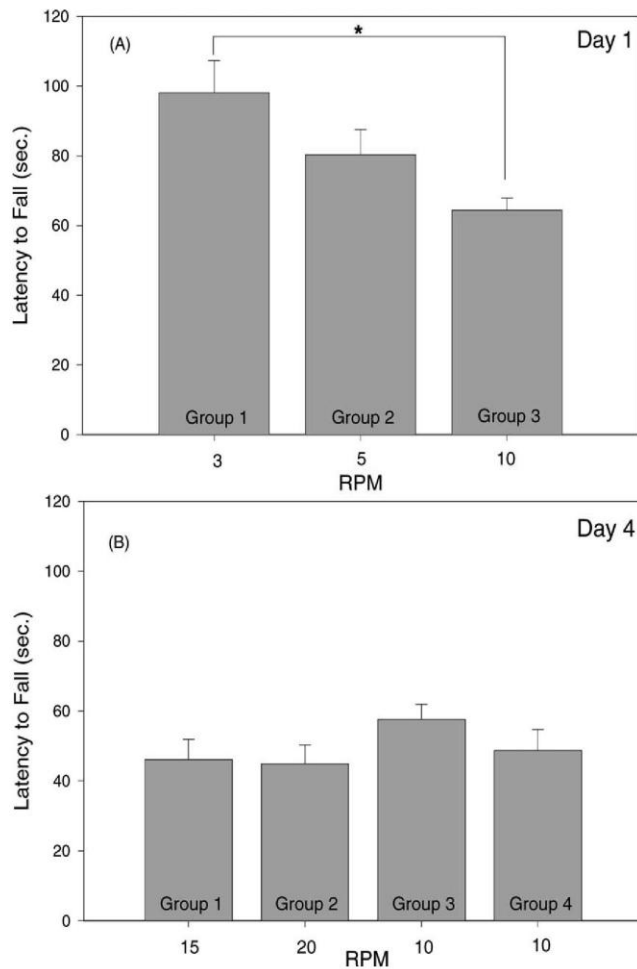


Fig. 7. Effect of rpm on FSRR performance. (A) Faster rpm lead to significantly shorter latencies to fall immediately after 2.0 g/kg EtOH. * $P < 0.05$. (B) The rpms higher than 10 did not decrease latency to fall immediately after 2.0 g/kg EtOH. Group 4 was only tested on Day 4 to control for repeated testing in Group 3. Values represent means \pm S.E.M. for $N = 12\text{--}13/\text{group}$.

3.6. Experiment 6: FSRR, ethanol dose

Two-way ANOVA (rpm \times dose) was used to examine effects of rpm and dose on latency to fall (Fig. 8). Mice tested at 10 rpm fell with significantly shorter latencies than those tested at 3 rpm ($F(1, 40) = 39.01$, $P < 0.001$). There was no significant effect of EtOH dose; however, mice treated with higher doses tended to show a decreased latency compared to those treated with 2.0 g/kg ($F(2, 40) = 2.97$, $P = 0.06$).

4. Discussion

Results from the ARR experiments showed that this apparatus can reflect substantial learning during initial trials, demonstrated by the significant increase in performance across training trials (Fig. 1). This is in contrast to one of the original reports on the ARR [13], which found stable performance across trials without pre-training. This is likely due to the fact that Jones and Roberts tested mice very infrequently—12 times over the course of 3 months. If testing is more frequent than this, we expect substantial carry-over, or savings, of performance as we saw (Fig. 1). In our studies, once stable performance was reached with pre-training, group basal ability remained remarkably consistent over repeated test sessions (see Figs. 2 and 3). This is an important consideration if one is repeatedly testing mice on the rotarod. Without pre-training until a plateau of performance is reached, repeated testing could result in longer latencies to fall which are due to learning, rather than the result of a drug treatment or other intervention. The pre-training regimen we used produced performance levels high enough to detect decreases in performance after sedative drugs. Interestingly, however, pre-training did not necessarily produce maximal performance, as a motor stimulant dose of ethanol (1.5 g/kg) served to increase performance in comparison to baseline (Fig. 5B).

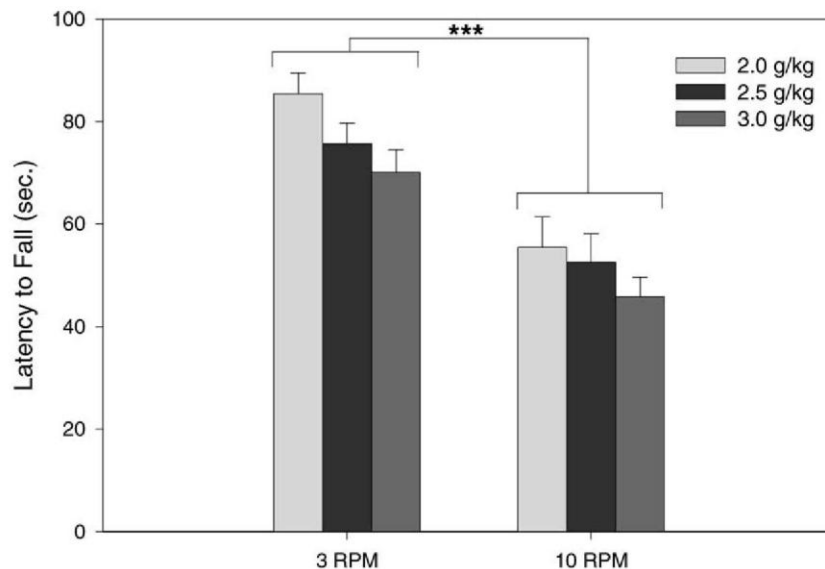


Fig. 8. EtOH dose effects on FSRR performance. Mice tested at 10 rpm had significantly shorter latencies to fall immediately after EtOH than mice tested at 3 rpm. *** $P < 0.001$. There was a trend for higher doses to decrease latency to fall ($P = 0.06$). Values represent means \pm S.E.M. for $N = 8$ /group.

Motor learning is thought to be controlled by complex interactions between the supplementary motor area, pre-frontal parietal cortex, basal ganglia, and cerebellum. Early in motor learning, attention to the motor task is required; activation of frontoparietal cortex and associative areas of the basal ganglia and cerebellum is seen in human studies during motor learning [14]. After practice, motor performance becomes a more automatic, unconscious behavior, controlled by motor cortex and motor divisions of the basal ganglia and cerebellum [11]. While the genetically heterogeneous mice we tested showed no change in performance across days, some inbred or transgenic mice may demonstrate increased performance with repeated testing. This is an important consideration if repeated testing with other mice is planned. Given the neurocircuitry involved in motor learning and performance, one may expect to see differences in learning and performance on the rotarod among a set of inbred strains. In fact, when we tested eight inbred strains on the ARR, we found differences in acquisition, peak performance, and carry-over performance among the strains (unpublished data). These results suggest that there may be differences in the underlying structure and/or function of the brain regions involved in rotarod performance among inbred strains.

In our analysis of different rod sizes, we found that two larger sizes did not produce different results. We do not know what the effect on performance would be for rod diameters >7.6 cm. However, the 5.1 cm diameter rod allowed a significant proportion of mice to cling to the rod and passively rotate at least once around the rod. We argue that, while quite possibly being another measure of coordination (or muscle strength), this is a different trait, and should not be confounded with a mouse's ability to balance and walk on top of the apparatus. Some studies have noted the existence of this passive rotation, and have analyzed it separately from balance and walking performance [12,22,25]. When we used dowels with 6.3 cm diameter or greater, the proportion of mice passively rotating was negligible for all practical purposes. This allowed us to analyze our data without the confound of passive rotation. Many of the current commercially available "mouse-sized" rotarod dowels are much ≤ 5.1 cm diameter. If dowels are to be purchased from one of these vendors, we recommend purchasing the "rat-sized" dowel (typically larger than 6.3 cm diameter) to eliminate the occurrence of passive rotation.

We also found large effects of acceleration rates on rotarod performance. As expected, faster acceleration rates led to decreased latencies to fall (Figs. 4 and 5). At the narrower range of rates tested (15–25 rpm/min), rates affected baseline latency to fall, but not post-EtOH latencies. This is likely due to the dose of EtOH we used (2.5 g/kg), which is well within the sedative range of doses on this apparatus. Thus, the lack of an effect of rpm on post-EtOH performance may be due to a floor effect. In the rpm-response study using higher rates, acceleration rate clearly affected fall times in the group given 1.5 g/kg (Fig. 5). One of the most important findings is the dependence of EtOH dose-response characteristics on acceleration rate. There was a clear

difference in performance of mice given 1.5 versus 2.5 g/kg at 20 rpm/min: 1.5 g/kg enhanced, and 2.5 g/kg disrupted performance. However, when acceleration rates reached 60 rpm/min, these dose differences were suppressed. Had we tested mice only at this fastest acceleration rate, we would have underestimated the effect of EtOH on ARR ability. Similarly, had we only tested 2.5 g/kg, we would have underestimated the effect of acceleration rate on post-EtOH performance, and we would have missed the fact that lower doses can improve performance at low acceleration rates.

In light of the previous results, we highly recommend testing multiple drug doses when using the rotarod. Many depressants, such as EtOH [8,20], barbiturates [8], benzodiazepines [4], and some glutamate antagonists [7,21], can have biphasic locomotor effects, producing stimulation at low doses and sedation at higher doses. In order to have a clear understanding of an individual mouse's (or genotype's) sensitivity, multiple doses need to be tested. In addition, we recommend using slower acceleration rates in order to avoid suppression of potential differences between groups. Twenty and 30 rpm/min were the most sensitive for detecting differences between dose groups in our experiments.

The results obtained on the FSRR support many of our findings on the ARR. We used the 6.3 cm diameter rod for all FSRR experiments and found that this diameter also prevented mice from being able to hold on and passively rotate at a fixed speed. The rpm had the largest effect on FSRR performance, with higher rpm leading to decreased latency to fall after EtOH (Figs. 6, 7 and 8). This effect of rpm was only significant up to 10 rpm, however. Rotation rates faster than 10 rpm did not significantly affect post-EtOH performance (Fig. 7). These results suggest that 10 rpm is the highest needed to measure performance immediately after a drug injection. Ethanol dose appeared to have an effect on FSRR performance, but it failed to reach significance (Fig. 8). Had more doses been tested on the FSRR, it is likely that a significant effect of EtOH dose would have been achieved. Although we did not test doses <2 g/kg, it is not clear from our studies whether lower doses would have impaired mice on the FSRR. If the blood ethanol levels do not reach intoxicating levels in the 3 min immediately following injection (the time period during which mice are tested on the FSRR), no impairment would be detected. The findings regarding EtOH probably pertain to any drug that is rapidly absorbed into the brain. However, different results may have been obtained if animals were tested later, after the absorbed drug had achieved stable distribution. It was clear that 30 min after 1.5 g/kg, mice were not impaired on the ARR (Fig. 5). If this were true on the FSRR, all mice may have been able to stay on for the full 3 min. At the doses tested, it did not appear that EtOH dose interacted with rpm. However, had we tested doses that did not impair mice at 3 rpm, these might have been effective at disrupting performance at the higher, more difficult rpm.

We believe it is likely that the current results will generalize to many mouse genotypes and intoxicating agents. Certainly, there will be genotypes that will not be testable on the rotarod due to their propensity to jump from the rod instead of running on top. Also, there are likely certain mutant mice with severe ataxia (e.g. some cerebellar mutants) which will not be able to perform on the rotarod. Perhaps testing these on other less demanding tests of ataxia (e.g. gait analysis) may be more appropriate for these exceptional mice.

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