Calibration of rotational acceleration for the rotarod test of rodent motor coordination

By: Martin Bohlen, Andy Cameron, Pamela Metten, John C. Crabbe, Douglas Wahlsten

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Abstract:
The latency of mice and rats to fall from the accelerating rotarod can differ markedly between laboratories using the same brand of rod as well as between studies using different kinds of rods. These discrepancies can arise from different rod diameters, surface textures, test protocols, or laboratory environmental factors beyond the test itself, but it is also possible that the actual acceleration rates of the different rods do not correspond to the nominal rates set on the devices. This paper describes a simple method to measure acceleration rate of the rotarod and to set the rate to a desired value for any brand of rod.
Keywords: Behavioral test, Motor coordination, Task parameters, Test standardization, Mouse, Rat, Rotarod

Article:

1. Introduction

The accelerating rotarod (Fig. 1A), where a rotating rod or drum functions as a treadmill for the rodent placed atop, is widely used to assess drug and genetic effects on motor coordination in rodents (Fig.1 B). Recently, when our two laboratories compared data for 20 inbred strains with ostensibly identical rotarods and test protocols, there was a noteworthy difference in mean fall latencies over 10 training trials (Fig. 1C). Such differences could arise from the laboratory environments extraneous to the test situation itself (Crabbe et al., 1999; Kafkafi et al., 2005; Lewejohann et al., 2006; Mandillo et al., 2008), but we could not rule out the possibility that the rotarods were actually accelerating at different rates in different labs or years, despite the identical parameter settings on the equipment. We have also noted substantial differences in fall latencies among published reports using the same inbred strains of mice but different commercial sources of rotarod. Here we present a method for determining the actual acceleration rate and describe a way to set any rod to the desired rate without the need for sophisticated test equipment.

Dunham and Miya (1957) first described the “rolling rotor” as a tool for measuring neurological deficits in rodents, adapting a kymograph drive to turn a rod at a fixed speed. Watzman et al. (1967) found that fall latency varied with several parameters, including rotation rate, but it was inconvenient to run separate tests at different speeds for the same animal. This problem was remedied by an accelerating version devised by Jones and Roberts (1968a,b). Studies on inbred strains of mice have provided empirical evidence that the latency between start and fall times is related to the rodent’s motor coordination, balance, and motor learning abilities (Chapillon et al., 1998; Crawley, 1999; Rustay et al., 2003a,b). In recent years, the rotarod has been used to investigate differences among inbred strains (McFadyen et al., 2003; Brooks et al., 2004; Bothe et al., 2005; Schneider et al., 2006), gene knockout and trans-genic mice (Crawley, 1999; Bolivar et al., 2000), effects of drugs (Karl et al., 2003; Monville et al., 2006; Bellum et al., 2007), recovery from brain injuries (Riess et al., 2007), and animal models for human disease (Carter et al., 1999; Van Raamsdonk et al., 2005). This shows the versatility of the tool, but diverse studies reveal many differences in the choice of rod diameter and rod surfaces—ranging from a ribbed rubber center rod to wood covered with sand paper. Several manufacturers offer rotarods of varying design (see Table 15-4 in Wahlsten and Crabbe, 2007). Nominal rates of acceleration as set on these devices range widely in the published literature.

As with any kind of aging apparatus, wear on parts of the drive mechanism can cause departures from desired results. The extent to which actual accelerations correspond to settings on the apparatus is not apparent in any publication we have read, including our own. Both the fixed speed and accelerating versions of the rotarod are now commonly employed. Calibration of the fixed speed of rotation is a very simple task, whereas determining rate of acceleration warrants some explanation.

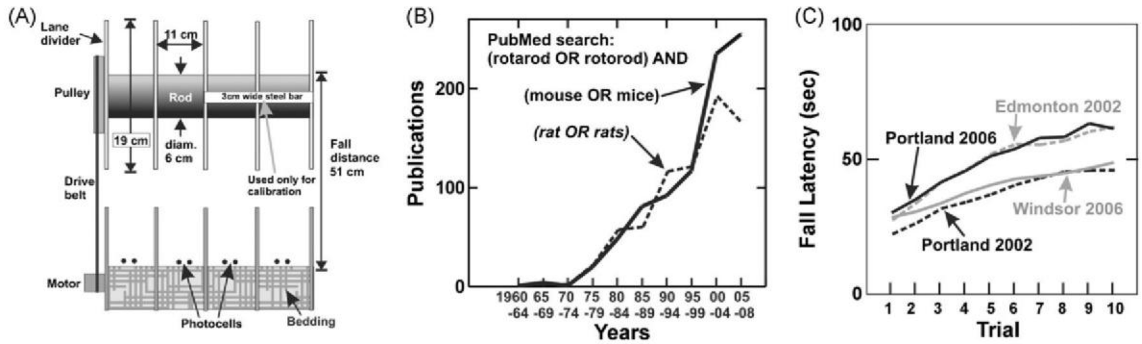

Fig. 1. (A) Diagram of AccuRotor rotarod from Accuscan Instruments Inc. that was used for measurements. Mouse remains atop the rotating rod until it falls into a trough of bedding and breaks photocell beams to stop a timer. A 3 cm wide steel bar was attached to the disks to provide a target for the ultrasonic beam device used here for calibration. (B) Number of publications determined from PubMed using title and abstract search terms "rotarod OR rotorod" and either "mouse OR mice" or "rat OR rats" in five-year periods. (C) Mean fall latencies for 20 or 21 inbred strains of mice over 10 trials in three different laboratories in two years. Equal numbers of males and females were tested in the 2006 study, but the sex difference and strain by sex interactions for fall latency were not significant ($P > 0.05$), despite a large sex difference in body weight ($F = 794.8$, df = 1/585, $P < 0.00001$). Complete data for 21 strains are published in Rustay et al. (2003a). Data from Portland are from the lab of J.C. Crabbe, while those from Edmonton and Windsor are from the lab of D. Wahlsten.

Fig. 2A portrays a smoothly accelerating disk where two points near the rim (A and B) are reached in times t_A and t_B after the disk has rotated by angular displacements θ_A and θ_B. The speed of rotation (ω) gradually increases when accelerated at rate α. Units of θ, ω, α are sometimes expressed in radians, radians/s and radians/s^2, respectively. When the initial displacement is θ_0 and initial speed is ω_0, the speed after t s is $\omega_t = \omega_0 + \alpha t$, and displacement is $\theta_t = \theta_0 + \omega_0 t + (1/2)\alpha t^2$. In the typical accelerating rotarod test, the rod begins at rest, so that $\omega_0 = 0$ and $\theta_0 = 0$. For point A, $\theta_A = (\alpha/2)t_A^2$, and $t_A = \text{sqrt}(2\theta_A/\alpha)$. For the case of one complete revolution of the disk, $\theta_A = 2\pi$ radians and time for K complete revolutions is shown in Eq. (1) for radians and α in radians/s2. If instead θ_A is given in revolutions and α in revolutions per min or RPM/min, then time for K revolutions is shown in Eq. (2).

$$t_k = 2\text{sqrt}(K\pi/\alpha) \quad \text{for radians and s} \qquad (1)$$

$$t_k = 60\text{sqrt}(2K/\alpha) \quad \text{for revolutions and min} \qquad (2)$$

The actual rate of acceleration can be estimated from times to reach two successive points A and then B along the rim from the relation $\theta_B - \theta_A = (\alpha/2)(t_B^2 - t_A^2) = (\alpha/2)\,(t_B + t_A)\,(t_B - t_A)$. Thus, $\alpha = 2(\theta_B - \theta_A)/[(t_B + t_A)(t_B - t_A)]$. For the first complete revolution, $\theta_B - \theta_A = 2\pi$, $t_A = 0$ and $t_B = T_1$, the revolution time; thus, $\alpha = 4\pi/T_1^2$. For the Kth revolution where the total elapsed time is ΣT and time for the Kth revolution is T_K, acceleration rate is given in Eq. (3) for times in s and radians/s^2, and Eq. (4) applies for times in min and RPM/min. The times for successive rotations can therefore be used to estimate acceleration rates and the variation in acceleration from one rotation to the next.

$$\alpha = 4\pi/[(2\,\Sigma T\text{-}T_K)T_K] \quad \text{for radians / s}^2 \qquad (3)$$

$$\alpha = 7200/[(2\,\Sigma T\text{-}T_K)T_K] \quad \text{for RPM /min} \qquad (4)$$

2. Materials and methods

2.1. Rotarod

The AccuRotor Rota Rod (Accuscan Instruments, Inc., Columbus, OH) model RRF/SP was used for all tests (Fig. 1A) in both labs. The OHSU lab had two rods whereas at UNCG there was one rod purchased in a different year than the OHSU rods. Acceleration was determined by setting the time to reach a maximum speed of 99.9 RPM, which for constant acceleration of 20 RPM/min is 5 min. Times for the first 10 rotations were determined by three different methods at UNCG, each repeated for at least 5 trials. At OHSU the times were determined by stopwatch for 10 trials.

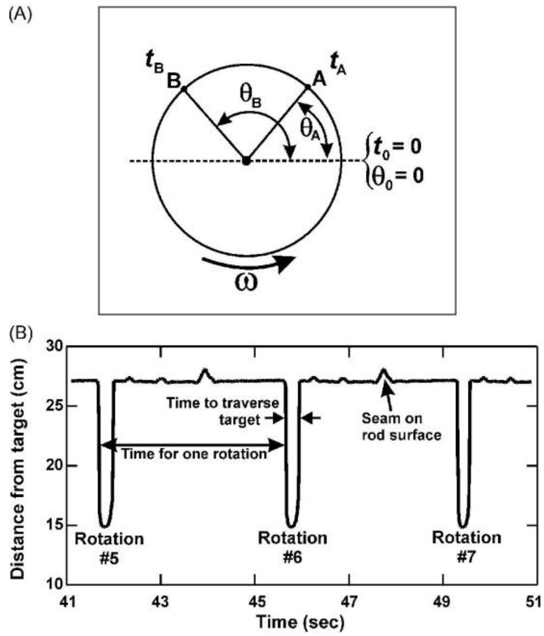

Fig. 2. (A) Diagram of rotating rod (end view) giving definition of symbols for time (t), angular displacement (θ) and velocity (ω) used in the text. (B) Record from three actual rotations of a rotarod, showing output from the Vernier motion detector 2 in terms of distance of an object from the transponder. One value is plotted every 0.05 s. The large transition when the ultrasonic beam encounters the steel bar (Fig. 1A) occurs in less than 0.05 s. The detector also indicates irregularities on the surface of the rod.

2.2. Ultrasonic motion detector

The first method used Vernier motion detector 2 technology and Logger *Pro* 3 software (Beaverton, OR). The motion detector emits ultrasonic pulses (50KHz) to measure distance based on the time taken for the pulse to reflect off an object and back to the device. Because 50 KHz is within the hearing range of many mice, this device is not suitable for work with live animals. Logger *Pro* 3 software presents the data numerically and graphically (Fig. 2B). The length of data collection was 70 s and sampling rate was 20 samples/s or 0.05 s/sample. The motion detector was placed 25–30 cm away from the rotarod and aimed at the center dowel. A 3 cm wide stainless steel ruler (Fig. 1A) was attached to the outer edge of the disks to provide a detectable difference in distance from the center dowel; as it rotated, the detected change in distance allowed for an accurate measure of the time taken for each rotation. In order to establish the rotarod's starting position, the motion detector and rotarod were turned on, and at the first indication of a change in distance (seen graphically in the Logger *Pro* 3 software), the rotarod was stopped and the motion detector turned off. The test was started by turning on the rotarod and hitting the "*start*" key in Logger *Pro* 3 to begin ultrasonic sampling. Data from Logger *Pro* 3 were then exported to Excel. The experimenter examined the raw data to determine the "*time on*" and "*time off*" points for each complete rotation, which were plainly evident in a distance change greater than 10 cm in 0.05 s (Fig. 2B). Rotation times were calculated in Excel.

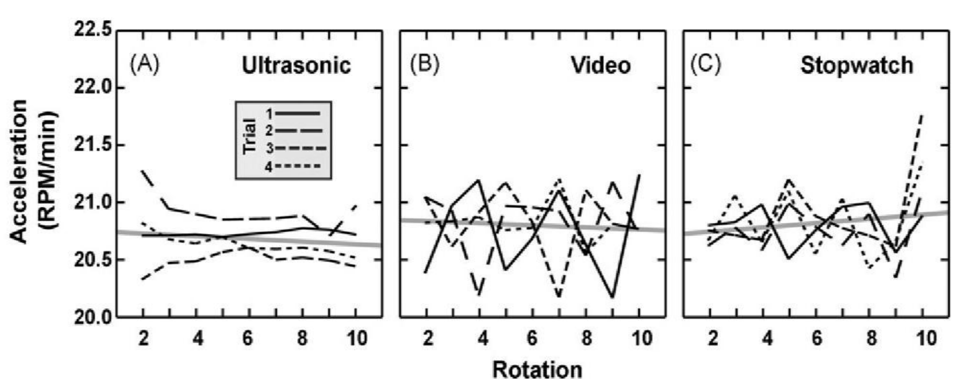

Fig. 3. Acceleration rates (α) in successive rotations of the rod on 4 trials using three methods. (A) is Vernier motion detector 2, (B) is AnyMaze video tracking, (C) is manual Nike lap timer. Heavy gray line is linear regression line computed across all trials, which indicates no appreciable upward or downward trend in acceleration rates for the AccuRotor device.

2.3. Video tracking

The second method used video tracking with two cameras (Any-Maze; Stoelting Co., Wood Dale, IL, USA). The first was aimed at the disk of the rotarod while the other was aimed at the timer on the front of the rotarod. Within AnyMaze, the test was set to run for 70 s with the highest allotted sampling rate of 30 frames/s. A black binder clip on the disk at one end of the rotarod was used as the target and a zone was made slightly larger than the binder clip. The record function within AnyMaze was used to save video records as they were captured live. The test was run by placing the 3 cm wide binder clip in the start zone, turning on the record function, and then starting the rotarod. After the initial data collection, the experimenter performed frame-by-frame video analysis to determine when the binder clip was closest to the center of the displayed zone. For each rotation that time was recorded from the video record of the display timer.

2.4. Stopwatch tracking

The third method used a stopwatch with a lap timer (Nike Oregon Series at UNCG, VWR Programmable model, NIST traceable and certified, at OHSU). The rotarod was started at the same time as the watch and, as a marker passed a fixed point, the experimenter pushed the lap button.

3. Results

3.1. UNCG rod/data

The first rotation time for each run was surprisingly variable, partly because of difficulty synchronizing the start of the timers and the rotarod. Additionally, there may have been some slippage of the drive belt as the apparatus, having considerable moment of inertia, first began to move. Consequently, calculations were based on the second through tenth rotations, using Eq. (4). While all three methods gave very similar results, the ultrasonic motion detector yielded the most consistent data from one rotation to the next (Fig. 3A). The video and stopwatch methods both resulted in more variability in recorded rotation times (Fig. 3B and C). The stopwatch method (Fig. 3C) became particularly inaccurate at higher rotation numbers when the experimenter encountered difficulty judging the precise moment when the marker passed the fixed point.

It happens that behavior on the very first trial on the accelerating rotarod, before there has been an opportunity for learning, is quite different from later trials. In large data sets from our laboratories, the average correlation of trial 1 fall latency on day 1 was only $r = 0.16$ with the first three trials on day 2, whereas the correlations between trials 2 and 3 on day 1 and the first three trials on day 2 were $r = 0.34$ and 0.40, respectively. Mouse behavior on trial 1 is not unusual because of mechanical imperfections in the rod itself or imprecise settings of acceleration rate, however. Our data (Figs. 3 and 4) indicate no substantial change in acceleration rate over a series of contiguous trials. Three studies of the same eight inbred strains were done in the D.W. lab at the University of Windsor from 2005 to 2007, and fall latencies did not differ significantly among them ($P = 0.14$; data not shown). Within a lab, it appears that acceleration rate is reasonably stable over trials and across studies when no major event intervenes.

Average rates of rotational acceleration from one rotation to the next over all trials were very similar for the ultrasonic motion detector (mean = 20.67, S.D. = 0.18), video recording (mean = 20.78, S.D. = 0.39), and stopwatch (mean = 20.8 1, S.D. = 0.29). There was a clear difference between the intended acceleration of the rotarod (20.0 RPM/min), as set by maximum speed and time to maximum, and the observed mean over several trials using a two-tailed *t*-test ($p < 0.0001$). Although the simple stopwatch method is not as stable and accurate as the ultrasonic motion detector, it yields data that are sufficient for most purposes and can be implemented in any laboratory.

3.2. OHSU rods/data

Acceleration rates determined from stopwatch data at 20 RPM/min were very similar for the two rotarods used there (Fig. 4A and 4B), whereas they yielded mean values ($\alpha = 20.13$ and 20.17 for rods 1 and 2, respectively) that were closer to the nominal 20.0 than the UNCG rod ($\alpha = 20.82$, Fig. 4C). For the rods at OHSU, 10 trials were also run at 5, 10, 15, 25 and 30 RPM/min. The rods were not able to operate at all at 5 RPM/min. At 10 RPM/min, rod #1 yielded elapsed times very close to theoretical values in Table 1, whereas rod #2 accelerated more rapidly than the setting (Fig. 5). At 15 RPM/min, Rod #2 was slightly faster than expected from theory, and at all higher accelerations the two rods were virtually the same and agreed closely with theory.

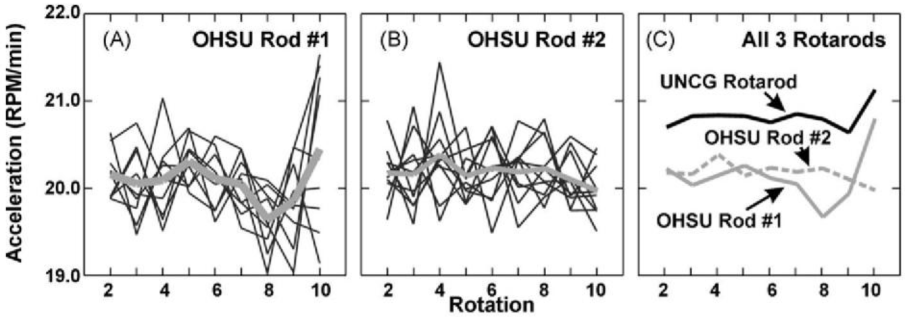

Fig. 4. Acceleration rates for three different rotarods on 9 trials determined with a stopwatch. (A) Rod #1 at OHSU. Fine lines are for individual trials and heavy gray line is average of the 10 trials. (B) Rod #2 at OHSU. (C) Average rates for 9 rotations of the three rods. Values for later rotations tend to fluctuate substantially because of variation in human reaction times when the rod is turning more rapidly.

Table 1
Elapsed times in s to complete different cumulative numbers of revolutions of the rod under different accelerations (α) expressed in RPM/min.

Revolutions	$\alpha = 5$	$\alpha = 10$	$\alpha = 15$	$\alpha = 20$	$\alpha = 25$	$\alpha = 30$
1	37.95	26.83	21.91	18.97	16.97	15.49
2	53.66	37.95	30.98	26.83	24.00	21.91
3	65.73	46.48	37.95	32.86	29.39	26.83
4	75.89	53.67	43.82	37.95	33.94	30.98
5	84.85	60.00	48.99	42.43	37.95	34.64
6	92.95	65.73	53.67	46.48	41.57	37.95
7	100.40	70.99	57.97	50.20	44.90	40.99
8	107.33	75.89	61.97	53.67	48.00	43.82
9	113.84	80.50	65.73	56.92	50.91	46.48
10	120.00	84.85	69.28	60.00	53.67	48.99

Besides documenting how great the deviation is between nominal and actual acceleration rates, the stopwatch method can be used to set a commercial rotarod to give the desired rate using Table 1, which displays theoretical rotation times for a variety of settings. The experimenter can run a series of trials with different settings on the device and continue the process until the desired rotation times are achieved. As indicated in Figs. 4 and 5, this needs to be done independently for each particular rod in a laboratory. Settings on one carefully calibrated rod cannot be copied to another rod from the same manufacturer to obtain identical acceleration rates.

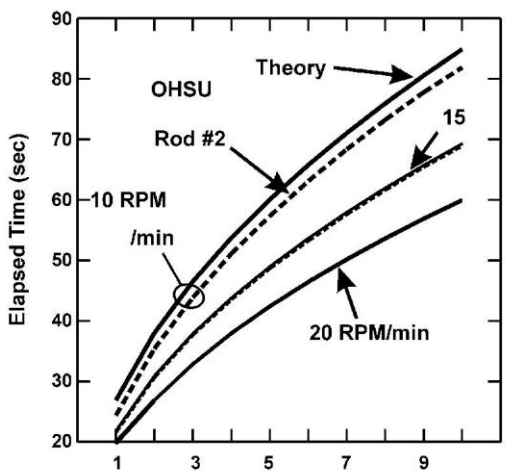

Fig. 5. Elapsed times for 10 successive rotations determined from theory (Table 1) and the stopwatch method at OHSU averaged over 10 trials. A clear discrepancy was seen for rod #2 at 10 RPM/min but the deviation from theory was very small at 15 RPM/min and negligible at all higher accelerations.

4. Discussion

These measurements reveal discrepancies between machine settings and actual acceleration rates at both labs, but the difference was very small at OHSU except at lower acceleration rates. The UNCG rod rate was almost one full RPM/min higher than the setting on the device. This deviation of about 5% from the nominal acceleration is considerably smaller than the difference between labs in both 2002 and 2006, however. There is no guarantee that the rate measured in 2008 was the same as in earlier studies, especially for the UNCG rod that experienced two long moves in the interim. It may be comforting to learn that acceleration rates did not shift appreciably when the apparatus remained in the same place in one lab. Unfortunately, we do not know precisely what the rate was for those convergent results. If the rate deviated from the nominal setting in one year, it was probably imprecise the next year as well.

In future studies, it would be good practice to adjust any rod so that the actual acceleration rate agrees closely with the desired rate before data collection commences. Then one could conduct a periodic check of rotation times during the course of a study to ensure the setting remains stable. Because a drive mechanism that uses a flexible belt may be influenced by environmental factors such as room temperature, it may be wise to check acceleration rate whenever there is a major change in local conditions.

In this study we only tested acceleration for full rotations. Smoothness of acceleration within a rotation could be assessed accurately with photocells (Bishop et al., 1996). Jerky acceleration might have an influence on latency to fall from the rod. The very smooth results for the ultrasonic detector (Fig. 3A) suggest that fluctuations from one rotation to the next seen in Figs. 3C, 4A and B arose from human judgments and reaction times, not jerky motion of the rod itself.

Perhaps the most important threat to validity of rotarod data is the occurrence of passive rotations that occur when the mouse can firmly grasp the rod instead of running on it (Wahlsten et al., 2003). These vexatious behaviors are influenced strongly by task parameters (Rustay et al., 2003b) and can be greatly reduced or even eliminated in mice by using a larger 6 cm diameter rod covered with fine (320 grit) sandpaper, provided there is no substantial seam joining the ends of the paper where mice can insert their claws. If this behavior cannot be eliminated by altering the task, the experimenter may decide to stop the trial after two passive rotations and perhaps exclude the animal from the study if this behavior persists.

In our extensive data on mice, we have never observed a sex difference or a body weight influence on accelerating rotarod performance among non-obese inbred mice. The mass of a mouse is small relative to the size of the mechanical apparatus of a rotarod, but rats are at least 10 times larger and could exert a considerable torque on the rod if they hold onto it and rotate passively, which in turn might induce slippage in the rod drive belt. Thus, body size may contribute to differences among labs that work with rats on the rotarod and it could interact with task parameters that affect passive rotations.

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