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

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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.

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. 1B). 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 transgenic 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 rota-

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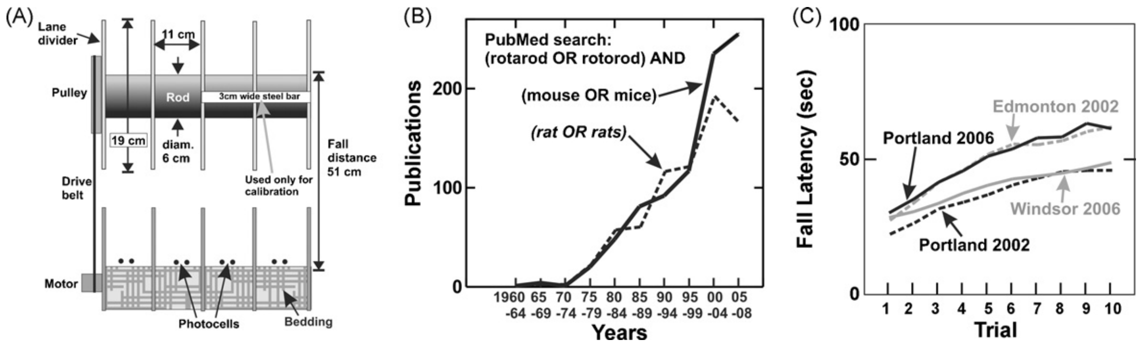

Fig. 1. (A) Diagram of AccuRotor rotarod from Accuscan Instruments Inc. that was used for measurements. Mouse remains atop the rotating rod until it falls into a trough of bedding and breaks photocell beams to stop a timer. A 3 cm wide steel bar was attached to the disks to provide a target for the ultrasonic 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.

tion is a very simple task, whereas determining rate of acceleration warrants some explanation.

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

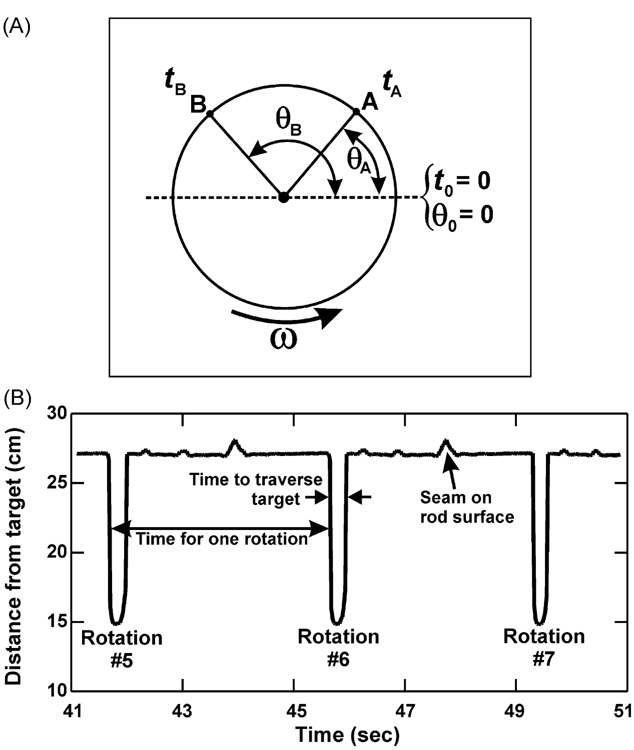

Fig. 2. (A) Diagram of rotating rod (end view) giving definition of symbols for time (t), angular displacement (θ) and velocity (ω) used in the text. (B) Record from three actual rotations of a rotarod, showing output from the Vernier motion detector 2 in terms of distance of an object from the transponder. 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.

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).

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

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

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.

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

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

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.

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 (50 KHz) 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).