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RAT ACCELEROD TRAINING PROCEDURES

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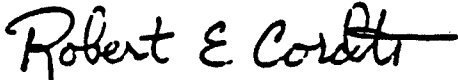
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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

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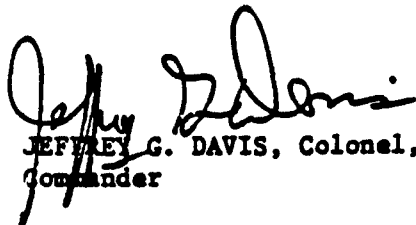
This report has been reviewed and is approved for publication.



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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Many researchers use rodents performing on a rotating rod to evaluate motor deficits from various physical or chemical agents. At the School of Aerospace Medicine, we have been evaluating radiation and anticholinesterase effects in combination on rats ability to remain on an accelerating rod. However, success in training animals has hovered below 60% and performance scores were too low to realize full advantages from the test. This paper outlines a new training method now in use employing a commercially available instrument using shock avoidance as motivation. In 2 sessions animals were trained to run at least 15 s with average training times reaching 27.4 s. This training was accomplished with a success rate of 99.8%. However, the standard deviation was large and must be planned for during experimentation.					
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RAT ACCELEROD TRAINING PROCEDURES

INTRODUCTION

In the military, several requirements exist for testing the ability of a subject to perform with relatively fine motor coordination. On the battlefield, personnel may be exposed to many types of radiation or chemical insults. Testing is also required when chemicals are proposed as prophylactic or treatment modalities. To maintain balance on a rotationally accelerating rod has been shown to demonstrate motor coordination. The process has, in fact, been used in comparing effects from different radiation sources as well as different chemicals (1, 2).

Researchers at the USAF School of Aerospace Medicine have been investigating performance decrement using rodents on a rotating rod (3). However, only 55% of animals learned the task adequately to be included for testing. Even then, generally low training scores (times) and experiment scores limited interpretive value of the data obtained. We have felt that the test was a good one, but that it was necessary to improve the percentage of trained animals and their demonstrated ability prior to testing. After receiving a standardized instrument to evaluate this type of performance, we have improved training procedures to yield nearly 100% animals trained to perform at least 15 s.

TRAINING PROCEDURES

Male Sprague-Dawley rats, weighing 200 ± 25 g, were kept 4 or 5 to a cage in filtered laminar air flow conditions. Food and water were available ad libitum. Tails were marked with indelible ink to separate animals so that individual records could be kept throughout training and experimentation.

Animals were trained on Columbus Instruments ROTAMEX V "EE". This instrument has a 7-cm-(2.8 in.) diameter rod divided into four 8.75-cm (3.5 in.) lengths by 11.5-cm (4.6 in.) high acrylic wafers. Each compartment has a door which adequately confines the rat while allowing safe rotation of the rod. The floor of each compartment, which is 12-cm (4.8 in.) below the rod, is a series of stainless steel rods capable of being electrified. When the rod was turned on, all activated timers started. When an animal jumped or fell to its grid floor, that compartment's timer stopped. The unit was made to rotate toward the operator so each subject was placed in the unit oriented away from the operator and in the direction it would have to walk. The rod was calibrated to accelerate to 116 rpm in 120 s from a standing start. Training was conducted with only 1 animal at a time. Approximately 50 rats were trained and fed in a session.

First day training was conducted after noon. The most important function of the first day was for the rat to realize the rod was safe from shock. Shock was used with a duration of 0.2 s and was started at the setting of 4.5 which was adequate to cause about 60% of the animals to move their forefeet off the grid without jumping or vocalizing. A shock-naïve rat was presented to the

apparatus so it would step up, out of the operator's hand, onto the rod. The chamber door was closed. Usually the animal quickly jumped off, in which case it was immediately given 4 rapid pulses of shock (operator manipulated) and as quickly as possible was placed back on the rod. The process was repeated every time the animal jumped off the rod. After a third attempt with little apparent impression from shock on the rat, the shock level was advanced about 1 unit. Occasionally an animal would become very excited and essentially jump over the rod to the grid. In that case, the animal was held gently by the tail and restrained from jumping until it calmed down because more shock was pointless. Each rat was required to stay on the rod 5 s or more (up to 10 s if it had become very excited) before proceeding to the next step. Records were kept of the number of times a rat jumped or fell from the rod at each step.

After the rat stayed calmly on the rod 5 s or more, the motor was turned on. Frequently, as the rod began to rotate, the rat would jump down. In that case the animal was also given 4 rapid pulses of shock and placed back on the rod. Animals had sufficient room to turn around on the rod. The rod was not activated if an animal was incorrectly oriented, but the rod was not stopped if the animal turned after it had been activated. On this first day, when each rat stayed on the moving rod 10 s or more, it was returned to its holding cage following that trial. In fact, an animal only had to move about 2 steps to stay on 10 s.

Training was continued the following morning. During this session, even a slow learning animal was required to walk several steps because the minimum criterion was raised to 15 s. Relatively the same procedure was followed. A rat was positioned so it would get on the rod. The animal had to stay on the stationary rod at least 5 s before the rod was activated. Most rats stayed on the stationary rod on the second day (Table 1). However, as Table 1 depicts, animals required essentially the same amount of reinforcement to stay on the moving rod to meet this new, minimum criterion. Only 1 of 438 rats subjected to this training failed to meet the minimum criterion of staying on the accelerating rod for 15 s.

TABLE 1. TRAINING SCORES

	First day of training		Second day of training	
	Average	Range	Average	Range
Returns to stationary rod	2.65	0-7	0.31	0-4
Returns to activated rod	1.11	0-7	1.07	0-8
Qualifying time on rod (seconds)	20.4	10-51	27.4	15-56

The time score by which each animal passed training on the second day was used to group animals. Forty-eight animals were needed daily (12 groups of 4

each). The total training scores of each group of 4 animals was within 12 s of the other 11 groups which were put together from that training session. By the end of the experiment (at least 16 animals in each of 24 groups) the greatest divergence of average training scores of a group compared to their control group was 2.3 s (9%).

Animals which were eliminated were more frequently left out because they jumped off several times before reaching the minimum criterion. Minimal qualifying scores was the next most common cause for elimination.

The morning following the second training day was the experimental day. Two accelerod trials in immediate succession were given each subject. Each animal was run individually, just as it had been trained. Prior to any testing it was decided that any animal would be allowed 1 and only 1 jump from the rod during experimentation. The rod was activated very quickly after each animal was quietly on the rod. Each animal's allowed jump could occur before 5 s elapsed after activation of the rod. Animals most decremented by the drugs of this experiment could generally stay on at least 5 s.

DISCUSSION

The accelerod, in the configuration listed (from 0 to 116 rpm in 120 s) did not perceptibly move in the first second. The first movements were somewhat jerky during the next 2 s. After that the acceleration was continual and smooth. Even at that the movement was less than one-fourth of a revolution during the first 5 s and about one-half revolution by 10 s.

During the first day of training rats had to walk just a couple of steps to stay on the rod for 10 s. As shown in Table 1, however, it is evident most rats walked well over 10 s. By the second day all knew how to walk on the rod and most did very well.

By the same token, animals which left the rod prior to 5 s rarely appeared to do so because they couldn't handle the pace of the rod. Instead, 2 processes were apparent as on experiment day animals jumped early:

a. Animals were not fully trained and were still searching for alternatives to walking on the rod; Bogo et al.(2) reported at least 6 training sessions in their method.

b. Occasionally an animal would overreact to the first movements of the rod and would lurch and lose its balance. These 2 features resulted in about 25 animals (6%) being replaced on the rod 1 time without recording a score which was less than 5 s. Bogo et al.(2) had allowed a "false start" fall for up to 10 s during their testing with a constant speed rod.

In a pilot study of training, giving the animals an added training session had resulted in markedly reduced experimental scores. Groups in the current experiment were being subdivided; we had therefore programed a large number of animals. While the impressively tight variance reported by Bogo et al. (2) would have been ideal, with less investment of time, we saw significant results based more on our numbers of subjects.

We also consider this approach to training to give a "motivational" component to the testing. The animals are not dogmatically forced to run until they can't keep up with the speed. If the stress of restraint, injection, and all other unique experiences of experimentation day affects their "desire" to run, this may be as significant as motor inability to perform. They also can show improvement; many animals did improve compared to their final training day. In fact, all 4 groups of experimental animals exposed only to radiation had average raw scores above their training scores. This is a typical effect of moderate amounts of acute radiation. Similarly, 1 of 2 control groups showed improvement on experiment day, as did 1 of 6 drug only groups and 2 of 12 groups given combinations of radiation and drugs.

Results which we previously obtained (3) were done with a different apparatus without shock. Animals required more training time but still attained lower scores. Animals in the control group of that earlier experiment averaged only 15.3 s on the rod on testing day. In the most recent experiment, 3 or 4 extra animals were trained each day and the poorest performers were eliminated. The result of this culling procedure is seen in Table 2; we also see that performance of controls in this experiment was double that of controls in the previous experiment.

TABLE 2. APPLICATION FROM TRAINING TO EXPERIMENTATION

	Second training day	Training score of selected rats	Controls (average of two trials)
Subjects (n)	437	401	35
Average score	27.4	27.9	30.6
Range	15-56	15-56	6-52.5

Table 2 shows that the 2 groups totaling 35 control animals had a "time on rod" range between 6 and 52.5 s. But, this is an average of their 2 trials. Their range of individual scores was from 4 to 65. Even though the group standard deviations (taken from each animal's 2 trials averaged together) were 10.7 and 12.3, there were 8 treatment groups with results significantly lower than controls. While this training method may not prepare animals as sensitive to mild decrement as other more extensive methods, it clearly has application if large numbers of subjects are to be used.

This training and testing paradigm has certain advantages including relatively quick, predictable training, and scores which clearly depict deficit but allow improvement. However, there is a major drawback in the variability of test scores and inability to predict whether an animal will do very well or very poorly on test day.

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