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A SWIMMING TASK USED TO ASSESS PERFORMANCE OF RATS

Linda B. Anderson, Airman First Class, USAF Thomas G. Wheeler, Ph.D.

November 1984

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FEB 8 1965

Final Technical Paper for Period December 1983 - May 1984

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USAF SCHOOL OF AEROSPACE MEDICINE Aerospace Medical Division (AFSC) Brooks Air Force Base, TX 78235-5000

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NOTICES

This final report was submitted by personnel of the Vulnerability Assessment Branch, Radiation Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-05-58.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder, or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

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.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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THE SWIMMING TASK AS A METHOD OF PERFORMANCE ASSESSMENT

INTRODUCTION

The purpose of this experiment was to ascertain the feasibility of using a swimming task as a sensitive measure of performance after a variety of insults. The rotarod task, although sensitive enough to demonstrate decreases in performance ability (Wheeler, 1983), has many limitations in its use; e.g., the time involved in training, the ability of only about 60% of the animals to learn the task, and the variability of the data which tend to have a bimodal distribution. These limitations initiated our search for a new task. In evaluating the swimming task, we investigated its limitations and sensitivity in comparison to the rotarod task. This study was conducted in two parts: first, training the animals; and second, testing the viability of the task for use in drug and radiation studies. During the training phase, we sought to learn the best training method and extent of memory for the task.

METHODS AND RESULTS

Evaluation of Training Methods

Twenty groups of Sprague-Dawley rats $(250 \pm 25 \text{ g})$, seven to nine per group, were housed in a room with environment control and a 12-h on/off light cycle. The training took place between 0900 and 1100 over 18 calendar days (also in the afternoon on day 7). It consisted of placing an animal in one end of an 8-ft-long, 6-in-wide (2.44 x 0.15 m) trough with 8 in. (20.32 cm) of water (water temperature 25°C), and recording the time it took the animal to swim to the other end where a ramp had been constructed as an avenue of escape.

Since one purpose of the study was to determine the best training method, we began by using only groups 1-10 to judge whether the length of the initial swim affected the time needed to learn the task. On day 1, groups 1-5 swam the full length of the tank and groups 6-10 swam from a barrier placed twothirds the length (6 ft, 1.83 m). On day 2, groups 1-10 swam the entire length. The distance of the initial swim showed no effect on the day-2 swim times (Figure 1). On day 3, groups 1-10 were retested and groups 11-18 (Figure 2) received their initial training, all groups swimming the entire length of the tank.



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Figure 1. Training times (square root of swim times \pm SEM) in seconds for groups 1-10.



Figure 2. Training times (square root of swim times \pm SEM) in seconds for groups 11-18.

On days 7-11, the animals swam the entire length. (On day 8, groups 15 and 16 were used for a preliminary test of dose response to pyridostigmine and were consequently eliminated from further testing.) The results from these swim tests showed no significant difference between the training methods we used. There was a significant (P<.05) increase in swim times after a 3-day rest period (Figure 1, groups 1-10) but not after a 2-day rest (Figure 2, groups 11-18). This observation led to the conclusion that the most convenient method of training would be once a day to avoid extended rest periods. To support this hypothesis, we trained groups 19 and 20 in this way the following week (days 14-18). Their mean scores (Figure 3) were slightly less (not significantly) than for groups 1-18, and the data showed the same trends.



Figure 3. Training times (square root of swim times \pm SEM) in seconds for groups 19 and 20.

All swim-time scores were recorded and statistically analyzed. The initial analysis produced a data distribution curve skewed to the right. To compensate for this and allow for more accurate analysis, the square root of all swim times was calculated, thus producing a normal distribution. A t-test analysis showed no significant differences between treated groups (pyridostig-mine injection and/or radiation) nor between control groups and treated groups (Table 1).

TABLE 1. RESULTS OF EXPOSURE TO PYRIDOSTIGMINE AND RADIATION (Mean ± SEM of square-root-transformed swim times)

⁶⁰ CO Exposure (rads)				
	0	700	_	
Pyridostigmine (mg/	kg):		•	
0	3.69±.28	3.74±.14	Test 1	
	3.06±.11	3.19±.12	Test 2	
0.29	3.75±.15	3.70±.14	Test 1	
	3.15±.12	3.0 ±.12	Test 2	

Determination of Task Sensitivity

This part of the experiment, pyridostigmine versus radiation exposure, was conducted 1 week after the last training swim. The animals (144) were randomly placed into four groups, 36 animals per group (Table 2), and exposed 16 at a time (4 from each group)-- 8 received 700 rads (midline tissue) of gamma radiation and 8 were sham exposed. Of these 8 animals, 4 received pyridostigmine (0.29 mg/kg, subcutaneous) and 4 received saline injections. The injections were given 15 min after exposure (radiation or sham); and 30 min after the injections, the animals were sequentially tested twice on the swimming task. Figure 4 shows no significant difference between the four groups.

TABLE 2. SENSITIVITY EXPERIMENTAL CONDITIONS

	60 _{CO}	Expo s ure O	(rads)[*] 700
Pyridostigmine (mg/kg)	:		
0 0.29		36 ** 36	36 36

*At 70 rads/min.

**Number of animals per group.



Day 25

Figure 4. Swim tests (in seconds) showing radiation/drug sensitivity. (Square root of swim times ± SEM)

DISCUSSION

We found that the swim task satisfied all of our training criteria (time involved, number of trainable animals, and data variability). The results show that this is a single-trial avoidance task. In general, the mean swim times decreased significantly after the initial exposure. Figures 1 and 2 show that the longer the rest between training swims, the greater the difference in mean scores; also, all four treatment groups (Figure 4) showed a 15% or greater decrease in their mean times between the first and second trials, suggesting that the animals' first swim after 7 days' rest was again an initial exposure to the task. Due to the nature of this swim task, the time involved in training could conceivably be cut to 1 day, making it possible to decrease both laboratory resources and time needed to complete the study. Because of their natural swimming ability, 100% of the rats are trainable (versus 50%-60% for rotarod testing), thus decreasing cost.

The last factor to be evaluated was data variability. Our concern with variability stemmed from the bimodal distribution of the data when rotarod testing is used. This was not, however, the case with the swim task data. Although raw data were skewed to the right, transformation of these data provided normalization and low variability. The swimming data indicate that the standard deviation was on the average less than 3% of the group's mean, as opposed to the rotarod standard deviation which was less than 15% of the group's mean. Training data also show a slight, but not significant, variability across groups (i.e., swim times for groups 1-10 during the final 5 training days averaged 3.44 s; 11-18, 3.56 s; and 19 and 20, 3.29 s). This

variability is probably due to the different training schedules; as a function of days, however, data trends (decreased swim time from first to second trial) were consistent across groups.

The satisfactory fulfillment of our training criteria appears to be the extent of this task's usefulness for our purposes. In past studies we have shown that radiation and pyridostigmine have a definite effect on rotarod-task performance (Cordts, 1983). This swimming task, however, lacked the sensitivity to show any effects of pyridostigmine and/or radiation. No significant difference is seen across these groups (Figure 4), nor is there a difference between the mean scores of the radiation/drug data and the training data; whereas under the same experimental conditions, the rotarod task demonstrated a performance deficit. Other investigators have used a more robust swimming task to assess the effects of various insults on performance. On one swim-toexhaustion task, irradiated rats swam with near-normal endurance (Pellegrino and Altman, 1979). This may be due to the natural swimming instinct of rats; to produce a change in performance may require a more debilitating insult or possibly a change in environmental conditions. In Kilmendorf's swim-toexhaustion studies (1961), a water temperature change from 14°C to 20°C caused the swim-trial duration to double. Molinengo and Orsetti (1976) showed that certain drugs could also affect the swim-to-exhaustion times, either increasing or decreasing the time. Although those studies used a different test than we did, the same variables (temperature and drugs) may affect an animal's ability to swim a certain distance and thus cause a performance deficit. To determine this, however, would take further study and was not the purpose of this particular investigation.

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