mals, and the respondent salivary conditioning obtained in this experiment was far more regular than that obtained with traditional procedures.

MARTIN M. SHAPIRO*
Department of Psychology, Indiana University, Bloomington

Notes

1. This investigation was supported by a postdoctoral fellowship (MF-10,166) from the National Institute of Mental Health, U. S. Public Health Service. I am indebted to Dr. D. W. Lauer for the generous use of his facilities and to Mrs. A. Radell for her skillful surgical preparations.

* Present address: department of psychology, University of Houston, Houston, Tex.

13 June 1960

Avoidance Learning and Relearning as a Function of Shuttlebox Dimensions

Abstract. Rats in a low-ceiling shuttlebox initially show a lower level of learning than rats in a high-ceiling box. After an hour's interruption of conditioning the performance of animals in low-ceiling boxes improves and avoidance is slightly more efficient than in the unimproved performance of animals in high-ceiling boxes. Box height also interacts significantly with length of box.

Recently, Kamin (*1*) attempted to measure the amount of retention of an incompletely learned avoidance response. Rats were given 25 trials in a typical shuttlebox and 25 retention trials after delays of 0, ½, 1, 6, and 24 hours, and 19 days. The results were unexpected; instead of a monotonic decreasing function with time, a V-shaped curve obtained which declined from 0 to 1 hour and then rose from 1 hour to 24 hours. Differences in retention at 0, 24 hours, and 19 days were not statistically significant.

Kamin interpreted the data in terms of an initial forgetting function followed by a jelling of the avoidance habit.

Denny (*2*) reinterpreted Kamin's V-shaped function (the "Kamin effect") in terms of an immediate incubation of anxiety followed by a dissipation of this anxiety after an hour or so. According to this interpretation, the anxiety, which is typically manifested in "freezing" behavior, incubates in the interval immediately after original learning and thereby interferes with the act of shuttling. Sometime after the hour delay the anxiety begins to dissipate, and retention of the avoidance response is clearly apparent after 24 hours. Although the results of Denny's study supported this hypothesis, in the first session of 25 trials and in the second session after a 1-hour delay the rats made more avoidances than found by Kamin (10.7 vs. 5.7 in session 1 and 10.1 vs. 6.6 in session 2). Since Denny's shuttlebox had a higher ceiling (14 vs. 4¾ inches) and a shorter gridway (26 vs. 36 inches) the present study was designed to investigate the importance of length and height of shuttlebox, both in original learning and in relearning 1 hour later.

Seventy experimentally naive hooded, black and albino rats, 35 female and 35 male, ranging in age from 90 to 150 days were used. The rats were randomly assigned to seven groups, except for a sex equation of five males and five females per group.

The shuttlebox was 4 inches wide, painted flat black, and had a glass front. The box was designed so that, with removable partitions, it could be adjusted to any of three lengths: 36, 26, or 16 inches. The fixed ceiling was 14 inches high; to lower ceiling height a sheet of clear glass was inserted 5 inches from the floor. Thus there were six different apparatus conditions which describe the six experimental groups: three groups with a 14-inch ceiling for each length and three groups with a 5-inch ceiling for each length.

In session 1 (25 trials) the rat was placed in the shuttlebox for 1 minute before the trials began. The buzzer was sounded 5 seconds prior to the shock (1.7 ma) and continued until the rat had crossed the midline (shuttled). The intertrial interval was 1 minute.

After session 1 the rat was returned to an outer room where it was placed in its living cage with its cage mates. One hour later the rat was again taken from its living cage and given 25 identical additional trials (session 2).

A control group run in a shuttlebox with a 26-inch gridway and a 14-inch ceiling underwent session 1 followed immediately by session 2 (no delay). The control was included solely to determine whether the present sample would perform like the rats in the earlier study (*2*).

The experimental design, excluding the control group, lent itself to a $2 \times 2 \times 3 \times 2$ analysis of variance with repeated measures. It was found that two main variables, ceiling height and sessions, and three two-way interactions were significant (see Table 1).

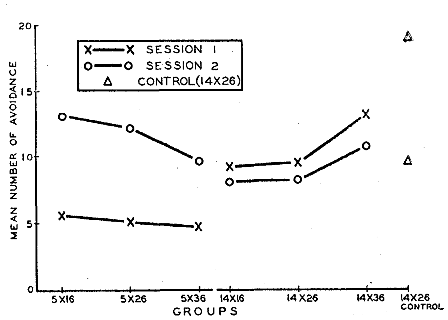

Fig. 1. Mean number of avoidance responses in each group for both sessions. The upper triangle represents session 2 for the control.

Table 1. Analysis of variance. The nonsignificant third- and fourth-order interactions (degrees of freedom = 9) are not included.

Source of variation	Degrees of freedom	Mean square	F
Between	59		
Sex	1	0.8	
Height	1	56.0	4.66*
Length	2	6.7	
Sex × height	1	90.2	7.52†
Height × length	2	80.5	6.71*
Sex × length	2	31.0	
Error	48	12.1	
Within	60		
Sessions	1	187.5	187.5†
Sessions × sex	1	0.0	
Sessions × height	1	480.0	480.0†
Sessions × length	2	9.3	
Total	119		

* $p = .05$ † $p = .01$

The mean number of avoidances for both sessions for the six experimental groups are plotted in Fig. 1. The means of the experimental groups were compared by a "studentized" distribution and yielded the following significant differences: (i) rats in high-ceiling boxes made more avoidances in original learning than did rats in low ceiling boxes; (ii) rats in low-ceiling boxes showed improvement after a delay of 1 hour, whereas those in high-ceiling boxes did not; (iii) performance in session 2 in a low ceiling, short (16-inch) box was better than performance in session 2 in a high ceiling, short (16-inch) box.

The significant interaction between height and length indicates that with a high-ceiling box learning tends to be better the longer the alley and that with a low-ceiling box just the reverse is true. The significant interaction between sessions and height indicates, at a minimum, that the "Kamin Effect" occurs to a greater extent with a high-ceiling than with a low-ceiling box. Finally, the significant interaction between sex and box height refers to the fact that females, which are especially prone to "freezing" in a low-ceiling box, make considerably more avoidances with a high ceiling than with a low, whereas males make a few more avoidances in the low-ceiling box than in the high. The control group performed in both sessions at levels comparable to Denny's controls, and performance during relearning was excellent (see Fig. 1).

The superiority in original learning of the rats in the high-ceiling box suggests that a low ceiling limits the number of avoidance-type responses in the animal's repertoire and in turn promotes the noninstrumental act of freezing. The resultant effect is a decrease in the probability of making the final shuttling response. The observation of relatively frequent freezing behavior with onset of buzzer in the low-ceiling groups supports this interpretation.

It further follows that to the extent a rat in the low-ceiling box learns the avoidance response, freezing must extinguish. This may account for the relatively better performance after a 1-hour delay for the low-ceiling rats. The 1-hour delay, according to the incubation-of-anxiety hypothesis, would, in general, increase the tendency to freeze, but less so in the low-ceiling conditions than in the high-ceiling because the animals in the high-ceiling box have not been freezing and thus the freezing response has had little opportunity to extinguish. Therefore, after 1 hour the rats in the high-ceiling box are at a disadvantage and show no improvement.

In contradistinction to the rats under low-ceiling conditions, rats under high-ceiling conditions can initially make more responses to the stimulus situation —leaping, jumping, standing, and such responses—that can readily chain in with the correct response. At first glance, the results pose a paradox. For by lowering the ceiling one might expect an increase in the specification of the correct response of shuttling. Instead it appears that a low ceiling promotes a response (freezing) which is incompatible with shuttling and incompatible with responses allied with shuttling.

The less important variable of length is more difficult to theorize about but may operate as follows. Under low-ceiling conditions the escaping rat can only run, which means it runs through shock to escape shock, being consistently punished during the early stages of making the correct response. Thus learning, despite the greater motivation for avoiding, tends to be poorer the longer the box (the greater the punishment). In high-ceiling boxes where escape is possible in a number of ways, this factor is not as critical, and the longer the alley the greater the motivation for avoiding.

M. RAY DENNY
JAY O. THOMAS
Department of Psychology, Michigan State University, East Lansing

References

1. L. J. Kamin, *J. Comp. and Physiol. Psychol.* **50**, 457 (1957).
2. M. R. Denny, *Am. Psychologist* **13**, 419 (1958).

2 June 1960

Dehydrogenase Activities in Dystrophic Mice

Abstract. The levels of activity of glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, glutathione reductase, lactic dehydrogenase, and α-glycerophosphate dehydrogenase have been studied in the gastrocnemius muscle of mice with "dystrophia muscularis." The activity of enzymes requiring triphosphopyridine nucleotide as a cofactor is increased relative to the control littermates, whereas the activity of those enzymes requiring diphosphopyridine nucleotide is decreased.

In 1955 Michelson *et al.* (*1*) described a hereditary disease in mice which is similar to human progressive muscular dystrophy. Since that time numerous investigators have attempted to determine whether or not this disease is related to a primary biochemical lesion. The present study is a report on the levels of activity of several dehydrogenases in the gastrocnemius muscle of mice with "dystrophia muscularis."

Dystrophic mice (genotype *dydy*) and control littermates (genotype *Dydy*) of the Bar Harbor strain 129 were used. At the time of sacrifice the gastrocnemius muscle was rapidly isolated, frozen in liquid nitrogen, and stored at −70°C. Transverse sections (15μ thick) were obtained and lyophilized at −30°C under vacuum, and the dry sections were stored at −20°C. At the time of analysis each transverse section (20 to 40 μg [dry weight]) was trimmed free of mounting material under a dissecting microscope, weighed on a quartz fiber balance, and placed in the appropriately sized test tube. Procedures for preparation and handling of the small pieces of tissue have been previously described (*2*).

The use of frozen-dried transverse sections of an entire muscle has several obvious advantages over the usual homogenate preparations. First, the stability of the preparation allows repeated analysis of the same tissue over a long period of time without deterioration of enzymatic activities or chemical constituents. Also, the morphology is preserved so that the extent of degenerative changes in each animal can be determined. Further, sampling errors due to a nonhomogeneous distribution of dystrophic fibers within the muscle are decreased. Whether or not the biochemistry of the "obviously dystrophic" fibers is markedly different from that of the "normal-appearing" fibers within an affected muscle, or whether the biochemical alterations are equally distributed among all of the fibers is a question for further study.

The present study confirms the fact that the lipid content of dystrophic muscle is increased (*3*). Therefore, the enzymatic results are based on the fat-free dry weight (*2*) determined separately for each muscle.

The activities of glucose-6-phosphate dehydrogenase (*4*), glutathione reductase (*5*), isocitric dehydrogenase (*6*), lactic dehydrogenase (*7*), and α-glycerophosphate dehydrogenase (*8*) were measured by the fluorescence of the oxidized or reduced pyridine nucleotides (*7*).

All of the enzymes requiring triphosphopyridine nucleotide (TPN) increased in activity in the muscle of dystrophic mice (Table 1). The activity of glucose-6-phosphate dehydrogenase was found to be approximately 400 percent ($P <$ .01) of the value observed in the control littermates. Preliminary results indicate that 6-phosphogluconic dehydrogenase, the second step in the glucose-6-phosphate shunt, exhibits a similar increase in dystrophic mice. The activities of isocitric dehydrogenase and glutathione reductase also increased, and were approximately 180 percent ($P <$ .02) and 170 percent ($P <$ .01) of the control, respectively.

In contrast, the activities of the dehydrogenases requiring diphosphopyridine nucleotide (DPN) decreased in dystrophic mice. The lactic dehydrogenase and α-glycerophosphate dehydrogenase activities were approximately 75 percent ($P <$ .01) and 50 percent ($P <$ .01) of the control, respectively.

Previous reports (*9*) have indicated that the activities of the Krebs-cycle enzymes are not markedly altered in muscular dystrophy. However, the activity of isocitric dehydrogenase, a TPN-requiring dehydrogenase in this cycle, increased almost twofold here. Rosenkrantz (*10*) previously reported no change in the activity of this enzyme in control and dystrophic mice. The reason for this apparent discrepancy may be due in part to different assay

Table 1. Dehydrogenase activities in mouse muscle. Activities are expressed as micromoles of product formed per gram of fat-free dry weight per hour, except for those marked * which are expressed as millimoles per gram of fat-free dry weight per hour. Results were obtained on five samples from each animal. G-6-PDH, glucose-6-phosphate dehydrogenase; ICDH, isocitric dehydrogenase; Gl-R, glutathione reductase; α-GPDH, α-glycerophosphate dehydrogenase; LDH, lactic dehydrogenase.

Dehydrogenase	Control	Dystrophic
TPN-requiring:		
G-6-PDH (10)	100 ± 3	387 ± 30
ICDH (4)	393 ± 48	603 ± 62
Gl-R (4)	551 ± 25	819 ± 35
DPN-requiring:		
α-GPDH (2)	7930 ± 14	3210 ± 14
LDH (4)	168 ± 7*	131 ± 10*