Biphasicity of the incubation curve*

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Two experiments were performed using a one-trial passive-avoidance task. In Experiment 1, different groups of mice were retested at different times after training. In Experiment 2, different groups were administered a footshock outside the conditioning apparatus and then were given the step-through experience at different times after the footshock. The time-response curve was found to be biphasic only in Experiment 1, while it produced a monotonic incubation curve in Experiment 2.

Time-response curves of conditioned avoidance responses have been found to be quite different from those that could be expected on the basis of the traditional concept of forgetting. Kamin (1957, 1963) first showed a U-shaped function for an incompletely learned active avoidance response. While several studies confirmed the "Kamin effect" (Denny, 1958; Denny & Ditchman, 1962; Denny & Thomas, 1960; Pinel & Cooper, 1966a), interest among those working in the field of memory was also aroused by the "incubation" phenomenon (McGaugh, 1966; Pinel & Cooper, 1966b, c; Robustelli et al, 1969), that is, by the initial gradual increase of response strength found in many passive-avoidance learning situations. Implications of this finding for the interpretation of the electroconvulsive shock gradient effect were suggested.

Recently Zerbolio (1969) showed a biphasic time-response curve for a one-trial passive-avoidance response, with a first peak at about 15 min and a second peak at 1 to 2 h after learning. The question arises, obviously, whether this biphasicity can be interpreted in terms of specific memory

processes or in terms of nonspecific aftereffects of footshock.

In Experiment 1 of the present investigation, the usual design with passive-avoidance retest given at different times after learning was used. In Experiment 2 animals were administered footshock outside the passive-avoidance apparatus and then were given the step-through experience at different times after footshock. The procedure of Experiment 2 was designed to provide a measure of nonspecific aftereffects of footshock, though, even so, some kind of generalized conditioning cannot be avoided.

EXPERIMENT 1

Subjects

The Ss were 192 naive female CF1 albino mice, approximately 9 weeks old, housed 8 to a cage, with ad lib access to food and water.

Apparatus and Procedure

Animals were trained in a step-through apparatus consisting of a trough-shaped two-compartment box (Jarvik & Kopp, 1967). In the smaller, transparent compartment Ss were exposed to a bright Tensor lamp. The smaller compartment was connected to the larger, dark compartment by an opening 3.2 cm high, 2.7 cm wide, and flush with the floor. The floors of both compartments consisted of a pair of parallel metal plates. The opening

between the compartments could be closed by a guillotine door.

The Ss were placed into the smaller, lit compartment facing away from the door, and they stepped spontaneously into the larger, dark compartment. A timer was started manually when the S was placed in the apparatus and was stopped automatically when the S crossed a photocell on the wall of the larger compartment 8 cm from the opening. A 350-microamp footshock was automatically delivered through the metal plates in the larger compartment at the moment the photocell beam was interrupted, and the Ss escaped from the dark compartment back into the lit compartment, from which they were removed. On the retest trials, Ss were placed in the lit compartment and their latency to step to the dark compartment was recorded. Those Ss remaining longer than 1,200 sec were removed.

The Wilcoxon rank sum test (Wilcoxon & Wilcox, 1964) was used for the statistical evaluation of the data.

The Ss were divided into six groups, with 32 animals in each group, according to the time of retest, which occurred 2 min, 5 min, 10 min, 30 min, 3 h, and 24 h after training. Ss in all groups were returned to their home cages for the training-retest interval.

Results

The results are summarized in Table 1. No significant differences were found in the training-trial latencies among all six groups. The latencies of the retest trial are significantly higher than those of the training trial for all groups, indicating that the passive-avoidance conditioned response was formed and retained at all training-retest intervals.

The retest trial latencies of the various groups show an initial increase to 5 min, a subsequent decrease, and another increase to 24 h. The retest latencies of both peak groups differ significantly from those of their adjacent groups. All significances are given in the table.

Table 1
Results of Experiment 1

Time between training trial and retest trial	N	Training trial latency median (seconds)	Retest trial latency median (seconds)	Probabilities of differences between training trial and retest trial latencies of each group (p <)	Probabilities of differences between retest trial latencies of various groups (p <)					
					2 min	5 min	10 min	30 min	3 h	24 h
2 min	32	21.1	133.5	0.005		0.005	0.05	n.s.*	0.005	0.005
5 min	32	20.9	639.6	0.005	0.005		0.05	0.005	n.s.	n.s.
10 min	32	23.4	249.8	0.005	0.05	0.05		n.s.	n.s.	0.005
30 min	32	21.2	220.0	0.005	n.s.	0.005	n.s.		0.005	0.005
3 h	32	21.0	365.1	0.005	0.005	n.s.	n.s.	0.005		0.05
24 h	32	24.3	680.3	0.005	0.005	n.s.	0.005	0.005	0.05	

*n.s. = not significant

Table 2
Results of Experiment 2

Time between footshock and step-through experience	N	Step-through latency median (seconds)
2 min	26	29.8
5 min	26	29.9
10 min	26	23.7
30 min	26	34.0
3 h	26	61.5
24 h	26	103.3
24 h (no footshock)	26	13.4

EXPERIMENT 2

Subjects

The Ss were 182 naive female CF1 albino mice, approximately 9 weeks old, housed 8 to a cage, with ad lib access to food and water.

Apparatus and Procedure

The footshock was delivered inside a metal cage 18 x 26 cm at the base and 16 cm high, similar to the cages in which the animals were normally housed. The floor of the cage was made up of four 11 x 7 cm metal plates raised 1 cm above a nonconducting Plexiglas base that rested on the floor of the cage. A footshock could be delivered when the S bridged the plates.

The Ss were placed on the metal plates and were allowed to remain there 15 sec without shock. Then a 1.5-sec, 350-microamp shock was delivered. The Ss remained in the box for a further 15 sec after the termination of the shock and were then replaced in their home cages. These parameters were chosen to approximate as closely as possible to the conditions obtained in the step-through training procedure described in Experiment 1.

The Wilcoxon rank sum test (Wilcoxon & Wilcox, 1964) was used for the statistical evaluation of the data.

The Ss were divided into seven groups, with 26 animals in each group, according to the time intervals between the footshock and the step-through trial, which were: 2 min, 5 min, 10 min, 30 min, 3 h, and 24 h (corresponding to the step-through training-retest intervals of Experiment 1). The seventh group was a control group, given an unshocked experience in the footshock apparatus and retested in the step-through apparatus 24 h later. The Ss in all groups were returned to their home cages for the interval between footshock and retesting.

Results

The results are summarized in Table 2. All the footshocked groups had step-through latencies significantly

($p < .01$) higher than those of the control unshocked group. The step-through latencies of the footshocked groups show an initial plateau and a subsequent gradual increase with a maximum at 24 h after the footshock experience. There is no evidence of biphasicity. The latencies of the 24-h group are significantly higher than those of the 2-min group ($p < .05$) and of the 10-min group ($p < .01$). The difference between the latencies of the 24-h group and those of the 5-min group approaches significance ($p < .1$).

DISCUSSION

The biphasicity of the time-response curve found by Zerbolio (1969) was confirmed in Experiment 1 of the present study. The differences in the loci of the two peaks can be accounted for on the basis of differences in apparatus, procedures, and animals. Also, those studies that have shown a monotonic increase in response strength after learning do not necessarily have to be considered in disagreement with our results and those of Zerbolio since in such studies the appropriate training-retest intervals may not have been chosen.

The results of Experiment 2 show the expected increase in latencies after the footshock experience, which follows the time course of the increase in latencies in Experiment 1, with an important exception. There is no initial peak at 5 min that would correspond with that found in Experiment 1. This suggests that the gradual increase in latencies following a footshock experience is some consequence of the footshock itself common to both experiments, whereas the early peak is a response to a specific conditioning situation.

To account for the two peaks of the time-response curve, Zerbolio has hypothesized the existence of two different memory systems. But there is a basic consideration which limits the legitimacy of his hypothesis. What are usually called retention curves are, in fact, just time-response curves. That is, what is measured is the strength with which the conditioned response manifests itself at each time interval after the learning experience, and it should be always remembered that this strength is not an unequivocal index of retention. On the contrary, a crucial problem in interpreting a time-response curve after a learning experience is to establish what is due to memory and what is due to factors, independent of memory, which can affect performance.

The present study has shown that

aftereffects of footshock not related to the specific conditioning situation could explain the second peak in the time-response curve, while they cannot explain the first peak. Of course, conceiving the retest latency as the expression of the animal's overall reactivity to the learning situation, does not imply denying the possibility that memory itself can vary at different times after learning.

If not determined by memory, however, the first peak could still be related to some short-lasting biochemical and physiological condition following administration of footshock and interacting with features of the specific conditioning situation to produce an increase of retest latencies.

Obviously, until the factors not specifically related to memory processes are appropriately investigated, very little can be inferred about memory from the phenomenological picture.

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