*MEMORY CONSOLIDATION OF ONE-TRIAL LEARNING IN CHICKS**

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The thesis that an enduring memory trace takes time to consolidate has not only been inferred from the phenomena of retroactive inhibition in verbal learning[1] and from retrograde amnesia after brain injury,[2] but is also supported by many direct experimental studies. Most of the experiments have been conducted on rats. The typical procedure is to subject the animal to electroconvulsive shock or some other "disruptive" treatment shortly after a learning experience, and later assess the effect on retention of the learning. In general, it has been found that retention is disturbed only if the treatments are given within a critical "consolidation period" that varied from a few seconds to a few hours in different experiments. Within this period, the sooner the treatment, the worse the retention (see recent reviews[3–5]).

The present study investigates the kinetics of memory consolidation of one-trial learning in day-old chicks, using electroconvulsive shock (ECS) as the treatment. Newly hatched chicks were used partly for comparative purposes and partly because of some special advantages involved in the particular experimental procedure. This procedure is free from possible "punishing" effects of the ECS, a complicating factor that has been difficult to control in many earlier studies. Also, its simplicity makes feasible the use of large numbers of subjects, thus yielding more reliable data and permitting the detection and clarification of certain subtle features of the problem, as will be indicated below.

Experiment 1.—Disruption by ECS of memory consolidation in chicks: The aim of the first experiment was to determine whether retention of learning in chicks can be disrupted by postlearning ECS, and if so, how retention deficit depends on the interval between the learning trial and ECS.

The learning involved inhibition of the chick's natural predilection to peck at a visually attractive lure. Chicks in the experimental groups were each given a learning trial followed after various intervals by an ECS. Retention of the learning was measured 1 or 2 days later. Some control groups provided data on either the normal retention level without ECS, or the basal pecking rate without learning and ECS. Other control groups served to check whether ECS functioned as a punishment in the learning situation, or whether an ECS alone would influence the normal pecking rate.

Method.—Day-old White Leghorn cockerels (*S*'s) were obtained in the morning from a nearby hatchery and housed individually in white, cylindrical, topless cartons (each 17 cm high, 9 cm in diameter) in the experimental room where the temperature was 85–95° and the humidity was 30–50%. S's that would later receive an ECS were anesthetized with halothane, and two electrodes were implanted under the skin, one on each side, behind the eye and above the ear. The electrodes were made of no. 16 Mustad-Viking hooks each connected with about 30 cm of fine, insulated no. 8340 Belden wire that led to the outside of the carton. Implantation of the electrodes was performed between 9 and 11 A.M., after which

all *S*'s had about 5 hr to adapt in their cartons before presentation of the learning trial or other experimental manipulations between 2 and 5 P.M.

Experimental groups: The lure used to elicit pecking was a shiny 3-mm stainless steel ball neatly soldered to a metal probe that was 30 cm long and $^1/_2$ mm in diameter, and was bent to a right angle 1 cm from the ball-tip end. For the learning trial, the lure was first dipped in methyl anthranilate (the MeA lure) before presenting to the *S*. The chemical was apparently unpalatable to the chicks. After pecking at the MeA lure, they typically shook their heads vigorously, turned away from it, and would not peck again. One experimenter (*E*) presented the lure to the *S* 1 cm in front of its beak for 10 sec, and reported when pecking and head-shaking occurred. Another *E*, with a stop watch, recorded the times and notified the end of the 10-sec presentation period.

S's in the 12 experimental groups were each given an ECS some time after the learning trial. The trial-ECS intervals in 11 groups were 0 sec, 5 sec, 10 sec, 30 sec, 1 min, 2 min, 4 min, 8 min, 16 min, 2 hr, and 24 hr, counting from the end of the 10-sec lure presentation period to the start of the ECS current. Each *S* in the "immediate" group received the ECS as soon as it started to shake its head, which on the average occurred at about the fourth sec from the beginning of lure presentation. For delivery of the ECS, the wires from the head electrodes were connected to the current source with small alligator clips. The *S*'s were apparently not aware of the manipulations involved which took place out of their view. The current was 60 cps AC of 28 ma and 0.45 sec in duration.

All *S*'s except those in the 24-hr group were given a test trial in the afternoon of the second day, about 22 hr after the learning trial. The 24-hr group had the test trial in the morning of the third day, about 44 hr after the learning trial. In the test trial, the same MeA lure was presented to each *S* 1 cm in front of its beak for 10 sec, and whether the *S* pecked at it or not was reported by a different *E*, who did not know the *S*'s previous records.

Control groups: To assess the normal retention level of the learning without an ECS, three control groups were used. One group was presented with the MeA lure for 10 sec during the learning trial in the afternoon of the first day, and had the test trial in the afternoon of the second day. The second group differed from the first one in that the lure was withdrawn as soon as the *S* started to shake its head during the learning trial. The third group differed from the first one in that the test trial was given in the morning of the third day.

To obtain the normal basal pecking rates, two control groups were each given the 10-sec "test" trial, in day-2 P.M. and in day-3 A.M., respectively, without any preceding learning trial or ECS.

To check whether an ECS alone would alter the normal pecking rate, one control group was given an ECS in day-1 P.M., and the "test" trial in day-2 P.M.

To find out whether the ECS functions as a punishment for pecking, five control groups were used. All of them were presented with a dry lure (a duplicate lure without methyl anthranilate on it) in day-1 P.M. One group had a 10-sec exposure to the dry lure without a subsequent ECS. One group was presented with the dry lure, and an ECS was given as soon as the *S* pecked at the lure. Three groups had 10-sec exposure to the dry lure, and received an ECS 0, 5, and 10 sec, respectively, afterward. All five groups had the standard test trial with the MeA lure in day-2 P.M.

TABLE 1

PERCENTAGE OF S'S THAT DID NOT PECK DURING THE TEST TRIAL (%), AND GROUP SIZE (N)

Control Groups			Experimental Groups		
"Learning-only" controls	(%)	(N)		(%)	(N)
MeA lure	79	289	"Immediate"	6	239
MeA lure, "Immediate"	85	108	0 sec	26	246
MeA lure, tested on day-3	85	124	5 sec	37	180
			10 sec	49	59
			30 sec	56	63
			1 min	67	55
"No-learning" controls			2 min	59	49
			4 min	54	63
Nothing on day-1	1	157	8 min	57	53
Nothing on days 1 and 2, tested on day-3	2	179	16 min	56	57
ECS only	2	136	2 hr	67	81
Dry lure	5	83	24 hr, tested on day-3	62	137
Dry lure, "Immediate" ECS	4	28			
Dry lure, 0-sec ECS	1	64			
Dry lure, 5-sec ECS	0	29			
Dry lure, 10-sec ECS	4	27			

Results and Discussion.—Both lures elicited a high incidence of pecking upon their initial presentation. Only about 10 per cent of the S's to which a lure was presented on the first day did not peck and these were excluded from the following data analysis. About 90 per cent of the S's given the ECS manifested tonic-clonic convulsion. The tonic phase of the convulsion started 2–4 sec after the onset of the current, and lasted for 9–13 sec. Other S's in which only clonic or no convulsion occurred were also excluded from the following data analysis. The responses of the various groups during the test trial are presented in Table 1. Since the learning comprised inhibition of pecking at the MeA lure, the percentage of S's in each group that did *not* peck during the test trial constitutes the index for retention—the higher the percentage, the better the retention.

It is clear from Table 1 that normally there was good retention of the one-trial experience. About 80 per cent of the S's did not peck at the MeA lure during the second or test trial. Whether during the learning trial the lure was immediately withdrawn when the S began to shake its head, or was left in front of it for 10 sec, made little difference; and the learning was remembered for at least 2 days without much forgetting. The pooled data from the three "learning-only" control groups that had a learning trial without a subsequent ECS, with a total of 521 S's, give a retention value of 81 per cent.

In contrast, the pecking rates were very high in all the other control groups. Among them, some S's had been given neither the learning trial nor the ECS before; some S's had only had the ECS alone without the learning trial; and the other S's had only pecked at the dry lure, with or without an ECS. Since the test results show no difference among these latter control groups, they suggest that one ECS unassociated with learning trial did not alter the normal pecking rate, and also that one ECS as administered in this experiment did not function as a punishment for the act of pecking. The pooled data from the eight "no-learning" control groups, with a total of 703 S's, shows that only 2 per cent of them did not peck during the test trial.

Results from the experimental groups indicate two findings. First, memory consolidation of the learning took place rapidly and was essentially finished in about 30 sec. This is evidenced by the fact that the retention values rise steadily only in

the four groups that had the shortest trial-ECS intervals, and level off at the 30-sec group. The retention value of the 0-sec group, in which 26 per cent of 246 *S*'s did not peck, indicates that an appreciable amount of memory had already been consolidated during the 10-sec learning trial!

The second finding is that ECS also seems to have caused a smaller but constant amount of retention deficit that was independent of the trial-ECS interval. This is supported by the results that among the eight groups whose trial-ECS interval ranged from 30 sec to 24 hr, the retention values varied from 54 per cent to 67 per cent, but did not show any trend toward the control level of 81 per cent. This finding might be explained in terms of some lasting brain damage caused by ECS,[6] and offers an explanation to the observations that at relatively long trial-ECS intervals where one ECS was apparently not effective, a series of ECS was still able to impair the memory.[7, 8] Another support for the brain-damage effect of ECS is that a series of ECS has been found to be detrimental even if it was given long before the learning tasks.[9, 10]

The difference in consolidation times reported in different studies that range from seconds to hours has been puzzling. Clearly, different experimental conditions and methods of data analysis can account for part of the inconsistencies. The present finding of the two different effects of ECS on memory may also help to explain the wide variation. Since many studies used only a few trial-ECS intervals with small numbers of *S*'s, it was not easy to differentiate the two effects of ECS on memory: some studies report retrograde amnesic effects of ECS given hours after learning as proof of long consolidation times, but this may reflect the aforementioned "brain-damage" effect of ECS.

The fact that day-old chicks can learn in one trial to inhibit the natural strong tendency to peck at a visually attractive lure is remarkable, especially when we consider that for many *S*'s the learning was achieved during as short a visual exposure to the lure as only 2–3 sec. The conclusion that learning in young chicks rarely takes place before the third day after hatching thus needs qualification.[11–13] The swiftness of the one-trial learning and the simplicity of the experimental procedure make it a promising tool for a wide variety of approaches to the study of physiological correlates of learning and memory.

Experiment 2.—The growth of the memory trace: Experiment 1 shows that when ECS was given within 30 sec after the learning trial, the sooner the ECS, the worse was the retention tested on day-2. As an alternative to a quantitative increase in the amount of memory consolidated, this observation might also be interpreted to be a reflection of wide individual differences in consolidation time. In other words, the better retention values obtained with the longer trial-ECS interval might simply mean that more *S*'s had their memory consolidated by the end of the longer interval. An important related question is whether the basic change involved in the memory trace is all-or-none, or whether it varies in degree. Since the behavioral index of peck or no peck is an arbitrarily chosen dichotomous measurement, the results by themselves do not reveal whether they reflect mainly (*a*) individual differences in consolidation time among the chicks, or (*b*) the increasing amount of consolidated memory in a typical individual, This second experiment was aimed to help decide between these alternative interpretations.

Method.—Three groups of *S*'s that on day-1 had had trial-ECS intervals of

TABLE 2

PERCENTAGE OF *S*'S THAT DID NOT PECK DURING THE DAY-3 TEST TRIAL (%), AND GROUP SIZE (*N*)

Day-1 trial-ECS (interval)	Pecked on Day-2 (%)	(*N*)	Did Not Peck on Day-2 (%)	(*N*)
"Immediate"	6	86	33	3
0 sec	18	63	55	20
5 sec	32	60	72	25
	Chi sq = 18.2; $P < 0.001$.		Chi sq = 2.52; $P < 0.30$.	

"immediate," 0-sec, and 5 sec, respectively, were used. If the memory trace of the day-1 learning experience is all-or-none, then all *S*'s that pecked at the lure on day-2 should have little or no memory of the learning, whereas all *S*'s that did not peck on day-2 should have approximately equal memory, regardless of their different trial-ECS intervals on day-1. Further, if their day-2 experience with the lure was prevented from undergoing consolidation by an immediately following ECS, then pecking or avoiding the lure on day-3 should depend on the day-2 response but not on the day-1 trial-ECS interval. On the other hand, if the memory trace does grow during the consolidation period, *S*'s that had longer trial-ECS intervals on day-1 should have more potent engram and hence should show better retention in the day-3 test than those permitted less consolidation time.

The procedure was as follows. During the day-2 test session, if the *S* pecked at the MeA lure, an ECS was given immediately after the peck response. If the *S* did not peck at the lure, the ECS was given at the end of the 10-sec test period. The same lure was presented again for a 10-sec exposure on day-3, and whether each *S* pecked or not was recorded. The percentage of *S*'s in each of the groups that did not peck at the lure on day-3 was calculated to give the day-3 retention values.

Results and Discussion.—The day-3 test results are given in Table 2. The data show that among *S*'s that pecked at the lure on day-2, the day-3 retention level was indeed better for those that had had longer trial-ECS intervals on day-1. This finding supports the interpretation that the memory trace is not all-or-none, but undergoes continuous strengthening during the consolidation period. If the "all-or-none" interpretation were correct, the *S*'s pecking at the lure on day-2 would imply that they all needed consolidation times longer than the trial-ECS intervals allowed in day-1. Since their day-2 pecking was followed immediately by another ECS, all three groups should have shown uniformly poor retention on day-3. To the contrary, the data indicate that *S*'s that had had longer day-1 trial-ECS intervals actually had more consolidated memory. The same relationship holds for the *S*'s that did not peck at the lure on day-2. Instead of showing uniformly good retention as would have been predicted from the "all-or-none" model, better retention was again obtained from the groups with longer consolidation intervals on day-1. Thus, although the *S*'s behaved similarly and were treated equally on day-2, the data indicate that *S*'s with varying day-1 consolidation intervals actually had different amounts of memory. The results favor the conclusion that the memory trace undergoes continuous strengthening during the consolidation period.

Summary.—A critical memory consolidation period of about 30 sec was demonstrated in day-old chicks by administering ECS at various intervals after one-trial learning that involved inhibition of *S*'s predilection to peck at a particular lure.

The degree of memory loss was inversely related to the trial-ECS interval within the critical 30-sec consolidation period. A smaller but constant amount of retention impairment was also found in groups with longer trial-ECS intervals of up to 24 hr. A total of 2606 *S*'s was used in 12 experimental and 11 control groups.

A follow-up experiment with 257 of the *S*'s indicated that memory for the one-trial experience was not all-or-none in the individual chick, but grew continuously during the consolidation period.

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