Hemicholinium-3 induced amnesia: Some temporal properties*

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Following one-trial passive avoidance training, rats were injected via intra-hippocampal cannulae with hemicholinium-3 or saline. Hemicholinium-3-injected rats showed little retention 18, 24, or 30 h later but complete retention after 6 h, 3 days, and 7 days.

Results of a number of recent experiments (Deutsch, Hamburg, & Dahl, 1966; Hamburg, 1967; Deutsch, 1969; Biederman, 1970; Wiener, 1970) have been interpreted as indicating that memory involves biochemical changes that modify conduction between the members of certain cholinergic neuron pairs. Essentially this thesis argues that synaptic bombardment results in a durable morphological or biochemical change that renders a particular junction or set of junctions more susceptible to subsequent activation via the same pathway.

A number of drugs are available which affect synaptic transmission by differing modes of action, offering the possibility of interrupting the memory process in the different stages. If, indeed, learning is followed by a transient release of ACh which initiates a long-term change, interfering with cholinergic synthesis might not prevent the development of long-term memory but could result in temporarily rendering the memory inaccessible due to presynaptic transmitter depletion. To test this possibility, hemicholinium-3, which inhibits ACh synthesis by competing with choline for access to the enzyme cholineacetylase (Gardner, 1961), was administered following learning.

EXPERIMENT 1

Subjects

Sixty-three naive male Wistar rats, 80-100 days old, were housed singly with ad lib access to food and water. One week prior to receiving avoidance training, they were bilaterally implanted with chronic indwelling 21-gauge cannulae in the hippocampus (2 mm caudal, 2 mm lateral to bregma, and lowered 2 mm from the skull surface). The cannulae were held in place by cranioplastic cement anchored to stainless steel screws drilled into the skull.

Apparatus

The rats were trained in a metal two-compartment step-through passive

avoidance apparatus, similar in design to one described by Jarvik & Kopp (1967). The outside dimensions of the compartments were 12 x 9.5 x 10 in. and 12 x 9.5 x 8 in. The inside walls converged toward the bottom at an angle of 60 deg, forming a trough-shaped floor which was 7 in. from the bottom drainage channel to the hinged transparent Plexiglas top in the larger compartment and 5 in. from the bottom to metal top in the smaller. The larger safe compartment was painted white and illuminated with a 30-W bulb. The floor of the smaller compartment was made of two parallel rows of eight 1.12 x 6.25 in. stainless steel plates which were wired to deliver a 1-mA shock when the rat bridged the floor. The compartments were connected by a 3-in.-diam sliding door.

Procedure

On the training day, rats were put in the start compartment facing the door; after 5 sec the door was opened. When the rat entered the dark compartment with all four feet, the timer was stopped and shock delivered until the rat returned to the light compartment and remained 120 sec. The rat was then removed, lightly anesthetized with Halothane, and injected, using a Hamilton microsyringe with a 30-gauge needle. The injection needle penetrated 1 mm through a rubber seal at the cannula guide tip. Bilateral injections were each 3.5 microliters of either HC-3 solution (.4 mg total) or the physiological saline drug medium. Retention tests were carried out either 24 h or 7 days following training and injection, except for some control groups who were injected 24 h prior to training or received HC-3 injections following training and 24 h prior to Day 7 retest.

Results

Results are shown in Table 1. Administration of HC-3 shortly after training produced amnesia on Day 2 but not on Day 7 (Mann-Whitney $U = 0$, $p \leq .01$).

The median step-through latency for naive animals (including preinjected controls) was 10 sec. Rats injected with HC-3 immediately after

training and on Day 6 showed retention on Day 7. Histological examination failed to reveal any damage other than cannulae tracks and a small amount of damage at the cannula guide tip.

EXPERIMENT 2

The purpose of this experiment was to replicate and extend the findings of Experiment 1. Animals were tested after various retention intervals.

Method

The surgical and training procedures were identical to those used previously. The injection procedure was modified so Halothane anesthetization prior to injection was unnecessary. The injection cannula was connected by polyethylene tubing to a Hamilton microsyringe mounted on a modified Davis Liquid Pump (Davis Scientific Instrument Company, Model LR-131), permitting automatic injection of the proper volume of drug solution.

The Ss ($N = 81$) were tested 6 h, 18 h, 24 h, 30 h, 3 days, or 7 days following training-injection. Saline controls were tested at 24 h, another control group was injected with HC-3 on Day 6 and tested on Day 7, and a final control group was injected with HC-3 6 h prior to training.

Results and Discussion

Results are shown in Table 2. Administration of HC-3 immediately following training produced amnesia on the 18-, 24-, and 30-h tests ($p \leq .05$, .01, .001, respectively). Complete retention was found for Ss tested 6 h, 3 days, or 7 days following training-injection. The saline controls showed retention, as did the Day 6 HC-3 group. Injection of HC-3 prior to training does not affect initial step-through latency.

HC-3 interferes with choline transport, and choline is necessary for the resynthesis of acetylcholine in presynaptic terminals; therefore, ACh cannot be resynthesized while the drug is present (Scheuler, 1955; Birks & MacIntosh, 1961; MacIntosh, 1961). However, neural transmission will continue for some time despite the

Table 1
Median Step-Through Latencies on the Retention Test Trials (Key, HC-3, Hemicholinium-3)

Drug Treatment	Retention Day	Median Test Latency (Sec)
HC-3	2	43
HC-3	7	120+
HC-3(1) & HC-3(6)	7	120+
Saline	2	120+
Saline	7	120+
Halothane	2	120+
Halothane	7	120+

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Table 2

Group	Median Training Latency (Sec)	Median Test Latency (Sec)
HC-3, 6 h retention*	4.5	120+
HC-3, 18 h retention*	4.0	78
HC-3, 24 h retention*	8.0	30
HC-3, 30 h retention*	4.5	11
HC-3, 3 day retention*	2.5	120+
HC-3, 7 day retention*	4.0	120+
Saline, 24 h retention*	5.5	120+
HC-3, 6 h training†	9.0	
HC-3, Day 6 injection, Day 7 retention†	5.0	120+

*N = 10 per group, †N = 7 per group

inhibition of ACh synthesis because of presynaptic ACh stores. The length of time a presynaptic ending will continue to release ACh varies inversely with the rate of neuron firing.

The U-shaped memory-loss function can be explained by the interaction of the drug's mode of action with hypothesized temporal changes in the memory substrate. Memory is in evidence 6 h following training-injection when the ACh stores

are not yet depleted; depletion between 18 and 30 h results in retrieval failure, and finally by Day 3, the drug effect has worn off. The injection on Day 6 has no effect on Day 7, possibly because activity in the pathways involved in memory was low.

The results of this experiment and others (Erickson & Patel, 1969) suggest that increased release of ACh following the learning event is the initial process facilitating or mediating the development of an enduring neural change subserving memory.

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