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BRIEF COMMUNICATION

Evidence for Adrenergic Neurons in a Memory Access Pathway[1]

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COHEN, R. P. AND M. D. HAMBURG. *Evidence for adrenergic neurons in a memory access pathway.* PHARMAC. BIOCHEM. BEHAV. 3(3) 519–523, 1975. – Injection of a beta-adrenergic blocker, propranolol, in rats within 5 min after training of a step-down passive avoidance response had no effect on performance either 2 or 6 hr later, however, when testing occurred 1, 3 or 7 days after training and injection a significant performance decrement was observed. If drug injection was postponed until 1 or 3 days after training and testing was conducted 2 hr later, again poor avoidance performance was obtained. No support for a state-dependency explanation [14] of the propranolol amnesia could be found. The amnesia that followed beta-adrenergic receptor block was identical to that previously reported when norepinephrine biosynthesis was reduced [9] and supports the hypothesis of a role for adrenergic neurons in memory formation and retrieval that is different from cholinergic neurons [4,10].

Memory Learning Retrograde amnesia Antiadrenergic Norepinephrine Beta-adrenergic block Propranolol

A TEMPORARY depletion of brain norepinephrine in rats produced by injection of a dopamine beta-hydroxylase inhibitor, diethyldithiocarbamate (DDC), 30 min prior to testing prevented performance of a trained passive avoidance response 1, 3, 5 or 7 days after training. Subsequent recovery in performance indicated that the memory itself was not destroyed but rather that the process of memory retrieval was affected. Animals treated with DDC up to 3 hr before training were capable of learning the passive avoidance task and of avoidance performance for a few minutes after training, however no long-term memory was produced and animals appeared naive if testing occurred longer than 6 hr after training [9].

Either intracerebral injection of the anticholinesterase, diisopropyl florophosphate (DFP) [4, 5, 19] or systemic injection of physostigmine [1, 6, 8, 10] also produced a retrograde amnesia of a previously trained response, however unlike the anticholinesterase induced amnesias, the degree of amnesia produced by DDC was not dependent upon the age of the memory at the time of injection [9]. In addition, injection of an anticholinesterase drug prior to training had no effect on memory formation nor subsequent performance of the trained response [4,10] while treatment with DDC prior to training prevented formation of a long-term memory [9].

Previous experiments have suggested a role for noradrenergic neurons in the formation and retrieval of memories. This hypothesis is based on the specific temporal pattern of the amnesia produced following a DDC-induced reduction of norepinephrine biosynthesis with a presumed drop in noradrenergic transmission [9,15]. This study supports this hypothesis by reporting a similar amnesia when the efficiency of noradrenergic transmission was reduced instead by injection of a beta-adrenergic receptor blocker, propranolol. The experimental method followed was identical to that previously employed [9,10] in order to permit direct comparison of the current data with that of the earlier studies.

Laverty and Taylor [12] and Singh *et al.* [18] have reported no change in Y-maze performance following propranolol treatment, however Richardson *et al.* [16] have clearly reported that daily injections of propranolol disrupt DRL performance (differential reinforcement of low rates) for food reward. Also memory formation of a shuttle avoidance task in mice was prevented by injection of another beta-blocker, dichloroisoproterenol (DCI) [7].

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METHOD

Animals

One hundred fourteen male albino rats (Sprague-Dawley, Holtzman Strain) were used. All animals were 2–3 months old upon arrival in the laboratory and were trained 7–21 days later. Rats were housed in community cages and had access to ample food and water at all times except while they were in the experimental room.

The task chosen was a step-down passive avoidance task [2, 3, 11, 13] in which the animals were taught to remain on a small raised platform in order to avoid foot shock. Length of time on the platform (step-down latency) served as a measure of task retention. The apparatus and procedure have been described elsewhere in detail [10]. Briefly, training consisted of 5 trials. On each trial the rat was placed on the platform and the step-down latency recorded. Animals were placed in a holding cage adjoining the experimental box for a 1-min rest period between each trial. On Trials 1–3 no foot shock was applied. On Trial 4, foot shock (approximately 0.4 mA) was administered for 15 sec. Trial 5 was conducted in a similar manner to Trial 4. On this trial, if the rat did not step down within 30 sec it was removed to the home cage and a step-down latency of 30 was recorded. On Trials 1–4 all animals stepped down within 30 sec (Mdn step-down latency for Trial 4 was 2.11 sec).

On the testing day, each animal was returned to the experimental room and placed on the platform for one trial. Upon step-down or after 30 sec on the platform the experiment was terminated.

Drug Injection

Each animal received either an intraperitoneal injection of propranolol (45 mg/kg; 2 ml/kg injected volume) (kindly supplied by Ayerst Laboratories, New York, New York) or similar volume of physiological saline. Drug injection occurred at different times relative to training and testing as indicated in the group procedures below.

EXPERIMENT 1

The purpose of the first experiment was to determine if a propranolol induced adrenergic block immediately after training of a passive avoidance response would prevent the formation of a long-term memory and subsequent performance of the trained response. Fifty-one animals were trained and randomly assigned to 1 of 6 groups. Six additional animals (Group 1.2) received the training experience, but no foot shock was delivered. Of the trained animals all but Group 1.7 were injected with propranolol within 5 min after completion of training. Testing occurred either 2 hr, 6 hr, 1 day, 3 days or 7 days after training and injection. Group 1.7 received an injection of saline 5 min after training instead of the drug and was tested 7 days later. The no foot shock control group was tested 2 days after training and injection.

Results

Table 1 presents the mean, median and standard deviation for Trials 4 and 5 of training and the Testing Trial for all groups. In all experiments, the scores of each group were individually compared with all other groups in the experiment by Mann-Whitney U test to determine if the differences were significant. Two-tailed p values were calculated by Siegel's method for direct critical values for Mann-Whitney U test [17]. In the case of multiple comparisons, the p value reported is the least significant value of the tests conducted.

Injection of propranolol within 5 min after training had no effect on performance either 2 or 6 hr later as compared with Trial 5 training scores ($p<0.01$). However, when testing was delayed until 1, 3 or 7 days after training and injection a significant difference existed between each group's Testing Score and its Trial 5 training score as well as between each group's Testing Score and the Testing Scores of Groups 1.2 and 1.3 (2 and 6 hr groups) (least significant difference, $p<0.02$). The slow step-down rates of animals tested 2 and 6 hr after treatment could not be due to a toxic effect of the drug as indicated by a comparison with step-down latencies of the no shock control animals (Group 1.2). Normal retention of the trained response for periods up to 7 days was demonstrated by the saline control animals (Group 1.7).

These results agree with previous data on DDC-induced storage amnesia [9]. The formation of a long-term memory appeared to be prevented by drug injection immediately after training. If animals were tested within the first 6 hr after training and injection short-term memory was sufficient to produce reliable performance of the trained response, but a significant amnesia was evident at longer testing intervals.

EXPERIMENT 2

In addition to preventing the formation of long-term memory (storage amnesia), DDC treatment prior to testing prevented the performance of a previously trained passive avoidance response (retrieval amnesia) [9]. Experiment 2 was conducted to determine if similar results could be obtained using propranolol.

Twenty-seven animals were trained in the passive avoidance response and randomly assigned to 1 of 3 groups. An additional 8 animals (Group 2.2) received the training experience but no foot shock was administered. Groups 2.1 and 2.2 were injected with propranolol 1 day after training. Group 2.3 received instead an injection of saline 1 day after training. Group 2.4 received propranolol injection 3 days after training. All animals were tested 2 hr after injection.

Results

Table 2 presents the mean, median and standard deviation for Trials 4 and 5 of training and the Testing Trial for all groups. Injection of propranolol either 1 day (Group 2.1) or 3 days (Group 2.4) after training significantly ($p<0.05$) impaired performance 2 hr later when compared to saline injected control animals (Group 2.3). The testing performance of the propranolol treated animals was not significantly different from animals receiving no foot shock during training (Group 2.2).

EXPERIMENT 3

A final experiment was conducted to determine if the above results could be alternatively accounted for as a state-dependency phenomenon. Overton [14] has reported that rats trained in a T-maze escape task following a sub-anesthetic dose of pentobarbital performed the task poorly when subsequently tested after the drug had worn off. The

TABLE 1

EXPERIMENT 1: MEAN, MEDIAN AND STANDARD DEVIATION OF STEP-DOWN LATENCIES IN SEC

	Group	N	Training Trial 4		Training Trial 5		Testing Trial	
			mean	mdn	mean	mdn	mean	mdn
1.1	Tr —5m— Prop —2h— Ts	12	1.94 (0.48)*	2.01	30.00 (0.00)	30.00	23.66 (10.71)	30.00
1.2	Tr_{NS} —5m— Prop —2h— Ts	6	1.86 (0.78)	1.52	3.81 (3.28)	2.51	2.40 (1.52)	1.79
1.3	Tr —5m— Prop —6h— Ts	8	2.13 (1.69)	1.53	30.00 (0.00)	30.00	23.37 (10.68)	30.00
1.4	Tr —5m— Prop —1d— Ts	9	3.49 (1.42)	3.31	30.00 (0.00)	30.00	9.24 (11.96)	4.25
1.5	Tr —5m— Prop —3d— Ts	8	2.08 (1.02)	1.62	30.00 (0.00)	30.00	5.11 (10.08)	1.32
1.6	Tr —5m— Prop —7d— Ts	8	4.03 (3.69)	2.89	30.00 (0.00)	30.00	6.73 (20.41)	1.79
1.7	Tr —5m— Saline —7d— Ts	6	2.25 (0.53)	2.09	30.00 (0.00)	30.00	28.82 (2.01)	30.00

*Figures in parentheses are standard deviations

converse was also true; animals trained normally performed poorly when tested under the influence of the drug. However, animals trained under pentobarbital and tested under the drug as well performed significantly better than either of the first two groups. Good testing performance was dependent upon reinstatement of the drug condition that was present during training.

Twenty-two animals were injected with propranolol, trained in the passive avoidance response 2 hr later and randomly assigned to 1 of 2 groups. Both groups were tested the following day. One group received an injection of propranolol 2 hr before testing thus restoring the drug-state that existed at the time of training while the other group received no injection prior to testing.

Results

Table 2 presents the mean, median and standard deviation for Trials 4 and 5 of training and the Testing Trial for both groups (Groups 2.5 and 2.6). Training was normal for both groups (not significantly different from all other training scores of animals receiving foot-shock) and both groups showed significant ($p<0.05$) and similar amnesias at the time of testing (compared with testing scores of Groups 1.4, 1.5, 1.6, 2.1 and 2.4). As previously reported for DDC-induced amnesia [9], no support for a state-dependency explanation of the propranolol amnesia could be found.

DISCUSSION

When noradrenergic transmission was reduced by beta-adrenergic block within 5 min of training, adequate performance was possible for a short period thereafter. However, the formation of a long-term memory appeared to be prevented and no evidence of the previous training could be found at longer than 6 hr after the training and injection. If drug injection was postponed until 1 or 3 days after training (presumably until after a long-term memory had been formed) a considerable decrement in avoidance performance was seen 2 hr after injection. A state-dependency explanation appeared inadequate to explain these results.

A role for adrenergic fibers in the access pathway of long-term memory is supported by this study. When the efficiency of adrenergic transmission was diminished either

TABLE 2

EXPERIMENT 2: MEAN, MEDIAN AND STANDARD DEVIATION OF STEP-DOWN LATENCIES IN SEC

	Group	N	Training Trial 4		Training Trial 5		Testing Trial	
			mean	mdn	mean	mdn	mean	mdn
2.1	Tr —1d— Prop —2h— Ts	11	2.14 (0.94)*	1.78	29.66 (1.14)	30.00	6.62 (11.57)	1.54
2.2	Tr_{NS} —1d— Prop —2h— Ts	8	1.35 (0.34)	1.36	1.62 (0.53)	1.68	2.29 (0.96)	2.15
2.3	Tr —1d— Saline —2h— Ts	8	1.94 (0.72)	1.62	29.85 (0.42)	30.00	27.99 (5.00)	30.00
2.4	Tr —3d— Prop —2h— Ts	8	1.95 (0.64)	1.94	30.00 (0.00)	30.00	5.62 (9.23)	1.91
2.5	Prop —2h— Tr —1d— Ts	12	2.16 (0.58)	2.11	29.89 (0.40)	30.00	7.51 (8.37)	3.80
2.6	Prop —2h— Tr —1d— Prop —2h— Ts	10	1.84 (0.82)	1.52	29.44 (1.42)	30.00	5.74 (8.92)	2.83

*Figures in parentheses are standard deviations

by the inhibition of norepinephrine biosynthesis [9] or by beta-adrenergic block, similar alterations in the processes of long-term memory storage and retrieval were observed. In addition, a recently completed study (Hamburg and Kerr, unpublished) has further demonstrated that injection of DL-threo-3,4-dihydroxyphenylserine (DOPS), a direct precursor of norepinepherine that does not require dopamine beta-hydroxylase for conversion to norepinephrine, prior to DDC treatment prevented a DDC-induced storage or retrieval amnesia. Of considerable interest is the fact that in none of the studies discussed in which cholinergic or adrenergic transmission has been modified has response acquisition (short-term memory) been prevented, nor normally trained responses (long-term memory) been destroyed; rather the functioning of the input and output pathways of long-term memory appear affected.

REFERENCES

1. Biederman, G. B. Forgetting of an operant response: physostigmine-produced increase in escape latency in rats as a function of time of injection. *Q. Jl exp. Phychol.* **22:** 384–388, 1970.
2. Carew, T. J. Do passive-avoidance tasks permit assessment of retrograde amnesia in rats? *J. comp. physiol. Psychol.* **72:** 267–271, 1970.
3. Chorover, S. T. and P. R. Schiller. A short-term retrograde amnesia in rats. *J. comp. physiol. Psychol.* **59:** 73–78, 1965.
4. Deutsch, J. A., M. D. Hamburg and H. Dahl. Anticholinesterase-induced amnesia and its temporal aspects. *Science* **151:** 221–223, 1966.
5. Deutsch, J. A. and H. Lutzky. Memory enhancement by anticholinesterase as a function of initial learning. *Nature* **213:** 742, 1967.
6. Deutsch, J. A. and N. I. Wiener. Analysis of extinction through amnesia. *J. comp. physiol. Psychol.* **69:** 179–184, 1969.
7. Dismukes, R. K. and A. V. Rake. Involvement of biogenic amines in memory formation. *Psychopharmacologia (Berl.)* **23:** 17–25, 1972.
8. Hamburg, M. D. Retrograde amnesia produced by intraperitoneal injection of physostigmine. *Science* **156:** 973–974, 1967.
9. Hamburg, M. D. and R. P. Cohen. Memory access pathway: Role of adrenergic versus cholinergic neurons. *Pharmac. Biochem. Behav.* **1:** 295–300, 1973.
10. Hamburg, M. D. and D. R. Fulton. Influence of recall on an anticholinesterase induced retrograde amnesia. *Physiol. Behav.* **9:** 409–418, 1972.

11. Jarvik, M. E. and W. B. Essman. A simple one-trial learning situation for mice. *Psychol. Rep.* **6:** 290, 1960.
12. Laverty, R. and K. M. Taylor. Propranolol uptake into the central nervous system and the effect on rat behavior and amine metabolism. *J. Pharm. Pharmac.* **20:** 605–609, 1968.
13. Lewis, D. J., R. Miller and J. R. Misanin. Control of retrograde amnesia. *J. comp. physiol. Psychol.* **66:** 48–52, 1968.
14. Overton, D. A. State-dependent or "dissociated" learning produced with pentobarbital. *J. comp. physiol. Psychol.* **57:** 3–12, 1964.
15. Randt, C. T., D. Quartermain, M. Goldstein and B. Anagnoste. Norepinephrine biosynthesis inhibition: effects on memory in mice. *Science* **172:** 498–499, 1971.
16. Richardson, J. S., P. D. Stacey, P. W. Cerauskis and R. E. Musty. Propranolol interfered with inhibitory behaviour in rats. *J. Pharm. Pharmac.* **23:** 459–460, 1971.
17. Siegel, S. *Nonparametric Statistics,* New York: McGraw Hill, 1956, p. 271–273.
18. Singh, K. P., D. S. Bhandari and M. M. Mahawar. Effects of propranolol (a beta adrenergic blocking agent) on some central nervous system parameters. *Indian J. Med. Res.* **59:** 786–794, 1971.
19. Wiener, N. I. Electroconvulsive shock induced impairment and enhancement of a learned escape response. *Physiol. Behav.* **5:** 971–974, 1970.