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Reversible Sodium Pentobarbital Amnesia in One Trial Discrimination Learning[1]

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WRIGHT, D. C., D. L. CHUTE AND G. C. MCCOLLUM. *Reversible sodium pentobarbital amnesia in one trial discrimination learning.* PHARMAC. BIOCHEM. BEHAV. **2**(5) 603–606, 1974. – Reversal of drug induced retroactive amnesia, previously observed in passive avoidance, is extended to a task measuring response choice rather than latency. Thirsty rats were given 3 drugged (D) and 3 non-drugged (ND) CRF bar-press for water sessions in a 2-bar box with one bar (B_1) present. During the seventh session, all animals were undrugged and both bars were present; 3 non-reversal (NR) groups received a single reinforcement after pressing the originally reinforced bar (B_1) while 3 reversal (R) groups received a single reinforcement only after pressing the previously unavailable bar (B_2). All animals were D or ND injected immediately after this session. All animals were again injected prior to the 24 hr test with both bars present in extinction. NR animals performed 80–84% of their test bar presses on B_1 as did R animals given D post-learning and ND pre-test (group R-D-ND). R groups given ND post-learning and pre-test (R-ND-ND) or D post-learning and pre-test (R-D-D) showed retention with 60-68% B_1 presses in test. The difference between groups R-D-D and R-D-ND suggests that the apparent amnesia shown by R-D-ND results from retrieval failure not consolidation failure.

Retrieval failure Retrograde amnesia reversal Memory consolidation Sodium pentobarbital Intrathoracic injection State dependent learning

RETROACTIVE amnesia, whether produced by electroconvulsive shock (ECS) or anesthetic drugs, has been shown to be reversible in passive avoidance tasks [2, 4, 7, 8, 9, 10]. Treatment with anesthetic agents after acquisition of a passive avoidance task results in performance decrements in later testing (e.g., [2, 6, 10]). However, the ability to induce better test performance in treated animals by pre-test redintegration of the drug state effective immediately after training, argues for an explanation based on retrieval failure rather than consolidation failure [2,10].

Although extensive work has shown that discrimination learning can be brought under drug state control (e.g., [5]), the reversal of retroactive amnesia produced by post-trial sodium pentobarbital administration has only been done within the context of a one-trial passive avoidance task [2,10]. Since the dependent variable is response latency, drug produced ataxia may artificially elevate response latency and artifactual alterations of fear or photophobia may occur. These side effects remain as alternatives to the state redintegration hypothesis of retroactive amnesia reversal. The use of an appetitive choice task in which the dependent variable is response distribution rather than response occurrence/non-occurrence should obviate such alternative explanations.

In the present study the task is a discrimination reversal with water reinforcement almost identical to that in which Bloch *et al.* produced retrograde amnesia with fluothane anesthesia [1]. However, an additional group is used which shows that the apparent retrograde amnesia, when produced by sodium pentobarbital, can be reversed by reinstating the same drug state during retention testing as was effective immediately after reversal learning. In the present study intrathoracic injection of sodium pentobarbital after the single reversal learning session was used to insure rapid induction of the effective drug state immediately after learning, presumably within the "memory consolidation interval," and to allow comparison with previous work on reversing passive avoidance amnesia [2, 9, 10]. Post-

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training treatment was chosen to parallel retrograde amnesia paradigms.

METHOD

Fifty male Sprague-Dawley rats (Holtzman, Madison, Wisconsin) weighing 350 to 400 g were used. The animals were housed individually and water deprivation was maintained each day for 23 3/4 hr, all animals being allowed 15 min free access to water in their home cage, 15 min after the termination of each daily session. Standard laboratory chow was available ad lib.

The apparatus consisted of 8 Skinner boxes with the left side white and smooth floored, and the right side black and rough floored. On each side a magazine and retractable bar were available. The entire apparatus was controlled by a DDP 116 digital computer.

Magazine training, with neither bar available, consisted of 6 daily 15 min sessions of alternating 15 left and 15 right reinforcements (0.15 ml water). Bar-press training on a continuous reinforcement schedule occurred for a subsequent 6 day period. During these sessions only one bar (B_1) was present. Within each group, B_1 was the left bar for half of the animals, and was the right bar for half of the animals. A session lasted until 17 reinforcements had been given, or 15 min had elapsed. To establish stable bar pressing behavior regardless of drug state, all animals were magazine trained and bar-press trained both drugged (D) and non-drugged (ND).

During magazine and bar-press training, animals received intraperitoneal injections 20 min prior to a session, for an equal number of alternating D and ND sessions. The alternating sequence of D and ND sessions began with D for half the animals and with ND for the other half of the animals within each group. Since multiple intrathoracic injections may result in pleural adhesions [10], intraperitoneal injection was chosen for this portion of the experiment to reduce the risk of unnecessarily compromising the animals. Throughout the entire experiment drugged animals received 12.5 mg/kg sodium pentobarbital while non-drugged animals received an equivalent volume of saline.

Following bar-press training, a single reversal or non-reversal learning session was given. The previously trained bar (B_1) and the opposite bar (B_2) were both present, and no injections were given until the session was completed. Reversal learning groups (R) pressed B_1 in extinction, and obtained one reinforcement for pressing B_2. For non-reversal learning groups (NR), B_2 was in extinction and the animals received one reinforcement after 7 B_1 presses. Pilot data had indicated that in the reversal condition, animals averaged 7 presses to B_1 prior to response on B_2. Thus an FR-7 for NR groups was chosen to provide equivalent partial reinforcement experience for both the R and NR groups. Within 10 sec of the single reinforcement in the R or NR condition, the animals were picked up, injected intrathoracically according to group designation, and returned to their home cages. For intrathoracic injections, a 25 g 5/8 in. needle was inserted into the thoracic cavity just medial and caudal to the right scapula. The onset of behavioral effects (mild ataxia, pupillary dilation) are more rapid (approximately 60 sec) with intrathoracic injections, compared to intraperitoneal injection. Variability between animals is reduced using the intrathoracic technique, as the possibility of hepatic-portal absorption with consequent drug degredation in the liver, does not exist.

A 20 min retention test was given 24 hours later with both bars B_1 and B_2 present, in extinction. Animals were injected intrathoracically 20 min prior to testing with D or ND according to groups. There were 3 groups receiving non-reversal training: Group NR-ND-ND (n = 9), received ND after training and prior to test, Group NR-D-ND (n = 8) received D after training, and ND prior to test while Group NR-D-D (n = 8) received D after training and prior to test. Three groups received reversal training: Group R-ND-ND (n = 9) received ND after training and prior to test, Group R-D-ND (n = 8) received D after training and ND prior to test and Group R-D-D (n = 8) received D after training and prior to test.

RESULTS

The mean number of bar presses in the three D and the three ND bar-press training sessions are shown in Table 1. One tailed Mann-Whitney U tests were used for all between group comparisons and one-tailed sign tests were used for all within groups comparisons [3]. There were no differences between groups for total number of presses during bar-press training (Mann-Whitney U tests [3]) and no differences for bar-presses within groups between D and ND training states (sign tests [3]).

Table 1 also shows the mean number of responses to B_1 and B_2 during R or NR learning. The number of responses to B_1 was fixed at 7 for NR groups and for the R groups the overall mean number of responses to B_1 was 7.56 with no differences among the groups. The number of responses to B_2 was fixed at 1 for the R groups and the NR groups showed an overall mean of 0.94 responses to B_2 with no differences among the groups (Mann-Whitney U tests [3]).

Retention test bar-presses are presented in Table 1. Mann-Whitney U tests [3] were used to make between group comparisons for the number of responses to the original training bar B_1, the number of responses to the reversal bar B_2, and the number of responses to B_1 expressed as a percent of total responses.

Only the ratio of responding to B_1 as a percent of total responses ($B_1 \div (B_1 + B_2) \times 100\%$) yielded significant differences between groups. As can be seen in Fig. 1, the 3 non-reversal groups did not differ from each other suggesting that post-learning and/or pre-test drug administration did not, by itself, influence response production or response distribution. Group R-D-ND showed apparent amnesia for the reversal learning, not differing from the three NR groups. Groups NR-ND-ND and R-D-ND performed almost identically to the equivalent control and reversal-amnesia groups of Bloch *et al.* [1], distributing 80–84% of their test bar-presses to B_1.

Groups R-D-D and R-ND-ND did not differ significantly from each other ($0.05<p<0.10$) and both showed evidence of the reversal training. Group R-ND-ND performs much like the equivalent reversal group of Bloch *et al.* [1] with less than 60% of their test responses distributed to B_1. Groups R-ND-ND ($p<0.01$) and R-D-D ($p<0.05$) differed from the NR groups and from Group R-D-ND.

DISCUSSION

It should be noted that in the present experiment, and the experiments of Bloch *et al.* [1], arbitrary test periods (20 min and 15 min respectively) were used and discrete choice behavior per se was not used as an index of discrimination. It is possible that the imposition of a time

TABLE 1

GROUP MEAN BAR PRESS RESPONSES DURING TRAINING, LEARNING AND TEST

Group	N	Mean Responses During Training*		Mean Responses During Learning		Mean Response During Test		
		D	ND	B_1	B_2	B_1	B_2	$B_1 \div B_1+B_2 \times 100\%$
NR-ND-ND	(9)	34.44	32.33	7	0.56	25.11	7.44	80.21%
NR-D-D	(8)	35.50	35.88	7	1.38	29.59	4.38	83.61%
NR-D-ND	(8)	32.88	36.50	7	0.88	22.25	6.63	82.36%
R-ND-ND	(9)	33.44	33.00	7.56	1	19.00	11.56	59.67%
R-D-D	(8)	37.00	37.87	8.38	1	31.62	15.88	68.19%
R-D-ND	(8)	36.00	33.38	6.75	1	28.13	9.50	80.36%

*average number of responses during the 3 drugged (D) and the 3 nondrugged (ND) training sessions

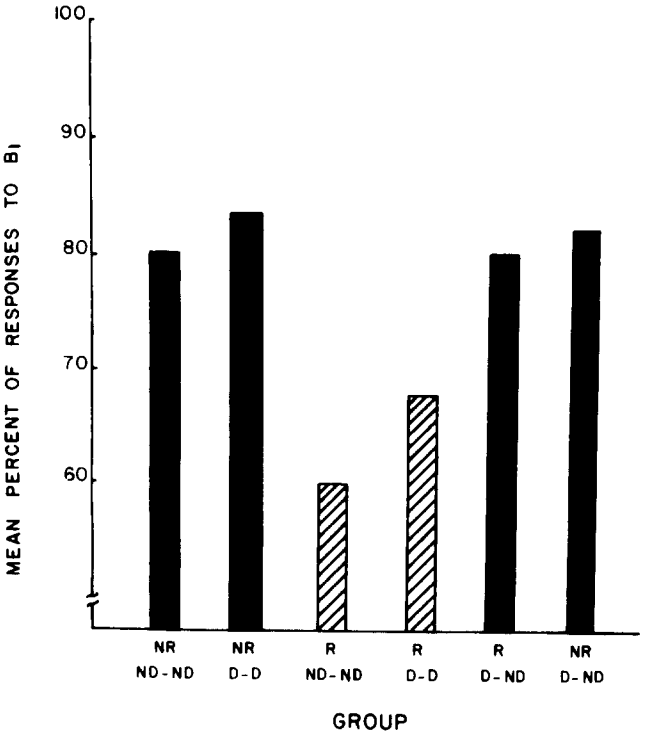

FIG. 1. Mean percent of bar press responses of trained bar (B_1) to total responses ($B_1 + B_2$) per group. Columns with same shading are not significantly different ($p>0.05$), while columns opposite in shading are different ($p<0.05$).

(and therefore a response number) ceiling could have inflated the discrimination index used. However, the responding interval allowed during test was equal to the maximum interval allowed in original CRF acquisition in the Bloch *et al.* experiment [1] and was 5 min longer than that allowed in original acquisition in the present experiment. In any respect, it is clear that the response distribution measure does yield reliable group test result differences attributable to training differences (e.g., NR-ND-ND vs. R-ND-ND) in both the Bloch *et al.* [1] and present experiments.

The demonstration of drug state dependent retrieval control in this appetitive task would seem to circumvent some of the problems common to passive avoidance tasks. Since response distribution rather than response latency is measured, drug artifacts affecting motor ability and motivation should be attenuated. This is especially important where treatment immediately precedes testing. The various drug manipulations had no statistically significant effect on response production in training and test, perhaps because both original magazine and barpress training were given in both the D and ND states. However, analysis of response distribution reveals that one trial discrimination learning is susceptible to drug induced retrieval control.

The amnesia produced by sodium pentobarbital administration after reversal learning in Group R-D-ND is impermanent. When the D state present immediately following reversal learning is redintegrated prior to testing, retention of reversal training is evident as shown by Group R-D-D.

While inadequate to test for symetrical state dependent learning of this task, the experiment does demonstrate that induction of the D state (Group R-D-D), is a sufficient condition to allow retrieval in presumably amnesic animals. Animals given only the injection procedure pre-test (R-D-ND) and animals given D treatment in the absence of R training (NR-D-D) do not show R behavior in test.

State dependent retrieval explanations have also been applied for both sodium pentobarbital and ECS induced retroactive amnesia reversals in passive avoidance tasks [2, 8, 9, 10]. Such post-trial treatments do not appear to prevent memory storage; rather, they appear to stipulate the CNS state that must obtain during testing if memory retrieval is to occur.

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