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Facilitation of Retention Performance in Mice by Posttraining Diethyldithiocarbamate[1]

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HAYCOCK, J. W., R. VAN BUSKIRK, AND J. L. MCGAUGH. *Facilitation of retention performance in mice by posttraining diethyldithiocarbamate.* PHARMAC. BIOCHEM. BEHAV. 5(5) 525–528, 1976. – These experiments examined the effects in mice of posttraining injections of diethyldithiocarbamate (DDC) upon retention (7 days after training) of active avoidance learning and upon whole brain catecholamine levels. When administered immediately following training, DDC enhanced retention performance. The degree of enhancement varied directly with the dose. DDC did not significantly affect retention performance if the injections were delayed 1 or 4 hours after training. Also, DDC administered 30 min prior to training did not affect retention performance. DDC (900 mg/kg) produced a large but transient increase in whole brain dopamine (DA) levels while norepinephrine (NE) levels were lowered.

Memory facilitation Diethyldithiocarbamate Retention Mice Active avoidance Norepinephrine Dopamine Brain

DIETHYLDITHIOCARBAMATE (DDC) produces deficits in retention performance (retrograde amnesia) in a variety of training situations [7, 11, 23, 24] if the drug is administered shortly following the training experience. In these studies, DDC inhibited dopamine-beta-hydroxylase [24] and decreased brain NE levels [23,24]. That is, the lowered NE levels and decreased conversion of dopamine to NE correlated, in a general sense, with the disruption of retention. A recent investigation [3] indicates, however, that DDC can enhance consolidation of shuttle box avoidance. This report suggests that DDC's effects upon retention may involve hippocampal heavy metals [3]. However, pharmacological evidence suggests a primary role for dopamine (DA) in shuttle avoidance performance [25]. This presents an interesting hypothesis for the facilitating effects of DDC upon shuttle box avoidance in that DDC also affects DA levels at short postinjection times [14,15].

The present study investigated the dose- and time-dependent effects of DDC upon retention of active avoidance learning and upon both brain NE and DA levels (not measured in previous investigations [23, 24, 27]).

METHOD

Animals

One hundred eighty-six naive, adult, male Swiss Webster mice (Ha/ICR from ARS/Sprague-Dawley, Wisc.; 55–65 days old upon arrival) were used. The animals were housed in groups of 8 in metal cages with food and water available ad lib and were maintained on a 12:12 light-dark cycle (7 A.M. on, 7 P.M. off). All animals were maintained in the laboratory for at least 5 days prior to training or biochemical analyses.

Procedure

Training. One hundred forty-four of the mice were given 50 training trials in a two-way shuttle box [19] and 50 trials on the retention test 7 days later. In each session the animals were placed in the shuttle boxes for a 5 min adaptation period. The number of shuttles made by each animal during the last 150 sec of this period was recorded (pretrain and pretest shuttles). The animals then received 50 trials with an intertrial interval of 30 sec. On each trial a light and buzzer were presented to the side which the animal currently occupied. Light and buzzer onset was followed, 5 sec later, by a shock (approx. 150 μA) delivered through the grid floor. The signals and shock terminated 10 sec later or upon the animal's performance of a shuttle response. The animals avoided the footshock if they shuttled within 5 sec after the onset of the light and buzzer. Failure to perform an avoidance response constituted an error. The same procedures were used on the 50 trial retention test. All training and recording procedures were automated.

To assess dose-response effects of DDC upon retention 64 mice were assigned randomly to 4 groups. All mice were given 50 training trials as described above. Immediately following training each animal was given one of the following treatments: saline, 100, 300, 900 mg/kg DDC. To assess the effects of time of administration of DDC upon retention, 80 mice were assigned randomly to 5 groups. Animals in one group received saline 30 min prior to

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training. Animals in the other 4 groups received 900 mg/kg DDC either 30 min prior to, immediately after, 1 hr after, or 4 hr after training. One animal in each of the DDC groups was eliminated due to apparatus failure, and 3 additional animals injected 30 min prior to training died during the training. Notably, no animals died which received 900 mg/kg after training.

Biochemical Analyses. Forty-two other mice were sacrified by cervical dislocation either 30, 60, 120, 240, 480 or 1440 min following injection of either saline or DDC. Whole brains (including cerebellum and medulla) were rapidly dissected out. Catecholamines were assayed by the method of Shellenberger and Gordon [27]. Each sample consisted of one mouse brain. Brains were homogenized in 0.4 N perchloric acid; oxidized and read on a Farrand spectrofluorometer. The data are presented in terms of nanograms of catecholamines per gram of brain wet weight.

Drug Administration. Sodium diethyldithiocarbamate trihydrate from Sigma Chemical Co. was dissolved in double-distilled water. All injections were administered intraperitoneally (0.1–0.3 ml/10 grams body weight).

All statistical comparisons are based on the Kruskal-Wallis one-way analysis of variance, two-tailed Mann-Whitney U-test, or two-tailed *t*-test [1,28] with an alpha for rejection of H_0 of 0.05.

RESULTS

Figure 1 illustrates the effects of immediate posttraining administration of DDC upon the 7 day retention performance. There was a main effect of dose on test errors ($H = 15.84$, $df = 3$). Animals which received 300 and 900 mg/kg DDC made significantly fewer errors than did the saline-treated animals (300: U = 75; 900: U = 30.5). There were no differences in errors during training ($H = 0.45$, $df = 3$), in shuttles during training or testing ($H = 1.31, 2.16$, $df = 3$, respectively), or in pretrain of pretest shuttles ($H = 4.92, 5.23$, $df = 3$, respectively).

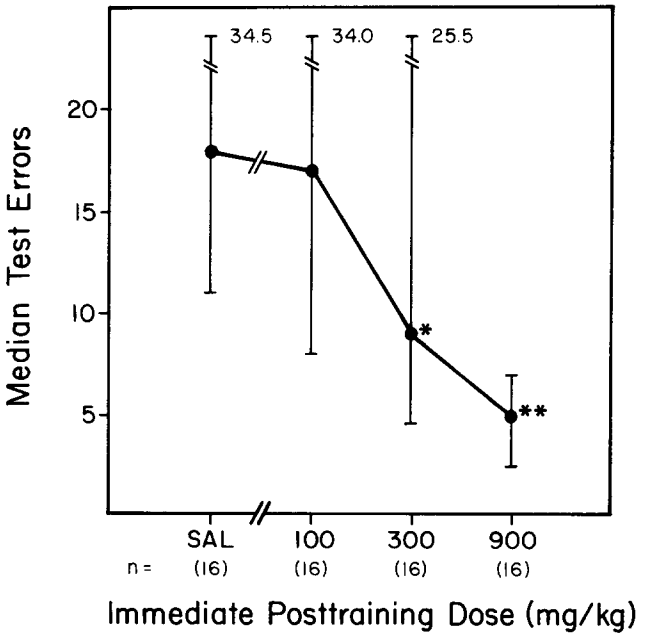

FIG. 1. Dose-response effects of immediate posttraining injections of DDC upon retention performance. Mice were trained and then different groups received either saline, 100, 300 or 900 mg/kg DDC intraperitoneally. Seven days later retention performance was tested. Error bars represent the interquartile ranges for each group. *$p<0.05$; **$p<0.002$; two-tailed Mann-Whitney U-test.

Delaying the time of DDC administration (900 mg/kg) after the training session abolished DDC's effects on retention performance. Animals injected immediately (n = 15, U = 66), but not 1 hr (n = 15) or 4 hr (n = 15), after training made significantly fewer errors than did the saline-treated animals (n = 16). Again, there were no differences in errors during training ($H = 3.72$, $df = 3$), shuttles during training or testing ($H = 1.77, 3.23$, $df = 3$, respectively) or pretrain and pretest shuttles ($H = 1.36, 12.4$ $df = 3$), respectively). As can be seen in Table 1, DDC impaired the acquisition of the active avoidance response when injected 30 min prior to training. The elevation in training errors was, however, accompanied by a significant decrease in pretrain and training shuttles (hence a decrease in escape and avoidance responses). Seven days after training there were no significant differences between the animals which received 900 mg/kg 30 min prior to training and saline-treated animals on any of the retention test session measures (Table 1), even if training-test error difference scores were compared (data not presented).

TABLE 1

EFFECTS OF DDC ADMINISTERED 30 MIN PRIOR TO TRAINING UPON ACTIVE AVOIDANCE PERFORMANCE

Treatment	N	Shuttles		Errors
		pretrain	training	training
saline	16	17.0* (15.0-21.0)†	54.0 (50.0-63.5)	34.5 (23.5-44.0)
900 mg/kg DDC	12	2.0 (0.0-3.0)	47.0 (40.0-52.0)	41.5 (35.5-49.0)
	U-value	0‡	38.5‡	53.0‡
		pretest	test	test
saline	16§	4.5 (2.0-7.0)	51.5 (50.0-55.0)	8.5 (6.0-16.5)
900 mg/kg DDC	12§	5.0 (0.0-8.0)	51.0 (50.0-52.5)	6.0 (4.5-18.5)
	U-value	93.5	84.0	84.0

*Median number of responses.
†Interquartile range.
§Same groups as above.
‡Denotes $p<0.05$, two-tailed comparison between saline and DDC groups.

The effects of DDC on whole brain catecholamine levels are presented in Table 2. Both 450 mg/kg and 900 mg/kg lowered NE levels significantly. Following 450 mg/kg DDC, NE levels were lowered for 2 hr but returned to control values essentially by 4 hr. The NE levels of animals which received 900 mg/kg were lower at all times investigated but had almost returned to control values at 24 hr. DA levels were slightly but insignificantly elevated following 450 mg/kg DDC. The 900 mg/kg dose of DDC produced a sharp rise in DA levels at 30 min followed by a rebound decrease at 2 hr which was over by 4 hr postinjection.

DISCUSSION

Centrally acting sympathomimetics (e.g., amphetamine [13]) and central catecholamine agonists (e.g., amantadine [5] enhance retention of some learning experiences when

TABLE 2

TIME COURSE OF DDC EFFECTS ON WHOLE BRAIN CATECHOLAMINE LEVELS

Time to Sacrifice (min)	Treatment saline	450 mg/kg DDC	900 mg/kg DDC
		dopamine levels (ng/g)	
30	771 ± 67	855 ± 27	1293 ± 166*
60	770 ± 28	824 ± 6	782 ± 99
120		801 ± 51	539 ± 110*
240		769 ± 7	704 ± 8
480		764 ± 36	739 ± 29
1440		766 ± 9	762 ± 16
combined	771 ± 32		
		norepinephrine levels (ng/g)	
30	380 ± 6	366 ± 4*	278 ± 47*
60	379 ± 4	202 ± 5*	213 ± 68*
120		286 ± 19*	95 ± 4*
240		362 ± 26	50 ± 3*
480		373 ± 9	141 ± 6*
1440		282 ± 32	350 ± 5*
combined	379 ± 3		

Each time point value represents the mean ± SEM of 3 determinations.

*Denotes $p<0.05$ compared to combined control group on two-tailed t-test.

the drugs are administered shortly after the training session. Conversely, posttraining administration of drugs such as reserpine and DDC, which disrupt central catecholamine metabolism, can produce retention impairment [7].

In the present studies, DDC produced a dose- and time-dependent enhancement of retention performance. Higher doses of DDC facilitated retention to a greater extent, and DDC's influence on retention decreased as the time between training and treatment increased. The procedure of administering drugs after training, but sufficiently long before testing for retention, allows the animals to be trained and tested while they are not under the direct influence of the drugs. With the use of this procedure, the effect of the DDC treatment upon retention performance can be interpreted in terms of the drug's influence on memory storage processes [16–18].

DDC also produced a relatively long lasting, dose-dependent decrease in whole brain NE and a relatively transient, dose-dependent increase in DA. DDC has, however, a variety of actions based on its ability to chelate metals [30] primarily in groups IB and IIB [29]. For example, chelation of copper inhibits the activity of dopamine-beta-hydroxylase and thus lowers brain NE levels [9, 10, 24]. But DDC's chelating action will also significantly inhibit other metallo-enzymes (e.g., liver aldehyde dehydrogenase [6]). And, chelation of zinc by DDC decreases sulphide-silver (Timm's) staining in the hippocampal mossy fiber system [4, 12, unpublished observations]. Clearly then, the correlation between enhanced retention performance and either NE decreases or DA increases cannot *a priori* provide an explanation of the cause of the behavioral effects. Modification of both catecholaminergic [23,24] and hippocampal function [2] by DDC may contribute to some aspects of modified retention produced by DDC. But the fact that DDC can produce either facilitation or disruption of retention performance when at least some of the same physiological effects occur (i.e., decreased NE levels, increased in DA levels, decreased hippocampal Timm's staining) makes it clear that an understanding of the bases of the effects of DDC on retention will require additional research.

Most investigations of the effects of DDC upon retention have reported that posttraining DDC disrupts retention (e.g., [7,23]; however, see [3]) And, studies from our laboratory have also observed that posttraining DDC disrupts retention of inhibitory avoidance training in rats and mice [in preparation]. The present results, however, demonstrate a facilitation of retention by posttraining DDC. One theory that might accommodate these task differences suggests that training elicits at least two distinct reactions, both of which are important for efficient memory storage: (1) transient activation of neuronal systems specific to the training situation (in terms of the information coded), and (2) transient activation of nonspecific systems (e.g., the pituitary-adrenal system) that modulate those specific neuronal systems [8]. Further, an optimal level of non-specific activation exists. Within this framework, a post-training treatment could influence retention by influencing those non-specific processes involved in modulation of the memory storage processes. One hypothesis, then, is that DDC disrupts the effects of nonspecific activation. In the case of a relatively weak (or short) training as in a one-trial inhibitory avoidance task, the nonspecific reaction is less than optimal and DDC disrupts retention by further reducing the non-specific effects. In the case of very intense training such as above (approx. 1 hr), the nonspecific reaction may be greater than optimal and DDC, by reducing the nonspecific effects, could actually facilitate retention.

Another possibility regarding the mechanisms of DDC's facilitation of retention preformance is suggested from Rech, Carr and Moore [25]. A low dose of α-methyl-p-tyrosine (which by itself was without effect on performance) disrupted conditioned avoidance responding of animals treated with reserpine 7 days prior. In this situation, α-methyl-p-tyrosine lowered brain DA, but not NE, levels. Thus the disruption of conditioned avoidance correlated with deficits in brain DA. A variety of other studies support this correlation [2, 21, 22, 26]. On the other hand, posttraining amantadine, a dopaminergic agonist, has recently been shown to facilitate retention of a conditioned avoidance learning task [5].

The failure of pretraining DDC to modify retention performance is at least consistent with a dopaminergic interpretation. In animals which received DDC 30 min prior to training, NE levels would have been lowered and Timm's staining would have been decreased in the posttraining period during which DDC administration enhanced retention performance. However, DA levels, although elevated at the onset of training, would not have been elevated following training. Thus, if the retrograde facilitation by DDC were due to an increase in central DA levels, the pretraining injections of DDC would not be expected to enhance retention. Thus these data suggest, although not conclusively, that multiple modes of action for DDC in altering retention may exist and that the predominance of any given mode of action may depend upon the training situation.

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