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BRIEF COMMUNICATION

Interacting Effects of Handling and *d*-Amphetamine on Avoidance Learning[1]

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GRIFFITHS, D. AND D. WAHLSTEN. *Interacting effects of handling and d-amphetamine on avoidance learning.* PHARMAC. BIOCHEM. BEHAV. 2(3) 439–441, 1974. – Rats were injected with either saline or 2 mg/kg of d-amphetamine 10 min prior to 50 trials of training with either the one-way, shuttle or shuttle-with-handling procedure. One-way training yielded more avoidances and shorter avoidance latencies than did either variant of shuttle training; it was not affected by the drug. The frequency of avoidances under shuttle training, on the other hand, was increased either by d-amphetamine or by handling the animal during the intertrial interval. When both the drug and handling were administered together, performance was similar to that during the usual shuttle training.

Avoidance learning Handling d-Amphetamine

IT HAS BEEN well established that handling an animal between trials during avoidance training can improve performance greatly [12, 13, 14]. The effect may occur because handling disrupts freezing behavior which would otherwise prevent the initiation of an active avoidance response. In two-way or shuttle avoidance training rats frequently anticipate shock by running in the wrong direction; the response is often punished and as a result freezing may become the predominant response in the hierarchy [1,2]. Handling then may disrupt this freezing. One-way avoidance, on the other hand, is an unambiguous task which is learned rapidly before intense freezing appears. Accordingly, handling has little effect on one-way avoidance learning [14].

A drug with excitatory effects such as *d*-amphetamine also improves shuttle avoidance learning [4,9], and it appears to act by disrupting freezing.

Since both handling and *d*-amphetamine may act by reducing freezing responses, the present study was run in order to make detailed comparisons of their behavioral effects in one-way and shuttle avoidance learning. Previous research had shown that avoidance latencies were much faster in one-way than in shuttle avoidance [14]. In addition, rats under one-way training were observed to approach the center gate prior to trial initiation, while those receiving shuttle training generally huddled in the far end of the compartment. It was therefore of interest to determine whether both handling and *d*-amphetamine would have similar effects on these behaviors as well as on avoidance probability.

METHOD

Animals

The 60 experimentally naive male Sprague-Dawley albino rats from Simonsen Laboratories were 60 to 90 days old and weighed 400 to 500 g at the beginning of training. All animals were housed singly and allowed free access to water and dry food. Each animal was handled for 5 min on each of 2 days prior to training.

Apparatus

The training apparatus was a 2 compartment shuttle box which has been described previously [14]. The conditioned stimulus (CS) was a 15 W frosted light bulb, which was centered on the side wall of each compartment. The unconditioned stimulus (US) was a pulsating, constant-current shock of 0.65 mA, which was delivered through a grid floor with every other bar wired in common.

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Procedure

On the day of training each animal was weighed and then assigned to one of 6 treatment groups to equate the groups for the animals' weights. The volume of fluid to be injected, 1.0 ml/kg, was also determined for each animal at this time. Thirty animals were injected with physiological saline (SAL) and 30 received 2.0 mg/kg of *d*-amphetamine sulphate (AMP) dissolved in saline; each animal was injected 10 min before its training session. The 30 animals in each injection condition were divided into 3 groups of 10 animals each which received either one-way, shuttle or shuttle-with-handling training.

Training for each animal consisted of a single session with 50 trials. The CS-US interval was 5 sec and the inter-trial interval (ITI) was 30 sec. A trial commenced with the raising of the middle door and onset of the CS. Crossing from the start to the safe box prior to US onset resulted in termination of the CS and lowering of the door and prevented the onset of the US. If the animal failed to cross before US onset, the US was applied until a successful crossing occurred. Half of the animals in each group began the first trial in the compartment to the experimenter's left side and the others to the experimenter's right side. Response latency was measured to 0.01 sec on a clock which was stopped by a microswitch under the grid floor of the safe compartment. Distance of the animal's head from the middle door at CS and US onsets was estimated on each trial by the experimenter, who used a scale in inches drawn on the far wall of each compartment for reference.

The animals receiving one-way training (OW) were required to run from the same start box to the same safe box on every trial. At 15 sec after entry into the safe box, the animal was removed by the experimenter and returned to the start box facing away from the middle door. During shuttle training (SH) animals were given trials with alternate start and safe boxes. The safe box into which the animal had just crossed became the start box for the next trial. At no time during the 50 trials was the animal handled by the experimenter. In shuttle-with-handling training (SHHA) the animals had alternating start and safe boxes as in SH training but were handled midway during the ITI as in OW training. At 15 sec after crossing into the safe box, the animal was removed by the experimenter and returned to the same safe box facing away from the middle door. That compartment then became the start box on the next trial.

The 6 groups of 10 animals apiece were designated OW-SAL, SH-SAL, SHHA-SAL, OW-AMP, SH-AMP and SHHA-AMP.

RESULTS

Several measures of avoidance learning are presented as mean scores for each group in Table 1. One animal in Group SH-SAL failed to perform even the escape response within 2 min on several trials, and its training was discontinued after the seventh trial. It was given a score of 0 avoidances and was excluded from further analyses. There was also one animal in Group SH-SAL which had no avoidances and therefore contributed no datum to the analysis of mean avoidance latencies. All other scores represented means for 10 animals.

Analysis of variance on the number of avoidances in 50 trials revealed no effect for Drug, a large effect for Training, $F(2,54) = 44.3$, $p<0.0001$, and a significant Drug × Training Interaction, $F(2,54) = 10.7$, $p<0.01$. Using the Newman-Keuls test on ordered means with $\alpha = 0.05$, it was found that OW-SAL and OW-AMP did not differ significantly; no differences existed between SH-AMP and SHHA-SAL or between SH-SAL and SHHA-AMP. All other comparisons yielded significant differences.

The latency of the avoidance response was not affected by the Drug condition, and the Drug condition did not interact with the Training procedure. There was, however, a large effect of Training on avoidance latency, $F(2,52) = 42.3$, $p<0.0001$; the OW procedure yielded shorter latencies than the SH or SHHA procedures, while the latencies under SH and SHAA training did not differ. Escape latencies were not differentially affected by any treatment condition ($F<1.0$).

The distance score at CS onset for each animal was the

TABLE 1

MEAN SCORES FOR EACH GROUP ON SEVERAL MEASURES OF AVOIDANCE LEARNING

Group	Avoidances in 50 Trials	Avoidance Latency (sec)	Escape Latency (sec)	Distance At CS Onset (in.)	Probability of Freezing, if No Avoidance
OW–SAL	44.0	1.58	7.26	4.11	0.536
SH–SAL	9.2	2.63*	6.79†	9.48†	0.626†
SHHA–SAL	23.4	2.59	7.19	9.88	0.701
OW–AMP	39.9	1.35	6.48	3.15	0.388
SH–AMP	23.1	2.43	6.84	8.22	0.453
SHHA–AMP	7.7	2.82	6.83	9.73	0.511

*n = 8 †n = 9

mean of the distance scores for the 50 trials, regardless of whether an escape or avoidance occurred on any trial. The animals which received *d*-amphetamine were generally closer to the center door than were those receiving saline, $F(1,53) = 4.21$, $p<0.05$, but the absolute difference, 0.79 in, was quite small. The Drug condition did not interact with Training. As observed for avoidance latency, a large effect on distances was observed due to Training type, $F(2,53) = 96.8$, $p<0.0001$; animals trained by the OW procedure were closer to the middle door than those trained with the SH or SHHA procedure, while distances under SH and SHHA training did not differ significantly. Examination of the frequency distributions of distance scores revealed that animals given OW training were within 2 in. of the door on over 50% of the trials while animals given SH or SHHA training were as close on less than 5% of the trials.

Freezing was detected by measuring the distance of the animal's head from the door at both CS and US onsets; freezing was said to occur on non-avoidance trials when the two distances were equal. Prior to analysis of variance, these proportions were transformed by $X' = 2 \arcsin \sqrt{x}$ to reduce heteroscedasticity. Analysis of these transformed scores revealed that animals receiving *d*-amphetamine were less likely to freeze on non-avoidance trials than those receiving saline, $F(1,53) = 13.7$, $p<0.01$. There was no significant effect on freezing due to either the Training procedure or the Drug × Training interaction.

DISCUSSION

As was expected, *d*-amphetamine had no effect under one-way training, while it improved avoidance under shuttle training. Other studies have found large changes in shuttle avoidance but no or slight changes in one-way avoidance using hippocampal lesions [5], cingulate lesions [6], and different shock intensities [11]. Altogether, these findings suggest that the mechanism which limits performance in shuttle avoidance is not important during one-way training.

That either *d*-amphetamine or handling alone improved shuttle avoidance constitutes a replication of previously mentioned studies. The surprising result was that handling animals which had been injected with *d*-amphetamine eliminated the facilitation usually resulting from either agent. When animals were injected with *d*-amphetamine and then were handled by the experimenter, they were prone to struggling and loud squeaking; their muscle tone seemed more tense than usual, too. It is conceivable that the two agents combined to yield a level of arousal or activation so high that they actually interfered with performance, as is often the case with very high doses of a drug [3,8]. That is, handling animals which receive *d*-amphetamine may act similarly to an increase in the dose of the drug. Several studies have reported that avoidance performance may actually decline under high doses of *d*-amphetamine [7,10]. Likewise, high levels of electric shock can interfere with shuttle avoidance [11]. It is thus important that researchers be aware of the possible interacting effects of handling with their experimental manipulation in behavioral studies.

Otherwise, handling and *d*-amphetamine had similar effects on behavior. Neither agent affected the avoidance latencies or the position in the shock box characteristic of the usual shuttle performance. Freezing on error trials was reduced during the CS-US interval only by *d*-amphetamine, but avoidance probability was increased equally by handling and the dose of *d*-amphetamine employed herein, so both must have reduced freezing in some way. Under the influence of both *d*-amphetamine and handling, freezing was less likely than without either agent, but the rats were not able to direct their responses properly.

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