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Effects of Alpha Methyl-para-tyrosine on the Recall of a Passive Avoidance Response[1]

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HALL, M. E. AND M. A. MAYER. *Effects of alpha methyl-para-tyrosine on the recall of a passive avoidance response.* PHARMAC. BIOCHEM. BEHAV. 3(4) 579–582, 1975. – Treatment with alpha methyl-para-tyrosine 4 hr before training on a passive avoidance task altered recall in mice tested 24 hr after training. The observed alterations were dependent on the intensity of the footshock used during training. Retention of the avoidance habit was reduced by drug treatment when a footshock of 1.6 milliamperes (mA) was employed, while retention by drug-treated mice was enhanced when a footshock of 0.16 mA was used. No significant differences in retention were noted when a footshock of 0.8 mA or no footshock was employed. These results could not be explained on the basis of drug-induced changes in activity or sensitivity to footshock, or to state-dependent learning.

Avoidance behavior Alpha methyl-para-tyrosine Memory Footshock

THE role of central catecholaminergic systems in the acquisition, retrieval, and performance of learned behaviors has attracted much attention in recent years, due in part to the rapidly expanding knowledge of such systems and to the availability of a variety of drugs capable of influencing these systems. Pharmacological reduction of central catecholamine (CA) content has been shown to disrupt the performance of well learned behaviors such as shuttle box avoidance [3, 8, 12]; conditioned suppression of drinking [16] and lever pressing [9,10]. In general, the greater the reduction of norepinephrine (NE) and dopamine (DA), the greater the disruption of performance. In the case of shuttlebox avoidance, for instance, at moderate levels of CA depletion, avoidance but not escape behavior is disrupted [12] while at higher levels of depletion both avoidance and escape behaviors are disrupted [3,12].

Several recent studies have examined the effects of CA depletion on the acquisition of learned behaviors. Cooper *et al.*, [2], for instance, observed that rats treated intracisternally with multiple doses of 6-hydroxydopamine in combination with pargyline showed no evidence of acquisition of an active avoidance response in the shuttlebox after as many as 100 trials over 4 days time. It must be noted, however, that rats preferentially depleted of NE actually displayed facilitated acquisition of the same task. Osborne and Kerkut [13], also using rats, noted that a dose of diethyldithiocarbamate sufficient to reduce brain NE by 40 percent, greatly retarded the acquisition of conditioned avoidance response. Randt *et al.* [14], observed that mice treated with diethyldithiocarbamate thirty minutes prior to training on a one-trial passive avoidance task showed little evidence of learning on retention tests given one, 6 or 24 hours later, while showing performance superior to controls at one minute and performance equivalent to controls at 5 minutes after training.

In another context, it has been suggested that the amnesic effects of puromycin [15] and acetoxycycloheximide [17] are perhaps due to some alteration in the availability of NE at adrenergic sites.

The present study is an examination of the effects of norepinephrine and dopamine depletion, achieved by treatment with alpha methyl-para-tyrosine (AMPT), an inhibitor of tyrosine hydroxylation [19], on the acquisition and retention of a passive avoidance response motivated by footshocks of different intensities.

METHOD

Animals

One hundred-sixty female mice (50 to 70 days of age) of the C57BL/6J strain were used. All mice were bred from breeding stocks obtained from the Jackson Laboratories, Bar Harbor, Maine. All animals were maintained with ad lib access to Purina Mouse Breeder Chow and tap water.

Drugs

DL alpha methyl-para-tyrosine (Sigma Chemical Company, St. Louis, Missouri) was dissolved in dilute NaOH (pH 11). The pH of the solution was adjusted to pH 7 by the addition of dilute HCL. The AMPT was still in solution when administered. All injections were given intra-

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peritoneally in a volume of 20 ml/kg and a dose of 35 mg/kg of body weight. Control animals were injected with an equivalent solution not containing AMPT.

Passive Avoidance Apparatus

A two-chamber apparatus similar to that previously described by Boggan [17] was employed. It consisted of a narrow Plexiglas straight alley 8 in. long by 1 in. wide by 7 in. high, which led into a larger Plexiglas shock chamber 6 in. square by 7 in. high having a removable Plexiglas top. The shock chamber had a grid floor through which a scrambled footshock could be delivered (GSC model 1064GS Shock Scrambler). The apparatus also had a hinged door at thee entrance to the straight alley and a guillotine door separating the straight alley from the shock chamber.

Procedure

Training consisted of placing each mouse inside the straight alley. Training scores for each mouse consisted of the latency, in seconds, measured between the time the mouse was placed in the straight alley, and the moment when the mouse placed all four paws inside the shock chamber. Following entry into the shock chamber, the guillotine door was lowered into place, preventing re-entry into the straight alley. Within 5 sec of entering the shock chamber, a scrambled footshock of 2 sec duration was delivered through the grid floor. The mouse was then promptly returned to its home cage. Retention of the avoidance response was tested by replacing the mouse in the straight alley and measuring the latency to re-enter the shock chamber. Mice failing to re-enter within 3 min were removed and given a latency of 180 sec. Raw data was in the form of net latencies (test latencies minus training latencies) for each mouse.

Three experiments were performed. In the first experiment, two groups of mice were treated with either AMPT or control solution 4 hr prior to passive avoidance training. At the time of training, each of these two groups was randomly subdivided into 4 treatment conditions. For the first condition, the training footshock was of 1.6 mA intensity. For the other conditions, the training footshocks were of 0.8 mA, 0.16 and mA, and 0.0 mA (no footshock) respectively, thus making a total of 8 separate treatment conditions. Retention of the passive avoidance response was tested 24 hr after training.

In the second experiment, two groups of mice were treated with either AMPT or control injections, and trained in the passive avoidance apparatus, receiving a footshock of 1.6 mA. Each of these two groups was randomly subdivided into three treatment conditions. In the first condition, the animals were injected 4 hr before avoidance training. In the second condition, the animals were injected immediately after training and in the third condition animals were injected 4 hr before testing for retention of the passive avoidance response. All animals were tested 24 hr after training.

In the third experiment, the possibility of state-dependent memory effects was examined. A 2 by 2 factorial design was used, involving two conditions of pretraining treatment (AMPT or control) with two conditions of pretesting drug treatment. All animals were given the appropriate drug treatment 4 hr before training and again 4 hr before testing. All mice were trained using a 1.6 mA footshock, and were tested 24 hr after training.

Open Field Maze

To measure spontaneous activity, two additional groups of mice were tested in an open field maze 28 hr after treatment with either AMPT or a control solution. This interval corresponds to the time of avoidance response testing used in the first experiment, described above. The open field was a 30 by 30 in. area, marked off into 36 five-in. squares, housed in a gray wooden box as previously described by McClearn [11]. The activity score for each mouse was the number of 5 in. squares entered during a 2 min test period.

Shock Reactivity

To measure reactivity to footshock, a variation of the jump-flinch test used by Eichelman [7] was employed. Mice treated 4 hr earlier with either AMPT or control solution were placed in a shock chamber identical to that used in the passive avoidance training and exposed to a series of footshocks of five different intensities (0.05, 0.08, 0.1, 0.2, and 0.3 mA). Footshock was generated by a GSC model 1064GS Shock Scrambler. Each mouse received 30 shocks, 6 at each of the 5 intensities, in alternating ascending and descending order of intensity. The mean intershock interval was 30 sec, and varied randomly between 15 and 45 sec. The animal's reaction to each shock was scored either as a zero (no visible response), a one (a flinch response, involving orientation, flinching, etc., but not including a jump) and a two (a jump, where all four paws left the grid floor simultaneously. Ten mice per group (either drug or control) were employed. The sound produced by the shock generator was masked throughout the experiment by white noise. Two additional groups of ten mice each were exposed to footshocks of greater intensities (0.8, 1.0, 1.3, and 1.6 mA). Each mouse received 24 shocks, 6 at each of the 4 intensities, and their responses recorded in a manner identical to that described above.

Biochemical Assay

For whole brain norepinephrine and dopamine determinations, two groups of mice (N = 4) were treated with either AMPT (35 mg/kg) or control solution injections. Four hours after injection, the mice were sacrificed by decapitation. Brains were quickly removed and weighed to the nearest 0.01 g. NE and DA determinations were made by a modification of the spectrophotofluorometric technique described by Shellenberger and Gordon [18].

RESULTS

The results of the first experiment are illustrated in Fig. 1. As can be seen, the net latencies for control subjects varied directly with the intensity of the footshock. Analysis of variance revealed a significant drug by shock interaction, $F(7,40) = 7.69$, $p<0.01$. The method of coefficients revealed that in the 1.6 mA footshock condition, mice treated with AMPT had significantly shorter latencies than control mice, $F(1,15) = 20.7$, $p>0.01$, whereas in the 0.16 mA footshock condition, mice treated with AMPT had significantly longer latencies than controls, $F(1,15) = 4.6$, $p>0.05$. Drug treatment groups did not differ significantly in the 0.8 mA and no footshock conditions.

In the second experiment, as in the first, treatment with AMPT 4 hr before training resulted in a significant latency difference 24 hr later ($t = 3.14$, $df = 14$, $p<0.01$). When

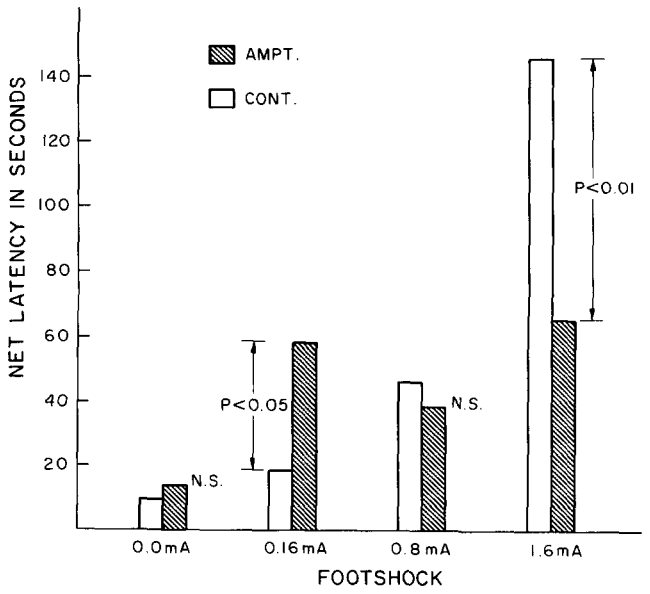

FIG. 1. Effects of drug treatment on net latency in seconds (day 2 test latency minus day 1 training latency) as a function of intensity of footshock used during training.

drug injections were administered immediately after training, however, latencies of AMPT and control treated mice did not differ significantly (t = 1.1, df = 14, p = n.s.), nor did they differ significantly when drug treatment was administered 4 hr before testing (t = 1.50, df = 14, p = n.s.).

In the third experiment, it was observed that mice given the same drug treatment prior to both training and testing, when compared to mice given different drug treatments prior to training and testing, revealed no evidence of any state-dependent learning effects, $F(1,35) = 2.77$, p = n.s.

The relatively low dose of AMPT used in all experiments did not seem to produce illness, and there was no difference in spontaneous activity, as measured in the open field maze, between mice treated with AMPT and those given control injections (t = 0.863, df = 39, $p>0.2$).

The results of the shock reactivity (jump-flinch) tests revealed no significant differences in reactivity to shock over the two ranges of shock employed (t = 1.3, df = 18, p = n.s. and t = 1.6, df = 18, p = n.s.; for the 0.05 to 0.3 mA range and the 0.8 to 1.6 mA range, respectively). These results would argue against the possibility that AMPT could have altered the mice sensitivity to footshock and in that way contribute to the differences in latencies seen in the first experiment.

Spectrophotofluorometric determinations revealed that treatment with 35 mg/kg of AMPT reduced whole brain norepinephrine content to a mean of 245.4 ng/g, as compared with a mean of 408 ng/g for saline treated control mice (t = 7.233, df = 6, $p<0.01$). Whole brain dopamine was reduced to a mean of 544.2 ng/g, as compared with a mean of 861.7 ng/g for control mice (t = 3.083, df = 6, $p<0.05$).

DISCUSSION

In this study, both the enhancement and the impairment of avoidance behavior were seen following treatment with alpha methyl-p-tyrosine. Several control experiments were conducted in an attempt to determine whether these results could be attributed to factors other than learning and/or memory. It was found that AMPT-treated subjects were neither more nor less active than control mice at the time of testing, as judged by open field maze behavior, and thus differences in test latencies could not be attributed to differences in overall activity. Likewise, the results of the shock reactivity test suggest that AMPT-treated mice found footshock of the intensities used herein neither more nor less aversive than did control mice, thus making it difficult to attribute the principle experimental results to drug-induced differences in motivation. Finally, in the absence of any state-dependent memory effects, it would seem most parsimonious to attribute the results reported herein as due to some alterations of learning and/or memory processes.

The data suggests, in fact, that AMPT may have exerted its differing effects on avoidance conditioning in accordance with the strength of the learned avoidance response. If one assumes that the latencies of control mice in the first experiment accurately reflect the relationship between intensity of footshock and strength of the conditioned avoidance response, then treatment with AMPT would appear to have enhanced the relatively weak avoidance response motivated by mild footshock while conversely impairing the stronger avoidance response motivated by strong footshock.

A possibly similar phenomenon was reported by Randt *et al.* [14], noted above. In their experiment, the strength of the passive avoidance response (as judged by the behavior of control mice) was seen to vary with the interval between training and testing, being weakest at one minute after training and stronger at longer intervals after training. Treatment with diethyldithiocarbamate, a dopamine beta hydroxylase inhibitor, was seen to enhance the avoidance response when tested one minute after training, when the response was weakest, while impairing the avoidance response when tested at longer training-test intervals, when the avoidance response was strongest in control subjects.

The only other reports in the literature on the effects of drugs on learning and memory where apparently weak responses were enhanced by drug treatments which conversely impaired stronger responses are described by Deutsch [4, 5, 6] regarding the effects of anticholinesterase drugs on memory. In these reports, DFP was reported to facilitate the recall of poorly learned responses, while impairing the expression of well-learned responses.

It would seem plausible, therefore, that the dual effects on passive avoidance behavior of a reduction in brain CA content, as achieved by AMPT in this experiment (and perhaps as achieved by diethyldithiocarbamate in Randt's experiment) indicate the importance of the strength of the avoidance response in determining the nature of the effect of such CA reduction on the processes of learning and/or memory. Furthermore, it is suggestive that the importance of response strength in determining the nature of pharmacological effects on learning and memory has generally been underrated.

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