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Time-Dependent Effects of Metrazol on Memory[1]

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PALFAI, T. AND P. KURTZ. *Time-dependent effects of Metrazol on memory.* PHARMAC. BIOCHEM. BEHAV. 1(1) 55–59, 1973.–The effects of Metrazol induced seizures on memory were studied in two experiments. In Experiment 1 it was found that Metrazol given at different intervals before a single passive avoidance training trial produced graded performance impairments 24 hr later. The degree of the resulting amnesia was shown to depend on the temporal proximity of the Metrazol seizures to the training trial. In Experiment 2 Metrazol was given immediately following the passive avoidance trial but amnesia was found to develop only after several hr following the Metrazol treatment. The data are similar to those obtained with ECS.

Amnesia Memory Metrazol Passive avoidance

ELECTROCONVULSIVE shock (ECS) will often produce memory deficits for a learning experience that just preceded this experience. It appears that the degree of this deficit is inversely related to the training ECS interval. Based on this observation it has been suggested that ECS interrupts [10] or slows [2] a hypothetical memory consolidation process that begins with the learning event and continues for a finite time thereafter.

Recently, Palfai and Chillag [16] have shown that the analeptic drug, Metrazol, given in doses large enough to induce overt seizures, will also produce time-dependent retroactive memory deficits, the gradient being comparable to that of ECS reported in a similar experimental situation [18]. Since there are considerable similarities with respect to the biochemical consequences of both ECS and Metrazol seizures [1, 3, 7], one might also expect further similarities in their various behavioral effects. From these similarities perhaps the understanding of the mechanism by which these agents produce amnesia may be improved.

In the following experiments we have investigated whether or not Metrazol induced seizures, like ECS, produce anterograde amnesia [8,19] for passive avoidance training. We also examined whether or not the amnesia resulting from Metrazol has a time course of development similar to that which has been reported for ECS [4, 5, 11, 12].

EXPERIMENT 1

It has been demonstrated [8,19] that ECS given prior to a passive avoidance training trial impairs retention of that trial 24 hr later. The data also show that the impairment is dependent upon the ECS training interval: shorter intervals result in greater performance deficits, longer intervals in smaller impairments. This phenomenon may be referred to as anterograde amnesia (AA) [13]. In the present experiment we studied the possible AA effect of Metrazol induced seizures on retention of a passive avoidance task.

METHOD

Animals

Animals were 55 male Swiss mice, 60–70 days old, obtained from Charles River Mouse Farms, Wilmington, Mass. They were housed in standard Econo plastic cages, five to a cage, with food and water available ad lib. A 12-hr day-night cycle was in effect; temperature and humidity were held constant at 72°F and 50%, respectively. All animals were kept under these conditions for at least seven days before the experiments, which were conducted in the afternoon.

Apparatus and Procedure

A step-through passive avoidance apparatus similar to that of Jarvik and Kopp [6] was used. In brief, the apparatus consisted of a covered V-shaped trough which was divided by a narrow guillotine door into a small illuminated start box and a larger darkened section. Stainless steel panels formed the walls and floor of the trough and served, in the darkened section, to deliver electric shock from a Grason Stadler Model 700 Constant Current Shock Generator to the animals' feet.

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The animals were randomly divided into five equal groups. Four of the groups received 50 mg/kg Metrazol intraperitoneally (IP) at either 15, 30, 60, or 240 min before being given a single passive avoidance training trial. One group of animals was given physiological saline 15 min before the training trial.

Passive avoidance training consisted of a single trial. Each animal was placed in the start box and 60 sec later the door was opened, starting an electric timer. The instant the mouse stepped through the door to the darkened compartment (defined as the passage of the hind legs over the threshold), the timer was stopped and an a.c. footshock (FS) (0.50 mA) was given which instantly produced an escape to the start box. The animal was then immediately removed from the apparatus and placed into the home cage.

Twenty-four hr after the training trial each animal was again placed in the start box and, as before, the door was opened after 60 sec and step-through latency was measured. This test latency was measured to an arbitrary maximum of 300 sec.

RESULTS

The Metrazol injections resulted in convulsions approximately 90% of the time. These convulsions were usually clonic; clonic-tonic convulsions were rarely observed. The onset of the seizures occurred between 72 and 219 sec after injection.

The median step-through latency for the training trial for all groups was less than 13 sec. Although statistically not significant, the step-through latencies of the 15-min Metrazol group during the training trial appeared to be depressed. This is consistent with the findings of Kurtz and Palfai [9], who found significant depression in general activity following Metrazol seizures.

Table 1 shows the median step-through latencies in the test trial. A Kruskal-Wallis Analysis of Variance indicated that the Metrazol produced a time-dependent treatment effect ($p<0.001$). Mice injected with Metrazol 15, 30, and 60 min before the training trial had significantly shorter step-through latencies than those injected with saline (Mann-Whitney U test, $p<0.001$). These data show that Metrazol induced seizures, like ECS [8,19], may produce anterograde amnesia.

TABLE 1

TEST LATENCIES 24 HR AFTER PASSIVE AVOIDANCE CONDITIONING

	Sal 15	Met 15	Met 30	Met 60	Met 240
n	11	11	11	11	11
$\bar{X}$	284	35	78	157	188
MDN	300	12	54	186	252
IQ Range	300–300	5–77	7–111	12–273	19–300

EXPERIMENT 2

It has been reported on several occasions that amnesia following ECS is not instantaneous but develops gradually over several hr [4, 5, 11, 12]. The common explanation of this phenomenon is that the trauma affects mechanisms responsible for long-term information storage [11]. In the present experiment we investigated whether or not this phenomenon can also be produced with Metrazol induced convulsions.

METHOD

Animals

Animals were 136 male Swiss mice of the same description as in Experiment 1.

Apparatus and Procedure

The apparatus and the training procedure were the same as those in Experiment 1, except that following the step-through response, the door was closed and the animals received a 0.50 mA a.c. FS for a 1-sec duration. One hundred twenty of the animals were assigned to 12 groups. These animals each received a single passive avoidance training trial, and were injected immediately with either saline or Metrazol (50 mg/kg) IP. Five groups received Metrazol; the other seven groups were given saline. Following this treatment, different groups of saline treated animals were tested for retention after 1, 10, 30, 90, 270, or 1440 min. One additional group of 10 mice was also given saline and tested after 1440 min but was given FS 1 min before testing, in order to control for the possible motor effects of FS. This control group will be referred to as FS + 1 Group. The five Metrazol groups were given retention tests 10, 30, 90, 270, or 1440 min following training. Two other groups, eight animals in each, were also used to control for the possible depressive or aversive effects of Metrazol seizures. These groups were given the step-through trial but were not given FS. Instead, they were kept for 1 sec in the darkened section, removed, injected with 50 mg/kg Metrazol, and tested for step-through at 30 or 270 min following the injection. They will be referred to as NFS + Met + 30 and NFS + Met + 270. The retention test and measure were the same as in Experiment 1.

RESULTS

The median step-through latencies for the training trial were less than 20 sec in all groups. The Metrazol injections resulted in convulsions, similar to those described in Experiment 1. The median step-through latencies obtained during the retention test are shown in Table 2 and Fig. 1. Using the Mann-Whitney U test, comparisons were made between groups that were given retention tests following the same posttraining interval but differing in drug treatment, and also between groups that received the same drug treatment but differed in terms of the posttraining interval when tested for retention. The results of this analysis indicated that Metrazol treated mice had shorter step-through latencies than saline treated controls if they were given the retention test 270 or 1440 min following passive avoidance training. No differences were observed between the saline and Metrazol groups at the 10, 30, or 90 min posttraining retention trials. The median step-through latencies of the Metrazol group tested 1440 min after conditioning were significantly shorter than the groups

given the drug but tested 10, 30, or 90 min following training. The step-through latencies in the NFS + Met + 30 Group were significantly shorter than the FS + Met + 30 Group, while this was not the case in the comparison of NFS + Met + 270 vs. FS + Met + 270. On this basis it was concluded that the long step-through latencies found at least in the 30- and 90-min Metrazol treated groups were not caused by the possible depressive or aversive effect of Metrazol seizures on step-through.

mg/kg Metrazol, which produced behavioral seizures, resulted in anterograde amnesia. Retention was inversely related to the temporal proximity of seizures to training. If the injection was given 15, 30, or 60 min before the training trial, retention performance was impaired 24 hr later. No deficits were observed if the injection occurred 240 min before training. A similar gradient has previously been reported [16] for retrograde amnesia following Metrazol for the same task.

TABLE 2

TEST LATENCIES AT VARIOUS INTERVALS FOLLOWING PASSIVE AVOIDANCE CONDITIONING

					p as compared among	
Treatment	n	Time of test after training (min)	Median retest latency	Interquartile range (sec)	Same intervals Met vs Sal	Same treatments 1440 vs all other
FS - Met	10	10	300	300–300	NS	0.001
FS - Met	10	30	300	300–300	NS	0.01
FS - Met	10	90	287	16–300	NS	NS
FS - Met	10	270	49	17–274	NS	NS
FS - Met	10	1440	34	8–272	<0.05	--
NFS - Met	8	30	23	22–39	--	--
NFS - Met	8	270	21	10–44	--	--
FS - Sal	10	1	43	12–229		0.01
FS - Sal	10	10	228	28–300		NS
FS - Sal	10	30	233	68–300		NS
FS - Sal	10	90	230	85–300		NS
FS - Sal	10	270	284	86–300		NS
FS - Sal	10	1440	300	101–300		NS
FS + 1	10	1440	300	300–300		NS

It was also found that the saline group tested 1 min after the avoidance training had shorter latencies than those tested after 30, 90, 270, or 1440 min. Since the FS + 1 Group showed good retention after 1440 min, the short step-through latencies in this group were probably not caused by possible activational effects of FS on the step-through response.

These data indicate that (1) Metrazol seizures do not produce amnesia instantly--amnesia becomes apparent only several hr after the trauma. This finding is consistent with those reported for ECS [4,12]. (2) Retention of a single passive avoidance conditioning trial is poor 1 min after training, but improves as a function of time and reaches asymptote approximately 30 min later. This phenomenon has been reported previously [17].

DISCUSSION

The results of two experiments indicate that Metrazol may produce behavioral effects similar to those reported for ECS [4, 5, 8, 11, 12, 19].

In Experiment 1 it was shown than an IP injection of 50

There may be three explanations for the data of Experiment 1: (1) Metrazol interferes with acquisition of a passive avoidance learning trial simply through its systemic effect. No obvious behavioral effects (e.g., longer shock escape latencies) were observed, however, that would support this notion. (2) Metrazol has a long lasting neurological effect which interferes with some stage of consolidation. Preliminary investigation on EEG (unpublished data) suggests that neurological effects may last as long as 30–60 min following the injection. (3) Metrazol produces dissociation of learning [14]. In this case, consolidation or part of it would occur in a brain state different from that state which exists during retention testing 24 hr later. Some recent evidence with respect to the dissociative effect of Metrazol has been reported from this laboratory [9].

In Experiment 2 it was observed that 50 mg/kg Metrazol given shortly after a learning trial does not instantly produce amnesia; passive avoidance performance impairments become apparent only several hr following the injection. Animals given Metrazol immediately following training and tested for retention up to 90 min have step-through latencies similar to saline treated controls.

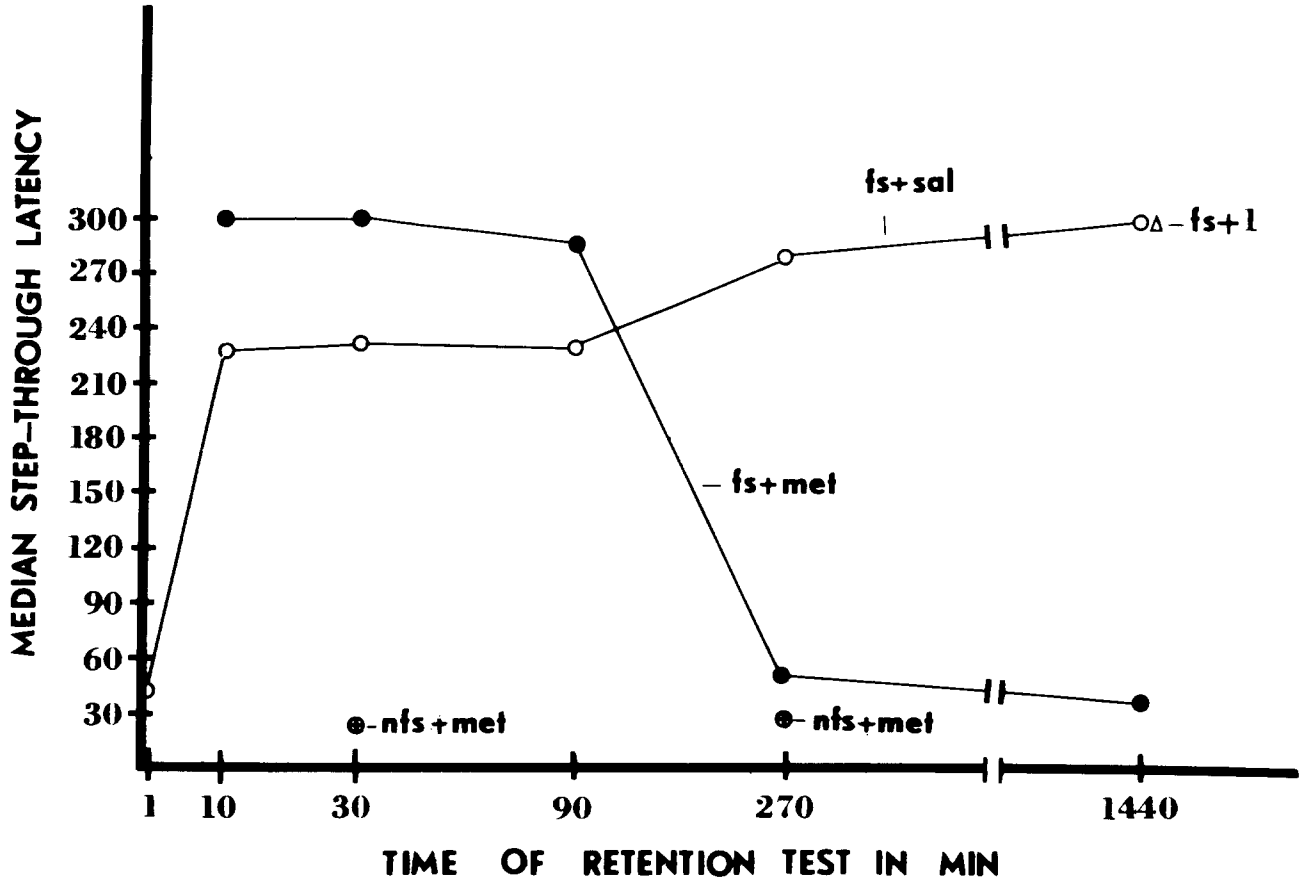

FIG. 1. Median step-through latencies of different groups tested at various intervals following training and treatment.

Metrazol treated animals that are tested for retention 270 or 1440 min following training, on the other hand, have significantly shorter step-through latencies than saline controls.

These results may be explained by assuming that (1) Metrazol interfered with some neurobiological mechanism responsible for long-term memory storage but had no effect on short term memory, and/or (2) Metrazol has dissociated consolidation in the long retention interval groups from the brain state in which these animals were tested. On the other hand, it had no effect on the short retention interval groups because the drug state in which consolidation took place was still in effect when these animals were tested.

The latter explanation implies that the dissociative effect of Metrazol may last as long as 90 min. Although such an interval would appear to be long in comparison to other dissociative agents [15], the fact that in Experiment 1 the 60-min pretraining injection group showed a significant amnesic effect suggests that Metrazol may indeed affect memory for a considerable time after its injection. Experiments are currently underway to clarify these time relationships.

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