Pharmacology Biochemistry & Behavior, Vol. 4, pp. 123–127.

Dose-Related Effects of Metrazol on Retention and EEG

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(Received 1 July 1975)

PALFAI, T. AND P. KURTZ. *Dose-related effects of metrazol on retention and EEG.* PHARMAC. BIOCHEM. BEHAV. 4(2) 123–127, 1976. – The effects of various dose levels of Metrazol on retention and electrocorticogram (ECoG) were investigated. Mice given a subconvulsive 30 mg/kg or a convulsive 50 mg/kg dose of Metrazol 15 min before reversal training in a discriminated escape learning task showed retention impairment of reversal training retention. Lower dose levels (5 or 10 mg/kg) had no effect. The 30 mg/kg dose produced asymmetrical dissociation. The convulsive dose (50 mg/kg), previously reported to result in symmetrical dissociation, produced ECoG changes that were still evident 15 min following the injection, i.e. at the time when training or testing usually took place. With lower doses (10 or 30 mg/kg), no apparent ECoG effects were observed at this interval. The implications of the findings were discussed with respect to the state-dependent learning hypothesis.

Dissociation State-dependent learning Metrazol Pentylenetetrazol

METRAZOL impairs retention of passive avoidance training when given in doses large enough to produce overt seizures [7, 8, 11]. The effect is time-dependent in that the drug must be given shortly before or after training to observe deficits in retention performance 24 hours later.

Recently, we reported [3] that proactive memory impairments seen when the drug is given 15 minutes before discriminated escape training, can be attenuated if the drug is readministered 15 minutes before retention testing. When training and testing occurred in different pharmacological states, test performance was impaired but when they were given in the same state no impairments were seen. On the basis of these data we concluded that convulsive doses of Metrazol may produce state-dependent learning, perhaps in a manner similar to that reported following electroconvulsive shock (ECS) or seizures induced by brain stimulation [4,10].

The phenomenon is not uniquely a consequence of convulsant agents, however. A variety of drugs have been reported to produce state-dependent learning without brain and/or overt seizures [1, 5, 6, 9]; therefore, it is possible that the dissociative effect of Metrazol is independent from its seizure-inducing properties. To elucidate this issue, a subconvulsive dose of the drug which produced proactive amnesia, was administered in a state-dependent learning paradigm. Since it has been suggested [4] that the dissociative effects of convulsions may be mediated by postictal neural depression, a further experiment examined the electrocortical (ECoG) correlates of subconvulsive and convulsive dose levels of this drug.

EXPERIMENT 1

A convulsive dose (50 mg/kg) of Metrazol given 15 min before an escape reversal training session, impairs retention of this training 24 hrs later [3]. To screen for potentially dissociative dose levels of the drug, the effects of this convulsive and various subconvulsive doses of Metrazol were investigated in the same escape reversal paradigm.

METHOD

Animals

One hundred-seventy male Swiss mice, 60–80 days old, were obtained from Charles River Mouse Farms, Wilmington, Mass. They were housed in standard Econo plastic cages, 5 to a cage, with food and water available ad lib. A 12 hr day-night cycle was in effect with temperature and humidity held constant at 72° F and 50% respectively. All animals were kept under these conditions for at least 7 days before the experiment.

Apparatus

A covered Plexiglas m-shaped maze, composed of three 10 x 20 cm arms at right angles to a 50 x 10 cm common alley was used. Guillotine type barriers could be inserted 10 cm from the end of all 3 arms. The metal grid floor of the maze could be electrified throughout except in the two safe boxes at the end of the two side arms. The center arm

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served as the start box and during training only one safe area was available for shock escape. Scrambled shock was delivered from a Grason-Stadler Model 700 shock generator set at 0.5 mA.

Procedure

The 170 animals were divided into 5 groups, 34 in each. Three animals died during the experiment but were not replaced.

On Day 1, each mouse was given 10 massed escape trials to one arm of the maze – half of each treatment group to one side, half to the other. The animal was placed in the start box for 10 sec followed by the simultaneous withdrawal of the barrier and footshock (FS) onset. The FS was terminated when the mouse reached the unblocked or correct safe area. Three sec later, the next trial began.

On Day 2, each mouse was given 10 reversal trials in which the procedure was the same except that the previously safe area was now blocked, and escape was possible to the previously blocked side.

On Day 3, a single trial was given during which both safe areas were accessible. The percentage of mice from each group going to the reversal side (Day 2) during testing (Day 3) was used as a measure of reversal trial retention.

Drug Treatment

On each day, 15 min before the first escape trial, mice were given an intraperitoneal (IP) injection. On Day 1, all animals received the drug vehicle which was distilled water (ND). On Day 2, one group received ND, the remaining 4 groups received either 5, 10, 30 or 50 mg/kg of Metrazol. On Day 3, again all animals were given ND.

RESULTS

Approximately 80% of the animals injected with 50 mg/kg Metrazol had overt seizures. These were mainly clonic; tonic seizures were not seen. Following the seizures, behavioral depression was apparent.

Two of the animals given 30 mg/kg of the drug exhibited seizures. The other animals initially appeared restless, showed slight tremors, and were, subsequently, less active following the injection.

Behavioral effects with the lower doses were not apparent. The dependent measure of retention was the percentage of animals turning toward the reversal side (Day 2) during the test on Day 3. Table 1 shows the results. As can be seen, both the 30 and 50 mg/kg dose levels impaired retention of Day 2 reversal training ($\chi^2 = 8.21$, $p<0.01$) when compared to the ND control group. The 10 and 5 mg/kg doses were not different from control. These data suggest that Metrazol given 15 min before training in high but not necessarily in convulsive dose may impair subsequent retention of that training, i.e., a subconvulsive dose of Metrazol may produce proactive amnesia.

TABLE 1

COMPARISON OF THE EFFECT OF METRAZOL DOSAGES ON REVERSAL RETENTION

Treatment Day				
1	2	3	N	% Animals to Reversal Side
ND	ND	ND	34	88
ND	5 mg/kg	ND	33	85
ND	10 mg/kg	ND	34	82
ND	30 mg/kg	ND	32	56
ND	50 mg/kg	ND	34	56

EXPERIMENT 2

The results of the previous experiment indicate that 30 or 50 mg/kg of Metrazol may produce proactive amnesia. The higher dose has been reported to produce dissociation of training [3]. Since a variety of drugs have been reported to produce state-dependent learning without brain and/or seizures [1, 5, 6, 9], it is possible that the dissociative effect of Metrazol is independent from its seizure-inducing properties. Therefore, in the present experiment, we investigated whether a dose which is subconvulsive (30 mg/kg) can also produce this phenomenon.

Based on the dissociation hypothesis, specific predictions may be made as to the performance of the various treatment groups. In general, animals trained and tested for retention in similar pharmacological states should show better retention than those trained in a drug state different from that of testing. In the Kurtz and Palfai [3] study, animals were trained to choose one arm of a T-maze on Day 1 (original learning). On Day 2, they were trained to reverse the response by choosing the opposite arm (Reversal learning). On Day 3, test day, both arms were accessible and the animal's choice was recorded. Our rationale was that animals tested for reversal training retention in a state similar to that of reversal training should demonstrate the best retention for this training. Those animals that are tested in a state dissimilar to both original and reversal learning should show less retention. Theoretically, they would be dissociated from all prior training. The poorest retention performance of reversal training, however, should be shown by animals that were tested in a state similar to that of original training and different from that of reversal training. While these animals might be expected to show poor retention of reversal learning, they would not be dissociated from original learning and should, therefore, exhibit a preference for the originally learned response. The results of the convulsive doses (50 mg/kg) were generally in agreement with these predictions. In the present experiment the same predictions were made.

METHOD

One hundred-twelve male Swiss mice, of the same description as previously, were housed and maintained as before. The apparatus and procedure were also the same as before.

Procedure

Drug treatment. On each day, 15 min before the first escape trial, mice were given an intraperitoneal (IP) injection of either 30 mg/kg of Metrazol (D) or the drug vehicle (ND). All injection volumes were 10 cc/kg body

TABLE 2

PREDICTED AND OBSERVED PERFORMANCE OF REVERSAL RETENTION OF THE VARIOUS TREATMENT GROUPS

Group	Treatment Day 1	2	3	N	% Animals to Reversal Side Predicted	Observed
A	ND	ND	ND	14	Good	71
B	ND	D	D	14	Good	79
C	D	ND	ND	14	Good	71
D	D	D	D	14	Good	64
E	ND	ND	D	14	Intermediate	50
F	D	D	ND	14	Intermediate	50
G	ND	D	ND	14	Poor	28
H	D	ND	D	14	Poor	71

weight. The combination of these 2 treatments over 3 days resulted in the 8 possible treatment groups listed in Table 2. Each group consisted of 14 mice.

RESULTS

One animal given the Metrazol injection had overt seizures; other animals showed behavioral effects from this dose similar to those described in Experiment 1. The behavioral results, that is the actual percentage of animals going to the reversal side on Day 3 for each group are shown in Table 2. In Groups A, B, C, and D, in which the pharmacological state was the same during both the reversal training and testing, reversal retention was apparent; no retention differences were observed among the groups. Group A (ND-ND-ND), 71% differed significantly, however, from Group G (ND-D-ND), 28% ($\chi^2 = 3.57$, $p<0.05$ one-tailed) indicating an impairment of reversal training retention in the latter group. This finding agreed with the prediction since reversal training of Group G occurred in a state different from that of testing. As it is apparent, the actual percentage of animals choosing the reversal side in this group is considerably lower than the analogous group in Experiment 1. However, the same applies to the control group (ND-ND-ND) as well. These differences may be due to the time lag and shipment differences between the experiments. In Groups E and F, where the pharmacological state during the test (Day 3) differed from that of both Days 1 and 2, 50% of the animals turned towards the reversal side. Although the lower retention performance of these groups are in the predicted direction, the difference between these groups and Group A was not statistically significant. The performance of Group H, 71% was different from that predicted by the hypothesis. Here the pharmacological states differed on Days 2 and 3, yet a high percentage of animals retained the reversal training, showing no evidence of dissociation. It appears that with the subconvulsive (30 mg/kg) dose of Metrazol, training in the non-drug state did transfer to the drug state but not vice versa; the dissociation from this dosage was incomplete or asymmetrical.

EXPERIMENT 3

The available data indicate that 30 and 50 mg/kg Metrazol may dissociate learning. The lower of these dose levels usually does not produce overt seizures; hence, convulsions do not appear to be necessary for the occurrence of what appears to be asymmetrical dissociation. However, it is possible that brain seizures independent of overt convulsions might underly the phenomenon of asymmetrical dissociation produced by this drug. The purpose of the present experiment was, therefore, to investigate the electrocorticographic (ECoG) correlates of various dose levels of Metrazol at the time of behavioral testing.

METHOD

Animals

Twenty male Swiss mice of the same description as in Experiment 1 were used. They were housed individually in standard plastic Econo mouse cages.

Apparatus

The ECoG was recorded with a Nihon Kohden Model RM-20 Multipurpose Recorder. Recording was done in the animal's home cage which was placed inside a larger electrically shielded cage. EEG voltage was integrated using a Model 23 EEG Integrator (Cold Springs Instrument Corporation).

Procedure

Each mouse was anesthetized with 75 mg/kg Nembutal (IP) and positioned in a stereotaxic apparatus. After a small incision the skull was exposed and dried. A pair of silver screws previously soldered to Amphenol connectors were turned into the skull. The screws were positioned unilaterally approximately 4 mm apart on the dorsal cortex. The screw tips were positioned to rest on the dura and the implants were cemented to the skull with non-conducting dental cement (Caulk Grip). At least 1 week of postoperative recovery was allowed before testing.

Following the recovery period, the animals were pretested to assure good quality records; mice with poor ECoG signals were replaced. Subsequently, each mouse was placed into the recording situation and following an initial 5 min adaptation period, three 20 sec ECoG segments were taken; each was separated by 120 sec intervals. This constituted baseline. The animal was then removed, injected and replaced promptly in the recording situation.

All injections were given IP. Three animals received distilled water (DW), three were given 10 mg/kg (Met-10), seven 30 mg/kg (Met-30), and seven 50 mg/kg (Met-50) Metrazol.

A 5 min period of continuous recording began immediately following replacement into the test cage. After this period, 20 sec ECoG segments were taken every minute for 20 min, and from then every 5 min up to 45 min after injection.

RESULTS

Six mice, all from the Met-50 group, convulsed. The seizures were mainly clonic; in two instances, however, tonic seizures were also observed. One of these animals died. The behavioral effects of the 30 mg/kg dose were similar to those described previously. The 10 mg/kg dose did not produce apparent behavioral effects.

Considerable individual differences were observed in the ECoG responses to Metrazol even within groups. When convulsions did occur, however, the onset and duration approximated the onset and duration of high voltage 1–2 sec ECoG spikes. These slow, high voltage discharges were followed by a period of low voltage, low frequency ECoG, commonly referred to as the postictal phase.

Figure 1 shows the mean integrated voltage/sec of 3 animals from each Metrazol group, and 2 from the DW group.

The integration procedure allowed an objective assessment of total voltage changes (amplitude × frequency) during the experiment. In order to minimize the effect of individual differences in baseline voltages, the data are presented as the difference between the baseline integrated voltage and the postinjection integrated voltage. Analysis of the results using this measure essentially agree with the subjective evaluation of the data.

The ECoG responses to the drug also varied considerably among animals in the Met-30 group. High amplitude, low frequency brain seizures were observed only in one instance. These discharges were sometimes accompanied by vocalization and a temporary loss of righting reflex, but overt convulsions were not observed, suggesting that this ECoG pattern may not always coincide with overt convulsions. In 3 of the other 6 animals, occasional high voltage, low frequency spikes were noted. In one of these mice, the period of discharges was followed by low voltage,

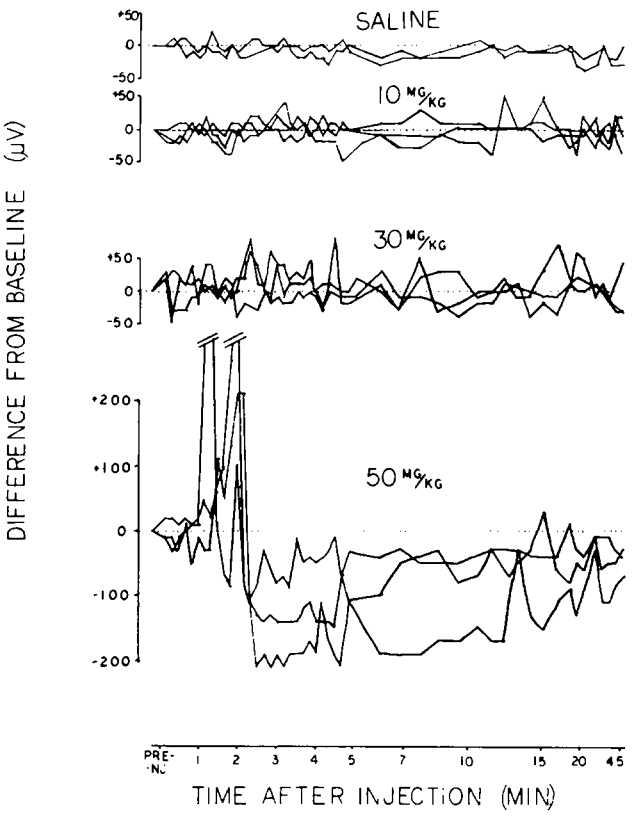

FIG. 1. Mean integrated voltage of 3 animals from each Metrazol group and 2 from the DW group. The difference between baseline and postinjection voltage is shown.

low frequency ECoG activity, resembling the postictal phase. Following the drug administration in the Met-10 group, no significant ECoG changes were noted.

The data indicate that both 30 and 50 mg/kg of Metrazol produce obvious, relatively short latency effects on ECoG. Since in the behavioral experiments, the drug was injected 15 min before training or testing, the nature of the ECoG effect, especially at this interval, may be important to characterize the dissociated state. Figure 2 shows the effect of 30 and 50 mg/kg dosages 15 min following injection together with the respective preinjection baselines, for 3 animals from each group. Although only 3 records are shown, the ECoGs of all animals in the Met-50 group were clearly distinguishable from preinjection baseline. This dose produces symmetrical dissociation [3]. In contrast, the ECoG of only one animal from the Met-30 group appeared to be affected 15 min following injection. No effects were noted in the DW or Met-10 groups. The integrated voltage data at this postinjection interval (Fig. 1) presents a similar picture.

DISCUSSION

The effect of various dose levels of Metrazol on retention and ECoG was studied. A subconvulsive dose (30 mg/kg) produced asymmetrical or imcomplete dissociation of reversal learning. Lower doses (5 or 10 mg/kg) did not significantly affect retention of this task. Since a convulsive dose (50 mg/kg) of the drug has been reported to produce more complete dissociation in the same situation [3], the

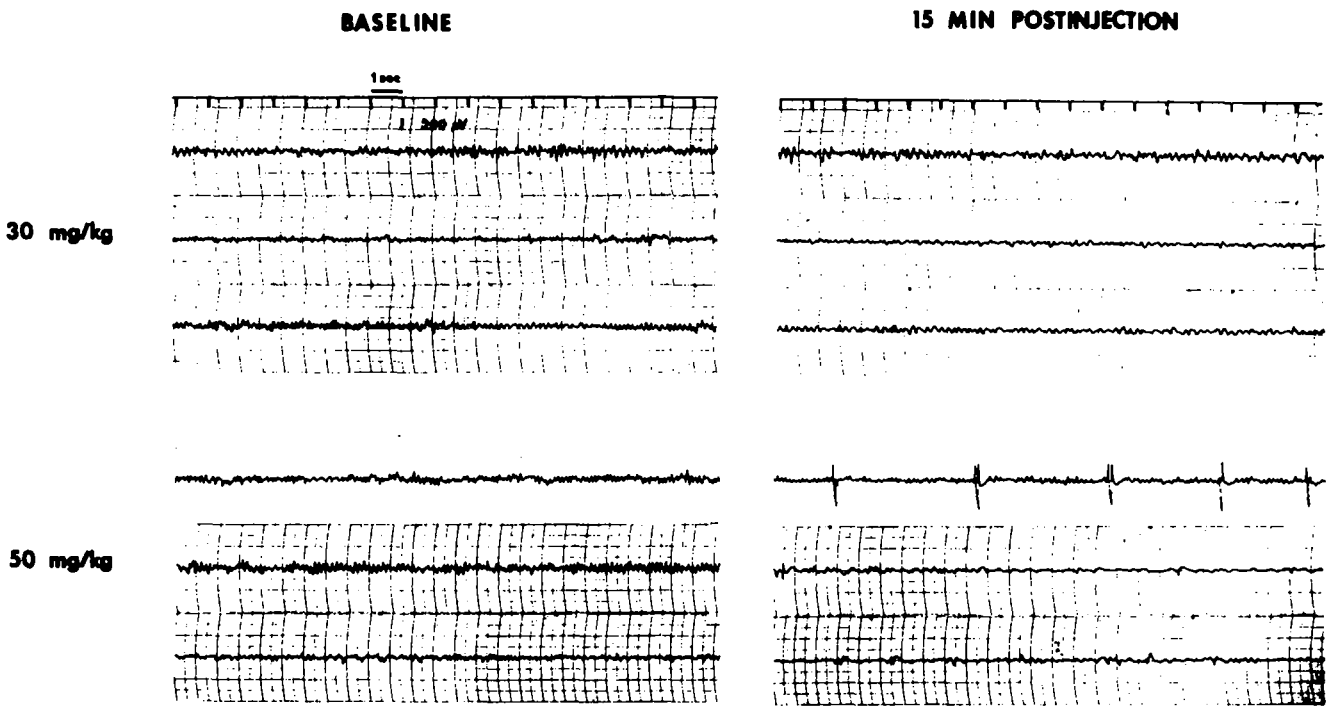

FIG. 2. The ECoGs of 3 animals from each of the 30 or 50 mg/kg groups are shown during baseline recording and 15 min following an IP injection of Metrazol.

effect of these differentially effective doses on ECoG was investigated. It was hoped that by describing some of the electrophysiological correlates of these drug dosages at the time of behavioral training and testing, a better understanding of the correlates of the dissociative state might be possible.

The data indicate that at the time of training and testing in our dissociation experiments [3], (15 min following Metrazol injection), the ECoG of 50 mg/kg of Metrazol is characterized by low voltage, low frequency electrical activity. Perhaps this could reflect and/or serve as a dissociative state; it is clearly distinguishable from preinjection baseline. The duration of this effect in most instances, was longer than 45 min. If the degree and duration of the ECoG effect is at all predictive of retention performance, Metrazol should have an effect on learning for at least this period, i.e. 45 min. The anterograde amnesia gradient reported by Palfai and Kurtz [8] suggests that this might be the case.

The electrophysiological data with 30 mg/kg are less conclusive. In only one instance, could the 15 min postinjection ECoG be considered as different from preinjection baseline. Since this dose level did produce asymmetrical dissociation at least two interpretations may be offered for these results. First, Metrazol may produce dissociation by neurobiological mechanisms other than those affecting cortical EEG. Thus perhaps the mechanism by which the drug produces dissociation might be independent from that responsible for brain seizures or the postictal phase. Second, the short duration but clearly evident effect of this dose on ECoG may produce a signal or cue preceding training or testing that is sufficiently discriminative for asymmetrical dissociation. The fact that reversal training in the non-drug state in Group H (D-ND-D) did transfer to the drug state does, however, suggest that the absence of the drug may be a stronger cue than its presence. That is, while the drug's pharmacologic consequences can be associated with a response choice, as the data indicate, this association might be less efficient than associations made to normal internal and external cues.

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