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Cycloheximide and Passive Avoidance Memory in Mice: Time-Response, Dose-Response and Short-Term Memory

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TUCKER, A. R., M. E. GIBBS AND M. D. STANES. *Cycloheximide and passive avoidance memory in mice: Time-response, dose-response, and short term memory.* PHARMAC. BIOCHEM. BEHAV. 4(4) 441–446, 1976. – The greatest loss of memory shown by mice 24 hr after learning was found to occur with cycloheximide (CXM) (120 mg/kg) administered subcutaneously 30 min before training. With injection at this time the extent of the amnesia was dose dependent (30–150 mg/kg) and the resultant amnesia was found to be relatively constant when tested at 1, 7 or 14 days. An attempt was made to follow the development of this amnesia with 100 and 120 mg/kg CXM. However, the saline controls showed an unexpectedly low avoidance 6 hr after training. This was interpreted as a possible interaction between the stress of the injection and the 6 hr interval. An experiment designed to test this possibility showed that mice injected with 0.1 ml of 1% lignocaine gave high avoidance at 6 hr but mice receiving only a needle puncture of the skin gave performances similar to mice receiving saline injections. It was felt that these findings cast doubt on the usefulness of the passive avoidance task in the assessment of drug action on short term memory.

Memory Short term memory Cycloheximide Lignocaine Avoidance conditioning Passive Avoidance

INHIBITION of cerebral protein synthesis by cycloheximide (CXM) has been shown to prevent memory formation in a number of species for a variety of learning tasks [10,16]. The passive avoidance task has been widely used to investigate memory and a number of studies have reported the effects of CXM on one trial step-through passive avoidance in mice [6, 7, 8, 11, 18, 19, 20, 22].

The findings of the above studies raise 2 issues which require clarification. The first is the effect of training and treatment parameters on subsequent amnesia, for example task variables may have to do with the rate of consolidation while treatment variables may directly determine how severely consolidation might be disrupted [2, 9, 14]. In relation to CXM-induced amnesia, it has been shown that the degree of amnesia is dependent upon time of injection [8,17], shock intensity [6,17], as well as shock duration, initial response latency [6,20], amount of training and strain of animal used [22]. Most of these studies have reported the effects of only one dose of inhibitor.

The second issue is the debate concerning the extent of recovery from CXM-induced amnesia [18, 19, 24]. Although it is maintained that spontaneous recovery of memory occurs within 7 days after training [17,24], other evidence suggests that recovery from CXM-induced amnesia occurs only under certain conditions such as repeated exposures to training apparatus, or the use of the reminder shock [18,19].

The present investigation examined the effect of variations in treatment procedures particularly variations in CXM dose and time of CXM injection and the effect of CXM on the development of amnesia up to 6 hr and its maintenance from 1 to 14 days after training.

GENERAL METHOD

Animals

Female Fullinsdorf mice, aged 4–6 weeks at the start of each experiment, were used. Initially supplies of mice were obtained from Hawthorn Park Farms (Sydney) and subsequently bred in the Psychology Department, La Trobe University. All animals were kept in the laboratory, on a 12/12 photoperiod, for at least one week prior to the start of an experiment. Food and water were freely available to the mice, which were housed in groups of approximately 15 in plastic cages. Animals were handled twice before the

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start of an experiment; once in order to number them and once in order to weigh them. Only those mice weighing between 20 and 35 g on the training day were included in the experiments. Assignment of animals to particular treatments was randomized within batches, with approximately equal numbers of mice in each treatment group.

Apparatus

A two-compartment, step-through, passive avoidance box, like that described by Jarvik and Kopp [13] was used to train and test the animals. The apparatus consisted of a trough-shaped acrylic plastic box divided into a small and a large compartment. The small compartment had transparent walls, allowing illumination by the room lights. The floor of this section measured 8.5 × 3.3 cm and was made from two stainless steel plates separated by a 2 mm gap. The partition separating the two compartments was made from black acrylic plastic with a 2.5 cm dia. hole at its base, the bottom of which was flush with the floor.

The floor of the large dark compartment measured 15 × 3.3 cm, and consisted of two pairs of stainless steel plates bent up to form the side walls. Both the plates nearer the opening (front plates) and the rear plates were 7.4 cm long. All the plates were 2 mm apart. The rear end wall of this compartment was also constructed from black acrylic plastic. The side walls of the two compartments were angled outwards so that their internal dimensions at the top were 10 × 10 cm (small compartment) and 17 × 10 cm (large compartment). The top of the large compartment was covered by a hinged black plastic lid while the small compartment was covered by a hinged transparent lid. A sliding guillotine door allowed the opening between compartments to be closed.

The two pairs of plates in the large compartment were connected to a direct-current shock generator. When the rear plates of the large compartment were bridged a 0.50 mA shock was delivered to both pairs of plates in the large compartment.

Drug Preparation and Administration

Cycloheximide (ACTIDIONE, Upjohn Co.) was dissolved in sterile 0.9% (w/v) NaCl. For each dose of CXM, one concentration was made and the exact volumes of injection varied according to the weight of the mouse (e.g., a 25 g mouse received 0.20 ml). Placebo treatments consisted of equal volume injections of 0.9% NaCl. In the final experiment 0.1 ml of 1% lignocaine (in 0.9% NaCl) was administered. In all experiments fully conscious mice were injected subcutaneously in the back of the neck. Only the experimenter injecting the mice knew each animal's treatment condition; experimenters training and testing were unaware of the animal's treatment condition.

Procedure

Training. Mice were taken from the plastic holding box and the feet of the mice were coated with a small amount of electrode paste to facilitate conduction of electricity. The animal was then lowered by the tail into the small (safe) compartment, facing away from the opening into the large (shock) compartment.

The latency to enter the shock compartment was the interval between the first contact with the floor of the safe compartment and bridging the rear plates in the shock compartment. This was signalled to the experimenter by the onset of a light. The animal was required to escape the shock by returning to the safe compartment. Consequently, each mouse was shocked for as long as it remained in the large compartment. The mouse was isolated in the safe compartment for about 15 sec and then removed by the tail. The apparatus was wiped with paper towel between trials.

Retention test. The retention trial was carried out in an identical manner to that of the training trial except that the shock output lead was removed, to preclude the possibility of the animal receiving shock again. The mouse's latency to step through into the shock compartment far enough to bridge the rear plates was timed to the nearest second. Animals failing to do this within 600 sec were removed and scored as avoiding. Retention was scored as percentage avoidance. The apparatus was again cleaned between trials. No animal was given more than one training or testing trial.

The memory measure used was percent avoidance since the nature of the task implies that if an animal remembers it will not step through. Although an arbitrary maximum of 600 sec was permitted, we do not claim that the latency reflected the degree of amnesia.

TIME–RESPONSE RELATIONSHIP FOR CXM-INDUCED AMNESIA

The purpose of this experiment was to investigate the effects of variations in the injection-training interval upon 24 hr memory.

Method and Procedure

Separate groups of mice were injected with CXM (120 mg/kg) at intervals of 180, 60, 30 and 5 min before training and 0.5, 10 and 30 min after training. Another 7 groups were injected with NaCl at the same intervals. The size of the 14 groups varied from 19 to 29 mice. All animals were tested 24 hr after training.

RESULTS AND DISCUSSION

The results are shown in Fig. 1. The proportion of NaCl- and CXM-treated mice avoiding was analysed for each time of injection with the Fisher exact test [12]. It was found that mice injected with CXM at the times −30 min, −5 min, +0.5 min and +10 min were amnesic ($p<0.05$ in each case). The greatest degree of amnesia was displayed by mice injected with CXM 30 min before training ($p<0.001$). These results show that 24 hr after training amnesia is dependent on the time of CXM administration relative to training. More particularly, the time course found in the present study is consistent with data obtained in previous studies [3,8], although a different strain of mice has been used.

Two studies have shown that CXM reduces the latency to enter the large compartment in the training trial [6,22]. Training trial latencies from the present experiment have been analysed to determine whether CXM affects the latency to enter the large compartment, and moreover to determine if this is a function of the time between CXM injection and training. Table 1 shows the training trial latencies of all groups. From this table it is clear that CXM does not significantly alter training latencies for any of the injection-training intervals employed. This result stands in

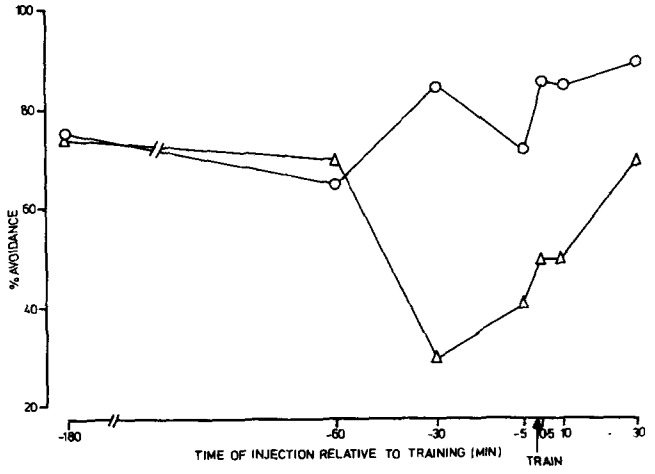

FIG. 1. Percentage of mice showing retention of the passive avoidance task by remaining in the small safe compartment. Different groups of mice were given subcutaneous saline or CXM (120 mg/kg) at various times relative to the training trial. Retention measured at 24 hr.

contrast to other reports [6,22]. The discrepancy may be due to strain differences as found by Randt *et al.* [22]. However, the finding that CXM does not affect training latencies when given up to 3 hr before training indicates that in the present situation, there are no effects upon step through behaviour, even at times of injection which result in substantial amnesia.

DOSE–RESPONSE RELATIONSHIP FOR CXM-INDUCED AMNESIA

The second experiment was concerned with establishing the effect of variations in dose of CXM on maintenance of a passive avoidance response over 2 weeks. On the basis of previous research [1, 8, 17] and the results of the first experiment, CXM was administered 30 min before the training trial in order to obtain the maximum treatment effect.

TABLE 1

MEAN STEPTHROUGH LATENCY (TRAINING TRIAL) AS A FUNCTION OF TIME OF INJECTION

Time of Injection Relative to Training (Min)	TREATMENT NaCl (N) Latency (sec)	CXM (N) Latency (sec)	t*	p
−180	22.9 (20)	22.7 (19)	0.051	≫.05
−60	35.7 (20)	37.6 (20)	0.285	≫.05
−30	34.6 (20)	25.4 (20)	1.485	>.05
−5	24.8 (21)	21.1 (29)	1.273	>.05
+½	29.0 (21)	29.8 (28)	0.226	≫.05
+10	37.3 (20)	32.6 (20)	1.25	>.05
+30	29.4 (20)	26.7 (20)	0.709	≫.05

*two-tailed t-test.

Method and Procedure

Separate groups were injected 30 min before training with either NaCl or 30, 70, 100, 120 or 150 mg/kg of CXM, and tested 24 hr after training. Groups with identical treatments were also tested 7 and 14 days after treatment. The size of each group varied from 19 to 25 mice.

RESULTS AND DISCUSSION

The results are shown in Fig. 2. Increasing dose of CXM has a significant effect upon avoidance at each retention interval (1 day $\chi^2 = 20.72$, $p<0.001$; 7 day $\chi^2 = 20.34$, $p<0.01$; 14 day $\chi^2 = 20.54$, $p<0.001$). Figure 2 indicates that amnesia is a monotonic decreasing function of increasing CXM dose for retention intervals of 7 and 14 days and for 1 day except for the 150 mg/kg dose of CXM. This is thought to be an artifact due to the systemic illness

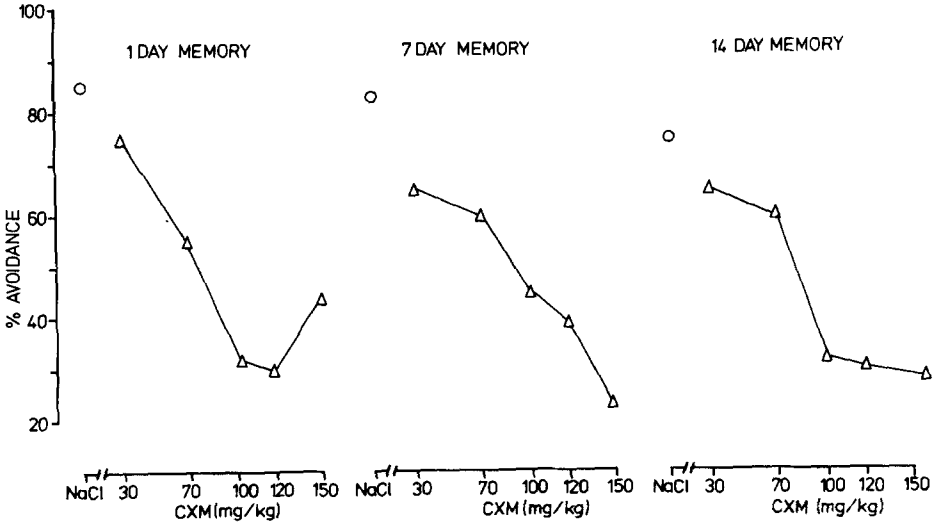

FIG. 2. Percentage of mice showing retention of the avoidance of the large compartment 1, 7 or 14 days after training, Mice were administered saline or CXM (30–150 mg/kg) 30 min before the training trial.

resulting from this high CXM dose. Evidence for this assertion comes for the observation that 15% of mice injected with 150 mg/kg CXM were either dead or too sick to test 24 hr after training, whereas 42% of mice similarly injected were dead after 7 days. Therefore about 27% of the mice actually tested at 24 hr were probably suffering from systemic illness. It was for this reason that Geller *et al.* [8], who used this dose, delayed testing for 7 days.

The training trial data from the first experiment revealed no effect due to 120 mg/kg of CXM on latency to enter the large compartment. Since it has been reported that CXM exerts a dose-dependent effect on the locomotion of mice placed in a box from 10–40 min after injection [25], the training trial data from the present experiment have been analysed to determine whether there is a CXM dose-dependent effect on latency. Training trial latencies for all mice given the same CXM dose, but tested at different intervals, have been pooled. An analysis of variance shows that there is no significant effect on latency due to varying doses of CXM, $F(5,333) = 0.148$. This finding supports the finding of no effect found in the first experiment and indicates that subsequent differences in avoidance cannot be attributed to differences in latency to enter the shock compartment during training.

DEVELOPMENT OF AMNESIA

It has been reported that CXM does not affect memory in mice for a multiple trial discriminated shock avoidance task 3 hr after training, although it induces amnesia 6 hr after training [1]. The same time course has been observed for CXM-treated mice trained in a multiple trial discriminated water reinforcement task [3]. The evidence on the development of amnesia in CXM-treated mice trained in one-trial passive avoidance of shock is conflicting. Quartermain and McEwan [17] reported that amnesia was evident within one min of training when low intensity (0.16 mA) footshock was used in training but amnesia did not appear until 24 hr after training when high intensity (1.6 mA) footshock was employed. However, Quinton [20] using a 2.0 mA footshock in training, found that amnesia developed 1.5 hr after training. The purpose of the present study was to investigate the development of amnesia up to 6 hr after training in a one-trial passive avoidance task.

Method and Procedure

Four groups of mice were injected with NaCl and trained 30 min later on the above described task. One of these groups was tested at either ½, 1, 3 or 6 hr after training. Four more groups of animals were similarly injected, trained and tested except that they were injected with 100 mg/kg CXM. A further 4 groups of animals were likewise injected, trained and tested except that they were injected with 120 mg/kg CXM. Each of the 12 groups contained 20 mice.

RESULTS

The most important aspect of the results of this experiment (Fig. 3) is the finding that the NaCl-injected mice do not exhibit a uniform degree of avoidance at all retention intervals ($\chi^2 = 11.81$, $p<0.01$). The 6 hr retention interval condition was repeated with a further 16 mice with the result that almost identical avoidance rates were obtained (45% first sample, 50% second sample). CXM-treated mice appear to respond differentially according to retention interval (see Fig. 3), however statements about the effect of CXM on memory retention intervals of less than 24 hr are rendered meaningless by the variability in response of the NaCl-injected mice.

The present experiment throws no light on the issue of the development of amnesia in the passive avoidance task [17,20] but rather indicates a serious problem for this paradigm when mice are tested for memory in the first few hours after training. It is noteworthy that Quinton's NaCl-injected mice showed a nonsignificant though very similar trend to the present results with the 5 hr-tested mice exhibiting the lowest median step-through latency for any retention interval [20]. It is possible that the 30 sec cut-off latency used in this study may have masked a significant trend in avoidance rates for NaCl-treated mice.

In the present experiment the majority of the group of mice tested for retention at 6 hours were tested between 3 and 6 p.m. Thus it is possible that, at this time of day, a

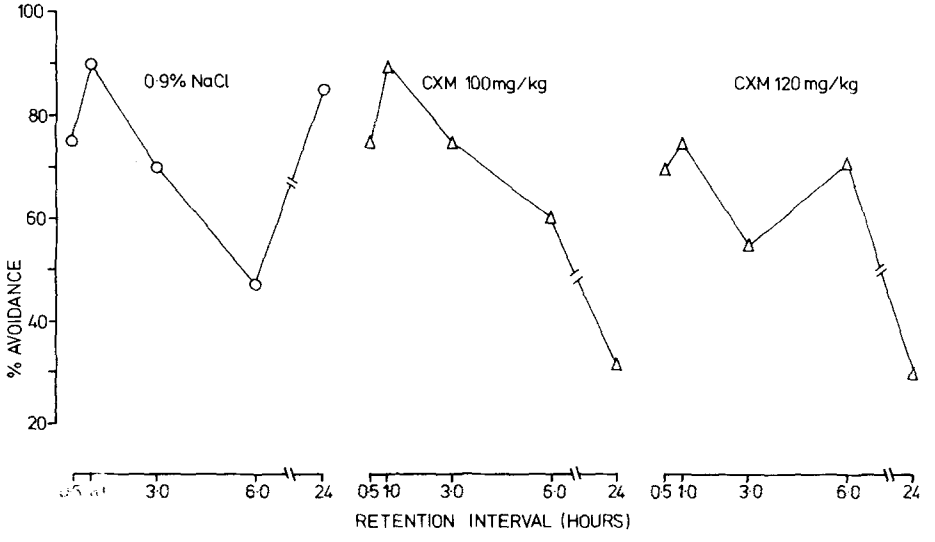

FIG. 3. Percentage avoidance of mice on retention tests ½ to 24 hr after training. Mice were given subcutaneous saline or 100, or 120 mg/kg CXM 30 min before the training trial.

natural increase in activity led to the observed decrease in avoidance. If an activity cycle effect was responsible for the low avoidance at this time of day, then it would be expected that NaCl-treated mice from other groups would also exhibit lower avoidance between 3 and 6 p.m. The three hour retention interval mice were tested over the period 12–6 p.m. Analysis of the proportion avoiding in the period 12–3 p.m. compared with 3–6 p.m. for this group shows no significant difference (Fisher Exact test, $p \gg 0.05$), although there was substantially more avoidance in the 3 hr group, taken as a whole, than there was in the 6 hr group (see Fig. 3). Analysis of the test data for 1 hr retention interval animals also shows no difference between 12–3 p.m. and 3–6 p.m. avoidance (Fisher Exact test, $p \gg 0.05$). These analyses show that the low avoidance of 6 hr-tested mice cannot be adequately explained by an increase in activity due to the time of day.

Another possible cause of the 6 hr retention interval effect is an interaction between the stress of the injection and the 6 hr interval. The next experiment was designed to test this possibility.

DETERMINANTS OF PERFORMANCE 6 HR AFTER TRAINING

The stress of injection by 6 hr interval explanation for the performance of NaCl treated mice would predict that mice not given any injection, or with the effects of injection masked by local anesthesia, would not display the low avoidance typical of NaCl treated mice but instead, a somewhat higher avoidance. Conversely, it would be expected that mice given a sham injection (needle puncture) would display a low avoidance if the most important noxious component of the injection was the pain associated with skin puncture and damage to underlying tissue.

Method and Procedure

Four groups of 18 mice were trained as in previous experiments and tested 6 hr later. Thirty min prior to the training trial one group was injected with NaCl as above. A second group was sham injected (i.e. needle puncture of the skin), and a third group was injected with the local anaesthetic, Lignocaine (0.1 ml of 1% solution). A fourth group was given no injection at all.

RESULTS

The results of this experiment are shown in Table 2. An overall χ^2 test reveals no significant difference in avoidance as a function of treatment ($\chi^2 = 5.07$, $p > 0.05$), however a significant difference in avoidance is obtained when NaCl + Sham groups are compared with Lignocaine + Not Injected groups ($\chi^2 = 4.94$, $p < 0.05$). This method of analysis [15] also shows that there is no significant difference within the NaCl and Sham subgroup ($\chi^2 = 0.132$, $p > 0.05$) or within the Lignocaine and Not Injected subgroup ($\chi^2 = 0.00$, $p \gg 0.05$). These findings support the hypothesis that the unexpectedly low avoidance displayed by NaCl treated mice tested 6 hr after training is a function of the interaction of needle puncture of the skin and the 6 hr interval.

TABLE 2

AVOIDANCE AT 6 HR AS A FUNCTION OF PRETRAINING TREATMENT

Treatment	NaCl	Sham Injection	Lignocaine	Not Injected
% Avoidance	50	56	78	78
N	18	18	18	18

GENERAL DISCUSSION

The present series of experiments has described the effect of systematic variation in injection-training interval and dose on CXM-induced amnesia for passive avoidance memory in mice. Our finding that the greatest degree of amnesia occurs for mice injected 30 min before training is consistent with the biochemical evidence that CXM produces maximum inhibition of protein synthesis within 30 min of injection [3, 6, 22]. The amnesia produced by CXM is clearly dose dependent and moreover this dose dependency is maintained over a 14 day period.

Recovery of memory after amnesia induced by a protein synthesis inhibitor has been reported in a number of papers [4, 5, 17, 18, 19, 23, 24]. In all of these studies the memory reappeared within 7 days. In contrast to this the present data and several other studies show no recovery of memory after CXM-induced amnesia for periods of 8–21 days after training [6, 7, 21]. Quartermain and McEwan [17] reported that an increase in shock intensity in one-trial passive avoidance led to the recovery of memory after CXM-induced amnesia. However subsequent work on the effect of variation of training strength has not shown any recovery of memory [6, 7, 21]. Furthermore, the present data show that when the strength of training is held constant and the amount of protein synthesis inhibitor is varied then the degree of amnesia remains relatively constant over a 14 day period. It is apparent that the conditions required to reliably produce recovery of memory are difficult to establish.

The methodologically important aspect of the present study is the finding that the interaction of the injection procedures with the 6 hr retention interval produced a level of avoidance indicative of a substantial amnesia (Figs. 2 and 3). The significance of this finding is that meaningful statements about CXM's amnesic action rest upon the assumption that control animals "remember". Clearly this result may be strain specific, however the observation of a similar, though nonsignificant, trend in a previous report [20] suggests otherwise. Another problem with this task is the host of environmental variables which can affect the animals' stepthrough behaviour without being discernable from the anmesic action of the drug. A discrimination task would alleviate this problem since the inference of memory is based on a choice rather than the occurrence or nonoccurrence of locomotion. In the light of these problems the authors suggest that it may be more profitable to use a discrimination task to assess short-term memory.

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