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Cycloheximide-Induced Amnesia for Taste Aversion Memory in Rats

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TUCKER, A. AND M. GIBBS. *Cycloheximide-induced amnesia for taste aversion memory in rats.* PHARMAC. BIOCHEM. BEHAV. 4(2) 181–184, 1976. – Male hooded rats were conditioned in one trial to avoid saccharin by pairing saccharin drinking with an intragastric injection of LiCl. A 24 hr water-saccharin preference test showed that conditioned rats exhibited a very low preference for saccharin whereas rats injected intraventricularly with cycloheximide (CXM, 400 μg) 5, 7, or 9 hr before training exhibited a greatly increased saccharin preference which differed significantly from NaCl injected controls. This 24 hr amnesia was found to be dependent upon the time of administration of CXM, since injection at 1, 3 or 17 hr before training did not confer amnesia. The nature of the task, a control measure and a control experiment indicate that the CXM-induced change in saccharin preference at 24 hr is not due to a CXM-induced aversion, nor a loss in drinking ability nor an inability to retrieve information whilst under the influence of CXM.

Saccharin aversion Cycloheximide Amnesia CXM injection-training interval

WHEN a rat drinks a distinctive tasting fluid such as saccharin, and is subsequently made ill (e.g. with LiCl) then it exhibits a strong, long-lasting memory for that particular taste [10]. This taste aversion is well suited to analysis of memory mechanisms since one trial is sufficient to establish the memory and moreover this information may be tested for in a discrimination situation.

Physiological investigations have shown that electroconvulsive shock (ECS), when interpolated between tasting and illness, has only a weak amnesic effect [14] unless fairly high current parameters are used [12]. A considerable problem with using ECS as a physiological treatment is that it is by no means clear which of the electrical and metabolic brain processes affected are crucial to the ensuing amnesia [11]. Cortical spreading depression has also been reported to produce amnesia for taste aversion memory when it is given between the taste and the illness experiences [7,8].

So far there is no information on the effects of metabolic inhibitors on the retention of such gustatory memories. Accumulated evidence suggests that the production of protein during or close to the time of learning is necessary for the encoding of memories in a number of species [5]. It follows then, that the cellular processes affected by protein synthesis inhibitors should be necessary for the establishment of taste aversion memories. The present study was designed, therefore, to investigate the effects of a protein synthesis inhibitor, cycloheximide (CXM), [1, 2, 4, 9, 13, 17, 18] on saccharin aversion memory in the rat.

METHOD

Animals

Seventy-seven experimentally naive male hooded rats aged approximately 80 days at the start of the experiment were used. All animals were bred in the Psychology Department, LaTrobe University, and were housed individually in the laboratory on a 12/12 light cycle for at least one week prior to the start of the experiment. All animals were maintained on ad lib food and water until 24 hr before the first training session when water was removed.

Apparatus

Training and testing was carried out in a 29 x 44 x 44 cm clear plastic box which had two 50 ml graduated cylinders fitted with copper drinking spouts attached to one wall. The spouts were accessible to a rat inside the box through 2 cm dia. holes set 13 cm apart at a height of 5 cm above the wire gauze floor of the box. The floor and the copper spouts were connected to an electronic circuit which counted tongue contacts with the spouts. The clear plastic box was housed in a larger sound attenuating box fitted with a one-way mirror.

Experimental Design

Prior to the experiment animals were assigned, on a random basis, to one of 14 groups. In the 6 experimental groups rats received intraventricular CXM at 1, 3, 5, 7, 9 or 17 hr before the start of the training trial. Animals in the placebo control groups received identical treatment to those in the experimental groups except that these animals received physiological saline intraventricularly, instead of CXM. Animals in the final 2 groups, control groups CC2 and CC3, received no intraventricular treatment. Group CC2 animals received the standard taste aversion training in which saccharin was followed by LiCl poisoning. Group CC3 animals were unconditioned controls which were given saccharin followed by NaCl.

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Procedure

Days 1–3 of the experiment were the pretraining phase in which rats were placed into the experimental box for daily 10 min sessions and trained to drink tap water from both bottles attached to the box. During these trials and all subsequent phases of the experiment each rat's total number of licks at each spout was recorded. Animals which did not register at least 100 licks at each spout on at least one of the 3 pretraining days were eliminated from the experiment. Throughout the experiment the only fluids received were those consumed in the training and testing trials and those fluids injected. On Day 4 at the appropriate time prior to training the CXM and NaCl groups received their intraventricular injections.

The freehand technique of injection used was a modified version of the one described by Noble and co-workers [16]. Rats were anesthetised with ether and placed on a small animal rack. A midline incision was made, the skin reflected and the cranial bone was cleaned. A small hole was bored by hand at a position 1.5–2.0 mm lateral to the saggital suture and immediately posterior to the coronal suture over the left hemisphere. A Hamilton dispenser and 500 μl syringe fitted with a 26 ga needle was carefully lowered into the hole. The needle was fitted with a stop so that it penetrated 4.0 mm below the cranial surface. Thirty microliters of solution was then injected into the ventricle. The hole was filled immediately with bone wax and the incision closed with skin clips. The entire operation usually took 5 min and animals were fully recovered from the anesthetic within 15 min. Experimental animals received 400 μg of CXM dissolved in 30 μl of 0.9% NaCl while the placebo controls received 30 μl of 0.9% NaCl.

In the training trial all rats were given a 0.1% sodium saccharin solution in place of the water in both cylinders. At the conclusion of this 10 min saccharin drinking session animals in all groups except CC3 were given an intragastric injection of 0.6% (0.15 M) LiCl (10 ml/kg body weight). Group CC3 animals received an intragastric injection of 0.9% NaCl (10 ml/kg body weight). Data from rats which licked less than 25% of their last pretraining trial lick total were not included in results.

On Day 5 all rats were given a retention test 24 hr after their training trial. In this session one cylinder contained tap water, the other 0.1% saccharin. The positions of the water and saccharin cylinders were decided by a random process. At the conclusion of the retention trial a saccharin preference score (%P) was computed for each rat, according to the following formula:

$$\%P = \frac{\text{number of saccharin licks}}{\text{total number of licks}} \times 100$$

Data from rats which failed to lick 50% or more of, their final pretraining trial lick total were not included in the results.

RESULTS

Figure 1 summarizes the main finding of this experiment, namely that CXM will cause amnesia for the memory

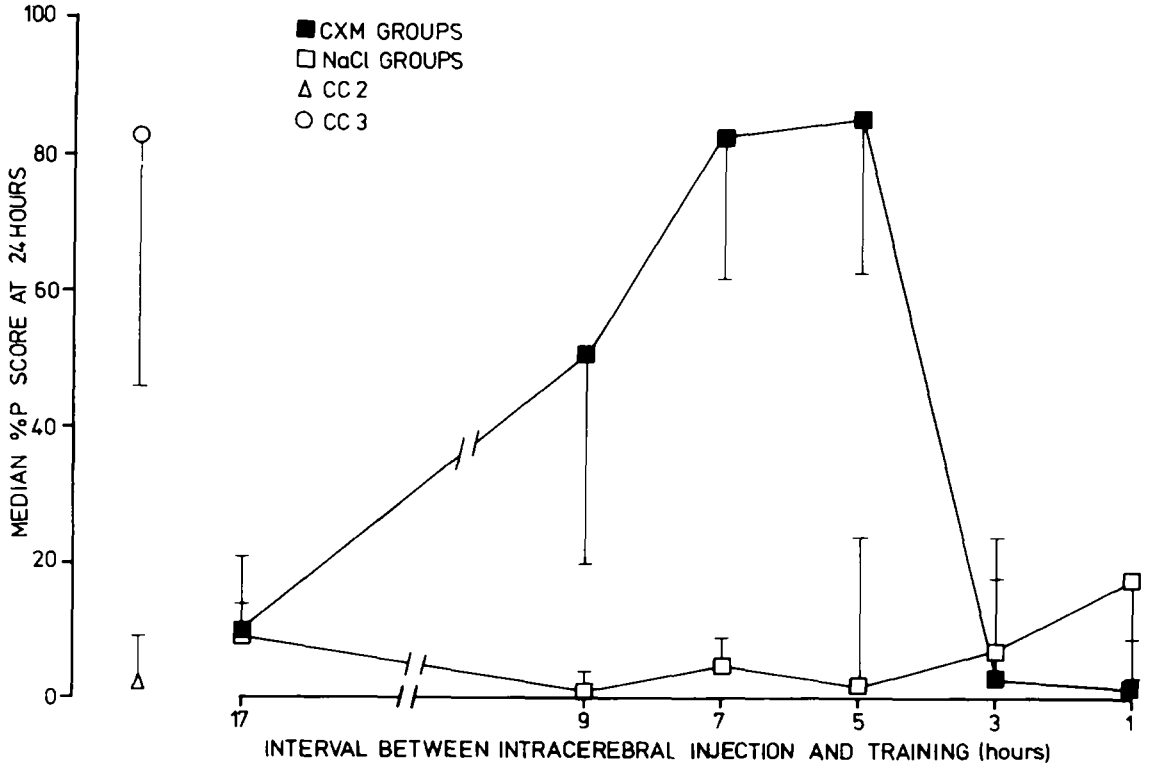

FIG. 1. Median saccharin preference scores 24 hr after training as a function of time of intraventricular treatment with CXM (400 μg) or isotonic saline (30 μl). Saccharin preferences at the level of untreated controls (CC2) indicate memory whilst saccharin preferences at the level of unconditioned controls (CC3) indicates complete amnesia. Vertical bars are half of the interquartile range.

TABLE 1

TOTAL NUMBER OF LICKS DURING 24 HR RETENTION TEST

Group	Median	$(Q_1 - Q_3) / 2$*	Group	Median	$(Q_1 - Q_3) / 2$*	p†
E1	2013	643	C1	2246	293	0.246
E3	2410	307	C3	2020	524	0.420
E5	2651	247	C5	1774	871	0.202
E7	2852	647	C7	2492	435	0.638
E9	2614	362	C9	2642	349	0.876
E17	3007	548	C17	2328	768	0.150
CC2	1734	378	CC3	2869	583	0.032

*Q_1 = lower quartile; Q_3 = upper quartile †Mann-Whitney Test, 2 tailed

of the taste of saccharin 24 hr after the learning trial when CXM is given intraventricularly from 5 to 9 hr prior to training. One-tailed Mann-Whitney U tests indicated that the CXM groups differed significantly on their %P score from the physiological saline controls at 5 hr (CXM median = 86, NaCl median = 2, $p = 0.003$), 7 hr (CXM median = 83, NaCl median = 5, $p = 0.024$) and 9 hr (CXM median = 51, NaCl median = 1, $p = 0.001$). Unconditioned controls CC3, though appearing to be quite different from the untreated conditioned controls CC2 were in fact not significantly different (CC3 median = 83, CC2 median = 2, $p = 0.075$, Mann-Whitney test, 1-tailed). However this nonsignificance may well be due to low group sizes, since one rat out of the 5 in group CC3 preferred water over saccharin to such an extent that he gave a %P score of zero. In spite of this it is worth noting that the median %P scores for the 5 and 7 hr CXM-treated animals are very close to that of the unconditioned controls (CC3). Also Fig. 1 reveals that the NaCl controls prefer saccharin at about the same level as rats which had received only the basic taste aversion conditioning.

The effects of the drug upon total number of licks during the retention test is shown in Table 1. The total number of licks recorded for each rat during its retention test may be taken as an index of general drinking behaviour. Statistical analysis of this data shows that only the controls CC2 and CC3 differ significantly from each other (Mann-Whitney test; $p = 0.032$, 2 tailed). None of the CXM groups differs from its appropriate NaCl control.

In order to test the possibility that CXM causes blockade of information retrieval during the retention test, additional data was collected. Four rats were injected with 400 μg of CXM 7 hr before the 24 hr retention test and compared with the 24 hr saccharin preference of another group of 4 rats injected with CXM 7 hr before training. Table 2 shows that the resulting saccharin preferences form nonoverlapping distributions ($p = 0.014$, Mann-Whitney test). From this it is clear that rats can retrieve previously stored information when cerebral protein synthesis is inhibited by CXM. This result also suggests that the performance change 24 hr after learning is not due to state dependent learning.

TABLE 2

24 HR SACCHARIN PREFERENCE OF INDIVIDUAL RATS UNDER THE INFLUENCE OF CXM

Treatment	% P			
CXM 7 hr before retention test	1	0	1	1
CXM 7 hr before training trial	53	88	55	9

DISCUSSION

Information on the biochemical time-course of inhibition of cerebral protein-synthesis, after intracerebral CXM injection, has been reported for mice but not, as yet, for rats. Barondes and Cohen [2] reported that the peak inhibition of cerebral protein synthesis (94–95%) in mice occurred 5–8 hr after injection. This biochemical time-course concurs with the time-course of amnesia found in the present experiment.

Although there are numerous reports of amnesia after subcutaneously injected CXM, there is only one report of intracerebral CXM treatment of mammals [2]. In this work no amnesia was found for mice trained in a multiple trial discrimination task after CXM injection. However there is evidence that this result may have been due to an excessive amount of training for the single-dose level of inhibitor employed [3].

Two recently published articles have reported that CXM can induce aversions to odors [6] and to water [15] when

administered intraperitoneally or subcutaneously (respectively). This raises the possibility that CXM-induced deficits of performance in appetitive tasks may not be due to amnesia but rather a CXM-induced conditioned aversion. The result of the present experiment does not support this non-memory interpretation. In the taste aversion paradigm a CXM-induced aversion to saccharin would lead to a reduced saccharin preference, not the reverse as is demonstrated. Also, CXM did not produce aversion to water when administered before the retention trial. Hence, intraventricular CXM would seem to be exerting a true amnesic influence on saccharin aversion memory.

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