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BRIEF COMMUNICATION

Midazolam Induces Amnesia in a Simple, One-Trial, Maze-Learning Task in Young Chicks

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GILBERT, D. B., T. A. PATTERSON AND S. P. R. ROSE. *Midazolam induces amnesia in a simple, one-trial, maze-learning task in young chicks.* PHARMACOL BIOCHEM BEHAV **34**(2) 439–442, 1989. —We report a simple, one-trial, learning paradigm which we have developed for use in young chicks. Chicks were separated from their brood mates and placed in a small isolation chamber. A 'T' corridor, or maze, connected the isolation chamber to the brood space, allowing the chick to escape isolation stress and rejoin the brood. When the chick successfully negotiated the corridor, the latency to perform this task was recorded. On a subsequent trial, any improvement in the speed of performance was reasoned to reflect the chick's memory of the task. Undrugged chicks always showed significant improvement in task latency if they were replaced in the maze 3 hours after a successful escape, suggesting that they had remembered the task. Chicks given midazolam (0.1 or 0.3 mg/kg, IP), a benzodiazepine, before the first escape, showed no improvement on their second escape. Improved performance was seen, however, if a second injection of midazolam was given before the second escape, suggesting a state-dependent effect.

Chicks Escape task Amnesia Midazolam Passive avoidance

FOR the past two decades there has been a blossoming of interest in the investigation of possible neural mechanisms associated with memory storage [e.g., (3, 6, 8)]. One approach to this theme requires animals to learn a simple, one-trial task, whose time parameters are well mapped and whose relationship to other forms of adult learning is reasonably secure. Several laboratories study this area using day-old chicks in a variety of learning tasks, ranging from the one-trial passive avoidance learning paradigm (PA), first reported by Cherkin (3), to the imprinting paradigm [e.g., (6)]. Briefly, in PA, chicks are presented with a small, shiny bead which they peck spontaneously. If the bead is coated with methyl anthranilate, a bitter disgustant, then chicks whose memories remain intact will avoid pecking the bead on subsequent presentations, but amnesic chicks will continue to peck the bead. Imprinting studies use the memory formed when the chicks recognise salient objects that are thought to represent their mother. Thus, in these memory studies, imprinting is useful, but could be argued to be a "special case," while PA is good but relies on a specific aversive response, giving rise to questions of the generality of the findings. Thus, it is advantageous, if not necessary, to the understanding of memory in young chicks to examine the problem with observations from other, nonpecking, tasks.

If young chicks are separated from those things which they have been close to, for example, their mother or brood mates, they show characteristic distress behavior. Therefore, we reasoned that chicks would work to escape isolation-induced stress, such that on one trial, say negotiating a maze, a memory for the task would be formed. Such a maze learning task would retain the advantages of being a one-trial paradigm, but would also have the benefit of having a quantifiable variable, the time taken to escape, as opposed to the all-or-none nature of a pecking task. Therefore, we designed and constructed a simple apparatus, consisting of an isolation chamber with a T corridor and, as an initial test of the apparatus' sensitivity to possible drug-induced amnesia, we gave chicks midazolam before running them through the maze.

Midazolam is a benzodiazepine. Benzodiazepines have been reported to induce amnesia in humans [e.g., (2,7)] and in animals [e.g., (11)], but it has been suggested that midazolam-induced amnesia may be state-dependent (11). As far as we can ascertain, midazolam has not been used before in young chicks. Midazolam is fast acting and quickly metabolised, lending itself well to studies where memory retention is tested within a few hours of the drug effect. We, therefore, selected midazolam as a drug with which we could test the sensitivity of the paradigm to putative amnestic effects. Further, to test the possibility that midazolam induces state-dependent amnesia in this model, a second injection was given prior to the second test run through the maze.

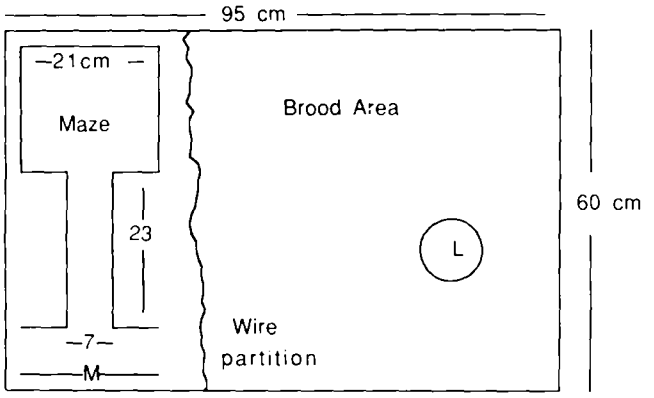

FIG. 1. A plan view representation of the apparatus. All dimensions shown are in centimeters. The brooder was 50 cm deep, while the isolation box and maze apparatus was 21 cm deep. The wire partition was to keep the brood from pecking at the apparatus, providing distracting cues, while the experimental chick was in the isolation chamber. A small 10×10 cm, mirror (M) was fixed just above the floor of the T maze. The circled L shows the position of the light and heat source, hence, the isolation chamber was less illuminated than the brood area.

METHOD

Subjects

A total of 96 Ross chunky chicks of both sexes served as subjects. The chicks were either hatched in our own laboratory or were purchased (Ross Poultry, Andover, Hants., U.K.). Chicks from our own laboratory were 36–48 hours old at testing, while those that were bought in were about 48 hours old. The chicks weighed 50 g ($\pm$ 10 g range) and before testing they were allowed free access to food (Biosure, starter chick crumbs) and tap water. They were housed in a large brooder, with a wood shaving floor covering, in a room with constant temperature (24°C) and humidity and a 12-hour dark 12-hour light cycle (lights on at 7 a.m.).

Apparatus

The apparatus (Fig. 1) consisted a T maze with isolation chamber, fashioned from stiff white cardboard (poster board) placed inside a large communal brooder, but separated from the brood area by chicken wire (for dimensions see Fig. 1). The floor of the isolation chamber was covered with a blue paper towel which was changed when it became fouled, while the brooder floor was covered with wood shavings. A small mirror (10×10 cm) was located at the T junction of the maze (Fig. 1.) The brood area was illuminated with a bright lamp (250 W) suspended immediately above it and food and tap water were available there ad lib.

The apparatus was kept in a small room ($9' \times 7'$) held at constant temperature and humidity during testing. So far as possible, external cues were kept constant during testing.

Drugs

Midazolam maleate (a gift of Roche, Welwyn Garden, U.K.) was dissolved in physiological saline. For a dose of 0.3 mg/kg a solution of 0.15 mg/ml, while for a dose of 0.1 mg/kg, a solution of 0.05 mg/ml were prepared as stock. Injections were given IP in a volume of 0.1 ml per bird 5 minutes before exposure to the apparatus. Control chicks received injections of 0.1 ml saline. The doses were chosen on the basis of published literature (5) and our own pilot observations (N.B. doses of midazolam above 1.0 mg/kg induced immobility and shivering, suggesting hypothermia). Drug solutions were coded by another person before administration and administered blind.

Procedure

Before training, all chicks were individually marked with spray dye and were placed in the communal brooder area for at least an hour to interact with each other freely. Training always commenced at 10 a.m. when chicks were randomly assigned to each of four drug groups (saline on the first and second escape; midazolam on the first trial, saline on the second; saline on the first trial, midazolam on the second; and midazolam on both escapes). Each repeat of the experiment was a balanced design using all 4 groups.

Each chick was injected 5 min before being separated from the flock and placed individually in the centre of the isolation box, facing away from the T corridor. The time taken for the chick to arrive at the exit of the apparatus was recorded, and the chick was then immediately replaced with its brood mates. A cut-off criterion of 600 sec was decided for any bird failing to find its way out, but in practice this criterion was not needed. After three hours, the chicks were again injected and placed in the apparatus. It was reasoned that chicks who remembered the way out from before would show an improved performance, leaving the isolation apparatus more quickly on the second trial.

Statistical Analysis

Where a reduction in time was predicted, one-tailed paired Student's *t*-tests were used, so each chick served as its own control. One-way analysis of variance (ANOVA) was used to check for any significant differences between each group of chicks with respect to their first escape latencies.

RESULTS

Figure 2a shows the changes in time recorded between the first and second trials for the four groups of chicks associated with administration of 0.1 mg/kg midazolam, while Fig. 2b shows the results seen with 0.3 mg/kg. One-way ANOVA revealed no differences between any of the 8 groups of chicks in their times to leave the maze on the first trial (i.e., the groups were all the same, statistically speaking, to start with).

The two groups (SAL/SAL) of birds that received saline before both exposures to the apparatus showed a significant reduction in their mean time to leave the isolation chamber, from 84.8 sec to 21.8 sec on their second trial, $t(13) = 2.98$, $p < 0.006$, one-tailed, and from 73.9 sec to 33.2 sec, $t(9) = 2.68$, $p < 0.05$, seen in Fig. 2a and b, respectively.

The two groups (SAL/MDZ) of birds that got midazolam before their second trials showed impaired performance on their second escape. Chicks which had the higher dose (Fig. 2b) failed to demonstrate a significant improvement, going from a mean time of 73.8 sec to 54.7, $t(9) = 1.29$, N.S., as did the group that was administered the lower dose (Fig. 2a) going from 53.3 sec to 26.8 sec, $t(13) = 1.59$, $p = 0.068$, N.S. The two groups (MDZ/SAL) of chicks which were given midazolam on the first escape only, also both failed to show a significant decrease in escape latency on their second trial. Birds which received the lower dose of midazolam left in 58.6 sec and then 47.8 sec, $t(13) = 0.73$, N.S., Fig. 2A, while chicks given the higher dose of midazolam (Fig. 2b) actually increased their mean escape time from 54.0 sec to 59.4 sec across the trials, $t(9) = 0.4$, N.S.

The final two groups (MDZ/MDZ) of birds were included because of the possibility that any midazolam-induced amnesia in this model is state-dependent. Thus, they received midazolam before both trials and both groups improved their performance. An

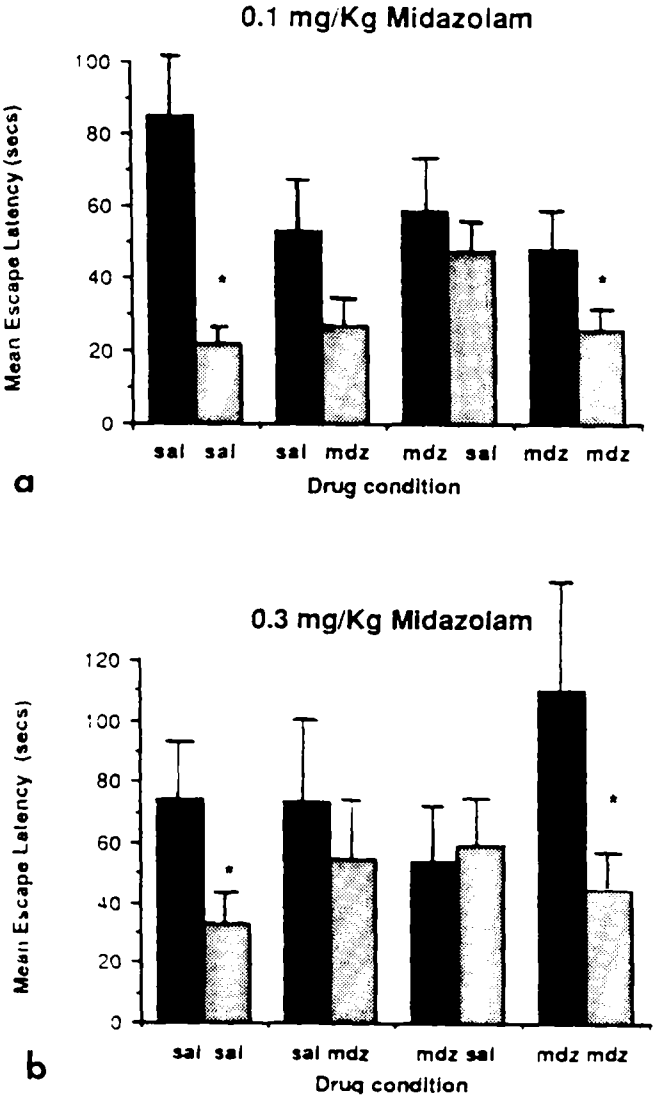

FIG. 2. The mean escape latencies (+SEM) are shown for those chicks who received 0.1 mg/kg midazolam (a), or 0.3 mg/kg midazolam (b). N = 14 chicks per group (a) and 10 birds per group (b). (*$p<0.05$.) One-way ANOVA revealed no significant differences between any drug condition on the first escape. Black bars: first escape; shaded bars: second escape.

improved time from 48 sec to 25.4 sec, $t(13)=3.44$, $p<0.003$, was observed for chicks receiving 0.1 mg/kg midazolam (Fig. 2a), and a similiar significant reduction in the mean time for the birds given a higher dose of midazolam, at 0.3 mg/kg, from 110.4 sec and 45.0 sec, $t(9)=2.26$, $p<0.05$, was recorded (Fig. 2b).

DISCUSSION

When chicks were separated from their brood mates and placed in the isolation chamber, they generally showed signs of emotional stress (e.g., distress calling, looking around and exploring the apparatus, in some cases defecating) but always negotiated the T corridor back to their brood mates. It was assumed that isolation stress motivated the birds to find their way out of the box, such that the task is a one-trial avoidance task. Birds who received only saline (or no injection at all in pilot studies), always improved their time to leave the isolation chamber on the second trial (Fig. 2a and b). The most parsimonious explanation of this improvement in performance suggests that the chicks had learnt something about, or formed a memory of the apparatus on the first trial. This spatial learning is undoubtedly different from the learning studied in, for instance, PA, in that it involves no pecking or obvious gustatory processing. Against a background literature of 'types' of learning, however, more work is needed to determine the type of learning involved in the escape task. This is particularly relevant in the chick where the relationship between at least two types of learning, imprinting versus associative learning, has been much discussed [e.g., (1)].

Administration of midazolam (0.1 or 0.3 mg/kg) before the first escape resulted in no significant improvement in performance on the second escape (Fig. 2). These observations are consistent with an hypothesis that midazolam-induced amnesia for the task is occurring and are in accord with previous reports of benzodiazepine-induced amnesia (2, 7, 11). Where midazolam was given only before the second trial, the results are less easily interpreted. Birds given either dose still failed to escape significantly faster than before (Fig. 2), but where there is some suggestion in the literature that amnesia associated with benzodiazepine administration may be mainly affecting long-term memory processes, seen only when benzodiazepines interfere with the formation of such processes, or with the formation of associations (2,7), in the current study, it is not clear to what extent the apparent amnesia seen with administration of midazolam on the second trial reflects true amnesia of the earlier exposure to the apparatus, or comes about indirectly. When midazolam was given before both trials the birds showed no amnesia (Fig. 2), suggesting that the amnestic effect of the drug is state-dependent in this task with chicks. Again, this phenomenon has been reported before with benzodiazepines (11).

The observations raise some interesting questions with respect to some of the contemporary thinking about how the benzodiazepines bring about their behavioral effects. For example, it has recently been shown that place preference can be induced with benzodiazepines (4, 9, 10). These studies raise the possibility that the lack of performance improvement associated with midazolam given before the first trial in the present experiments may come about as a result of the chicks lingering longer in the presence of drug-paired environmental cues. Conversely, the current observations bring into question to what extent benzodiazepine-induced place preferences in rats come about as a result of amnesia.

A second anomaly comes from the widely held belief that benzodiazepines are anxiolytic, or stress attenuating in their effect. If that were always the case, it would be expected that midazolam would reduce the isolation stress in the chicks so that they would not be as motivated as control birds to escape and rejoin their brood mates. This clearly was not the case. If anything, birds injected with 0.3 mg/kg of midazolam before the first trial escaped from the apparatus quicker than the others (Fig. 2). More work is needed to determine if midazolam reduced any neophobia that may be generated by the T corridor, but left the isolation stress intact. The data, however, do not support the idea that midazolam allowed the birds to perform at an optimal level on the first trial, so that they could not improve on the second trial and would appear amnesic. All other groups of chicks escaped in much faster mean times (Fig. 2) on the second trial than the birds given 0.3 mg/kg midazolam on the first trial only. We should stress, however, that the benzodiazepines are an extremely complex class of drugs at both the molecular and behavioral level. Much more work is required fully to investigate the effects of midazolam in this paradigm, where we were interested, at this stage, to show only that the model would detect drug-induced amnesia if it were present.

In summary, the results suggest that escape from the novel apparatus is a behavior capable of being learnt by young chicks, that this learning is sensitive to the induction of midazolam-induced amnesia; and that this amnesia is state-dependent with respect to recall. The method may well prove a valuable tool in exploring neural mechanisms of memory and learning.

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