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Pharmacology, Biochemistry and Behavior 70 (2001) 133–139

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Chlordiazepoxide specifically impairs nonspatial reference memory in the cued radial arm maze in rats

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Received 22 May 2001; accepted 29 May 2001

Abstract

Anxiolytic benzodiazepines, at low doses, reportedly impair the radial arm maze with nonspatial visual/tactile but not spatial cues. We replicated the former result controlling for changes in drug state and cue effectiveness. Rats learned an eight-arm radial maze with reward in only four arms. The reward varied in spatial position from trial to trial but was always cued by a piece of sandpaper at the entry to the arm. Chlordiazepoxide (5 mg/kg, ip) impaired acquisition. Rats that switched from saline during acquisition to chlordiazepoxide showed an impairment of performance that only lasted for 1 day. Removal of the cues reduced the performance of controls and switched rats to the level of the rats that received chlordiazepoxide during acquisition but did not affect the latter. These data suggest that chlordiazepoxide does indeed impair nonspatial reference memory in the radial arm maze while leaving working memory, and, possibly, spatial reference memory, intact but that the previous report of this effect was the result of a change in drug state rather than of the drug itself.

Keywords: Anxiolytic; Benzodiazepine; Chlordiazepoxide; Radial arm maze; Working memory; Reference memory; State dependency

1. Introduction

The classical anxiolytic drugs (e.g. barbiturates, benzodiazepines) and novel anxiolytic drugs (e.g. buspirone) share clinical effectiveness in treating anxiety but have quite distinct side effects. Their common anxiolytic action has been proposed to result from their common effects on the electrophysiology of the septo-hippocampal system (Gray, 1982; Gray and McNaughton, 2000). Given the sensitivity of spatial tasks to hippocampal damage (O'Keefe and Nadel, 1978; Morris et al., 1982), this theory predicts that anxiolytic drugs should affect spatial tasks like Morris's water maze (Morris, 1984) and this has proven to be the case (McNaughton and Morris, 1987, 1992).

The parallel effects of hippocampal lesions and those of anxiolytic drugs appears to break down, however, in the radial arm maze. This task was devised by Olton and Samuelson (1976) to assess spatial learning ability in rats and, although it can be solved using nonspatial strategies, appears as sensitive to hippocampal lesions as the water maze (Gray and Rawlins, 1986; Cassel et al., 1998; Floresco et al., 1997; M'Harzi and Jarrard, 1992; Winocur, 1982). Gray and Rawlins (1986) report that substantial interference with performance in an eight-arm radial arm maze was obtained only with 20 mg/kg chlordiazepoxide and not with the 5-mg/kg dose that is normally effective both in the Morris water maze (McNaughton and Morris, 1987) and on hippocampal theta rhythm (McNaughton et al., 1986). McNaughton et al. (1983) report a similar failure of 5 mg/kg chlordiazepoxide in the 16-arm radial maze.

Hodges and Green (1986), by contrast, reported that 5 mg/kg chlordiazepoxide impaired the efficiency of responding in a version of the radial arm maze where only half the arms were baited at random and correct arms were indicated by the presence of coarse sandpaper. If the baiting and sandpaper remained in a fixed position from trial to trial, the drug was less effective. If taken at face value, these results suggest that it is some factor such as level of complexity of the task, rather than anything to do with space, that renders the water maze sensitive and the conventional radial arm maze insensitive to chlordiazepoxide at 5 mg/kg.

This interpretation of Hodges and Green's results is, however, open to question. They did not administer the

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0091-3057/01/$ – see front matter
PII: S0091-3057(01)00590-1

drug until after the rats had reached a level of 60–70% correct and then did so only on a single trial. The decrease in performance that they observed could therefore have been the result of a change in drug state on learning (see e.g. McNaughton, 1985) rather than a specific interaction of the drug with the type of learning being tested. The present study, therefore, investigated the effect of chlordiazepoxide on the task used by Hodges and Green (1986) but with the drug administered throughout acquisition. We also tested the effect of introducing the drug for a number of trials in rats that had learned the task in an undrugged state to a level of 60–70% correct to replicate Hodges and Green's paradigm. Finally, we finished testing with a probe trial in which the rewards were present but the cues removed so as to determine the extent to which correct behaviour was under the direct control of the specific sandpaper cues. We did this because Olton and Samuelson (1976) had found that undrugged rats did not use intramaze cues in solving a simple uncued radial arm maze and extramaze cues could have contributed to the working memory component of the cued version of the task.

2. Method

2.1. Subjects

The subjects used were 36 male Sprague–Dawley rats. They were 320–465 g (10 months old) at the beginning of training and were kept on 23-h food deprivation with water freely available. The rats were caged in groups of four at a temperature of 22°C with a natural light–dark cycle. All of the rats had been tested for ambulation and rearing in an experiment 7 months earlier using an open field. During this previous study, 1/4 had been dosed with 5 mg/kg chlordiazepoxide, 1/4 with 1 mg/kg Naloxone, 1/4 with chlordiazepoxide and Naloxone, and the remaining 1/4 were the control group on saline. In addition, 16 of the rats (Numbers 1 to 16) had been used in an experiment with a T-maze. Position discrimination and reversal was assessed using 10 trials per day over a period of 15 days. Each animal received the same drug as in the open-field experiment. The latter study was carried out 5 months prior to this present study. The experiments were approved by the University of Otago Animal Ethics Committee.

2.2. Apparatus

The apparatus was an eight-arm radial maze constructed of clear, colourless Perspex. The arms were 9 cm wide, 68 cm long, and extended from an octagonal centre, which measured 33 cm in diameter. The arms were numbered 1 to 8 in a clockwise direction. A 6-cm-high edge ran around the outer sides of the arms and central platform. At the end of each arm was an 8.5-cm square of Perspex. A hollow area at the centre of these squares acted as a receptacle for the reward pellets (45 mg, Camden Instruments, UK). Four strips of coarse sandpaper (5×9 cm) were used as cues. Each was placed 5 cm from the central platform across the mouth of the selected arms. The entire maze was elevated 52 cm above the ground and was illuminated by a 15-W bulb that was positioned 68 cm above the centre of the maze. Additional materials included a 6.5-cm-high aluminium ring with a 25-cm diameter, a digital stopwatch, 0.5×16 mm hypodermic needles, and 1-ml disposable syringes. Boxes made of cardboard with Perspex lids were used to hold the rats between trials and between the injection time and training time. The injecting and training was conducted in a 2.3×5-m room that was thermostatically maintained at a temperature of 22°C. The windows were blacked out and objects in the room, such as a door, cupboards, a desk, and other equipment provided a variety of extra maze cues.

2.3. Group assignment

The rats were assigned to the three conditions in a counterbalanced fashion, which ensured each condition contained equal numbers of rats from each of the experimental groups they had been assigned to in the previous studies. The three conditions were:

- CDP–CDP: chlordiazepoxide hydrochloride (CDP; 5 mg/kg; Roche);
- SAL–SAL: saline (SAL; 0.9% NaC1) for the control group; and
- SAL–CDP: SAL until Trial Block 13 and then CDP (5 mg/kg).

The CDP was dissolved in 0.9% saline. All injections were given intraperitoneally at 1 ml/kg half an hour before training. For half of each drug group, the cue signalled the presence of food at the end of the selected arms, and for the other half of each drug group, the cue signalled absence of food at the end of the selected arms and pellets were at the end of the uncued arms.

2.4. Training

Subjects were first familiarised with the radial arm maze. This involved placing three reward pellets at the end of each of the arms. One rat at a time was placed in the maze and remained there until all pellets had been located and consumed or until 30 min had elapsed The time taken for each rat to collect all reward pellets was recorded. Because eight of the rats did not move freely within the maze and locate all pellets within the 30-min time limit, they were administered the familiarisation procedure two more times.

Following familiarisation, the animals were given two sessions of additional pretraining. The first session involved injecting half the concentration of drug or saline half an hour before each rat was placed in the maze to locate and consume three pellets from each of the eight arms. The

second session was similar except full doses of drug or saline were administered. In both sessions, cues were not used and each rat was removed from the maze after consuming all of the pellets. A time limit was not imposed. Acquisition trials were initiated the day after pretraining ceased and occurred between 6 AM and 12 NN each day. Six rats were randomly selected from each drug group and the two sets of 18 rats were trained on alternate days with the order of training for each day being determined randomly. A training session for each rat consisted of three consecutive trials (analysed as blocks).

The rats to be trained were placed in holding boxes with their cagemates and taken to the testing room before being injected. Half an hour before each rat's scheduled training time, it was injected. Before each trial, the maze was wiped with water and disinfectant to eliminate scent trails, and one cue and two pellets were placed in each of the selected arms. There were six cue patterns used that followed the criteria of Hodges and Green (1986) where no more than two cues were adjacent (see Fig. 1). Their criteria allow 26 possible cue patterns. The two patterns where either all of the even or all of the odd numbered arms were cued were excluded. This left a pool of 24 patterns, and from this, the six cue patterns used in the study were randomly selected.

For the rats where the cue signalled food, they were allocated Patterns 1–6. For the rats in the groups where cues signalled no food at the end of these arms, they were allocated Patterns 7–12. Patterns 7–12 were identical to Patterns 1–6 in terms of the arms in which the cues were placed, but pellets were placed in the uncued arms. Allocation of cues (hence, pellets) was such that all of the six patterns were used over two blocks of three training trials.

At the beginning of the trial, each rat was placed within the aluminium ring that enclosed the central part of the maze. The aluminium ring was used to reduce the tendency of a rat to run down a particular arm solely because it had been placed near or facing the arm while being lifted into the maze. The aluminium ring was removed approximately 2 s after the rat had been placed in the maze and at the same time the digital timer was started. Every arm entry was recorded by arm number and an entry was defined as being when the entire body of the rat (including the tail) passed the line that indicated where the particular arm of the maze was attached to the central platform. The trial ended when the rat had picked up all of the pellets. The time for the rat to complete the trial was recorded. The rat was returned to a holding box alone between trials when the maze was rewiped, the cue pattern was changed, and pellets and the aluminium ring were replaced. After three consecutive trials, the rat was returned to its cage with its cagemates. Average running time for each rat was calculated as the time taken to run the three trials divided by the total arm entries for the three trials. The acquisition training continued for 13 blocks of three trials, at which point the saline-treated groups reached over 60% efficiency. Efficiency was calculated as the percentage of reward arm entries for the three trials (always 12 entries) in the total number of entries for the three trials (variable depending on errors). The 60% level of efficiency was chosen as the criterion needed for group SAL–CDP to be changed from SAL to CDP since Hodges and Green (1986) had used a criterion of 60–70% efficiency. For Trials 40–45, group SAL–CDP rats were injected with 5 mg/kg CDP. The experimental procedure and treatment for the other groups continued as it had for the previous trials.

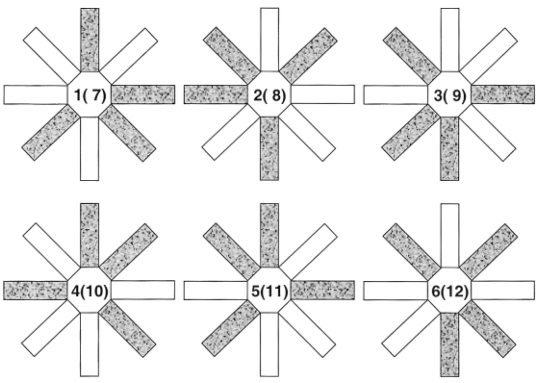

Fig. 1. Diagrammatic representation of arms of the maze with sand paper placed at the mouth of the maze on any given trial. Half the rats received Patterns 1–6 in which sandpaper signalled the presence of food. Half received Patterns 7–12 in which the sandpaper signalled the absence of food.

2.5. Probe test

At the end of the experiment, a probe test was conducted in order to determine if the rats had in fact employed the sandpaper cues to guide them to where the pellets were located. The probe test involved three consecutive trials for each rat. No cues were used, but, otherwise, the procedure and reward allocation was the same as for the previous trials. Drugs were the same as they had been for Trials 40–45.

2.6. Data analysis

Running times and number of different types of responses were collected for all trials. Initial entries into reward arms were classified as correct responses. Three types of errors were recorded; first entries into nonreward arms, reentries into reward arms, and reentries into nonreward arms. The Numbers of correct entries and of the three types of incorrect entries were each converted to a percentage of the total arm entries for each of the three trials of a block.

Before the ANOVAs were performed to compare the three groups on these response measures, an angular transform (arcsine of square root) was carried out to normalise the distributions of the % values (Zar, 1974). In order to assess acquisition, the first 39 trials (13 blocks) for each group were submitted to ANOVA. The GENSTAT statistical package was used to provide split plot analyses with Groups

tested as a between-subjects factor and Blocks and Groups × Blocks tested within subjects. Linear, quadratic, and cubic components of blocks effects were extracted (Snedecor and Cochran, 1967). Separate ANOVAs were also performed on Trials 37–45 and on Trials 43–48.

In addition to the response data, the running speeds for each group were analysed over all trials. Before the ANOVA was performed to compare the three groups, reciprocals of the average running times were calculated to obtain running speed values and to produce homogeneity of variance. Similar to the response data, ANOVAs were performed to compare the running speeds between Trials 1–39, Trials 37–45, and Trials 43–48.

3. Results

3.1. Acquisition

All rats learned the task steadily over the first 39 trials (Fig. 2). The two groups receiving saline (SAL–SAL and SAL–CDP) reached the criterion of 60% correct choices at this point. The rate of learning in the CDP-treated animals (CDP–CDP), although it appeared equally steady, was under half the rate of the other groups and significantly lower than them (Drug × Blocks, Dev × Lin: $F = 6.30$, $df = 2/396$, $P = .002$).

Nonspatial reference memory errors, as indexed by the percentage of incorrect choices made on the first arm, decreased steadily during acquisition for both saline groups but not for the CDP-treated group (Drug × Blocks, Blocks, Dev × Lin: $F = 6.86$, $df = 2/396$, $P < .001$; Fig. 3).

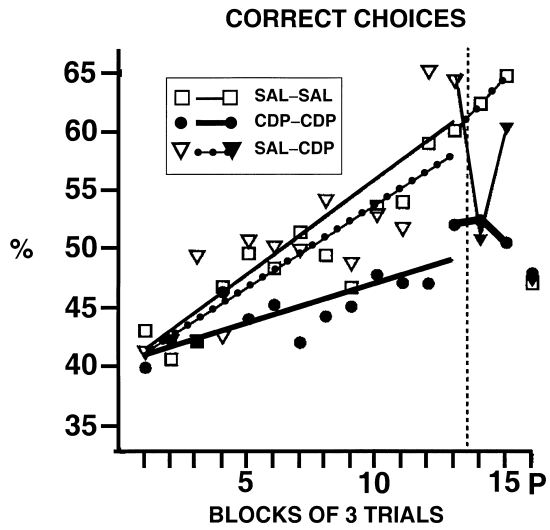

Fig. 2. Performance of rats during acquisition of the cued radial arm maze (see Fig. 1) as assessed by the number of correct choices (i.e. the first entry into a reward arm) expressed as a percentage of total arms entries during a trial. Filled symbols (●, ▼), trials in which animals received chlordiazepoxide hydrochloride (5 mg/kg, ip); open symbols (□, ▽), trials in which animals received saline. The broken vertical line marks the transition of one group of rats from saline treatment to chlordiazepoxide treatment (▽–▼). Each rat received one block of three trials per day. The letter P indicates the day in which a probe test was run in which the visual cues were removed. The nonlinear vertical axis is the result of angular transformation.

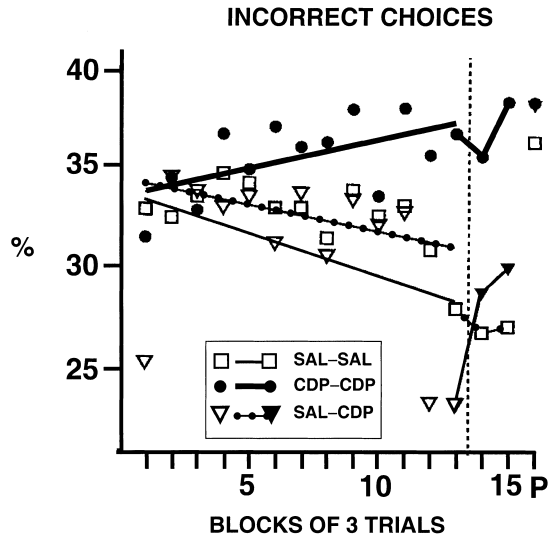

Fig. 3. All details as for Fig. 2 but showing the number of incorrect choices (i.e. the first entry into a nonreward arm) expressed as a percentage of total arm entries during a trial. This measure assesses the level of reference memory errors.

Working memory errors (which could be nonspatial and/or spatial), as indexed by the percentage of reentries into reward arms, showed a steady decline in all groups (Fig. 4). The CDP-treated group were, if anything, more efficient than the saline-treated groups, but this trend was not significant.

Reentries into nonreward arms could be the result of either working or reference memory errors or some interaction of the two. These errors decreased steadily in all groups (Fig. 5), but the rate of decrease was much lower in

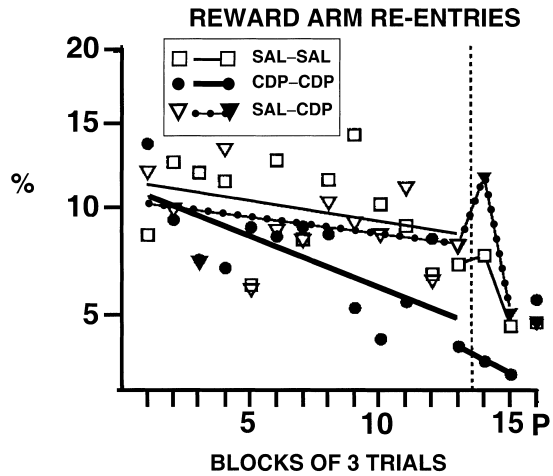

Fig. 4. All details as for Fig. 2 but showing the number of reentries into reward arms expressed as a percentage of total arm entries during a trial. Since the animals had already collected the reward from these arms, this measure assesses the level of working memory errors.

the CDP-treated group (Drug × Block, Dev × Lin: $F = 8.19$, $df = 2/396$, $P < .001$). The largest difference between the groups on this measure occurred on Block 12 and the data from this block were used to estimate the contribution of reference and working memory errors to nonreward arm reentries. The ratio of reentries to first arm entries in the CDP group was calculated for both reward arms and nonreward arms and was found to be 0.21 and 0.19, respectively. Thus, the proportion of pure working memory errors on reward arm entries is essentially the same as the proportion of errors on nonreward arms (where working memory errors are compounded by reference memory errors).

3.2. Drug state change

Over Blocks 13–15, the two groups continuing to receive their acquisition drug (SAL–SAL and CDP–CDP) continued to improve their performance at much the same rate as in previous acquisition blocks (Fig. 2). The group in which the drug state was changed (SAL–CDP) showed a decrease in efficiency on trial Block 14 (when the drug was first received) to the same level as the CDP–CDP group, but showed a marked rebound on Trial Block 15 (Drug × Blocks, Dev × Quad: $F = 3.27$, $df = 2/66$, $P = .044$).

Inspection of Figs. 3–5 suggests that this significant overall change in percent correct trials is a compound of impaired reference memory and working memory (with working memory showing greater rebound). However, none of the apparent changes over Trials 13–15 in Figs. 3–5 reached conventional levels of statistical significance, and it seems likely that all contributed to the weakly significant overall state-change effect.

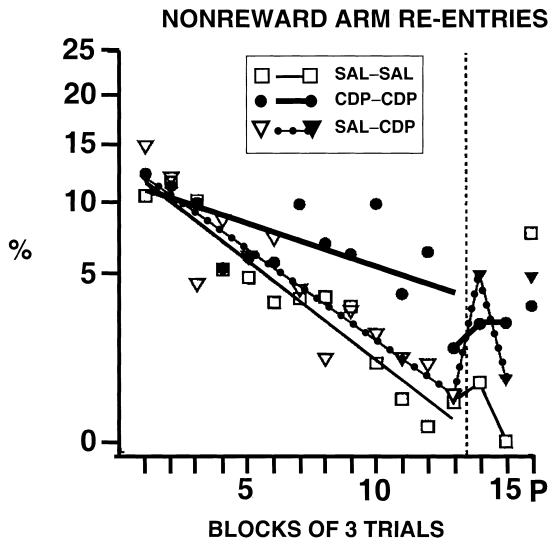

Fig. 5. All details as for Fig. 2 but showing the number of reentries into nonreward arms expressed as a percentage of total arm entries during a trial. Since the animals had already entered these arms, this measure assesses the level of working memory errors potentially confounded with reference memory errors (see text).

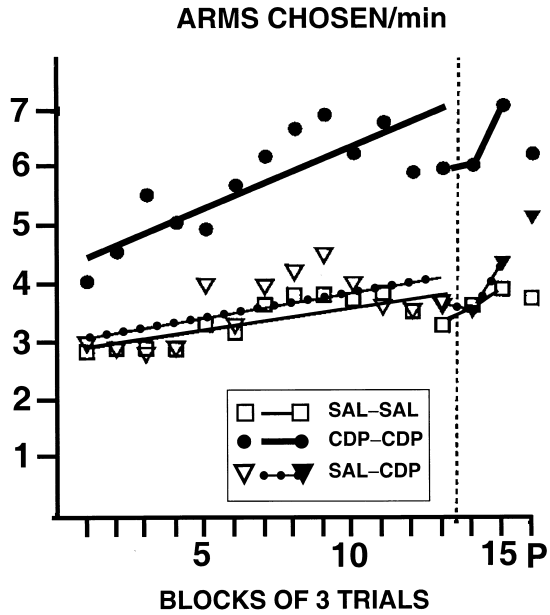

Fig. 6. Choice speed (including running speed) assessed by the total number of arms entered divided by the total time taken for each block of three trials. Other details as for Fig. 2 except that the data were not transformed.

3.3. Probe test

Between Block 15 and the probe test, all groups showed a decrease to the same level of efficiency. Given their different scores on Block 15, this involved a smaller decrease in performance for the CDP–CDP group than for the other two (Blocks × Drug: $F = 40.89$, $df = 2/330$, $P < .001$; Fig. 2). Unlike the other two groups, the CDP–CDP group showed no change in reference memory errors (Blocks × Drug: $F = 4.53$, $df = 2/33$, $P = .018$; Fig. 3), suggesting that the cues had not controlled the drugged rats behaviour. Reentry errors appeared to increase somewhat in all groups (Figs. 4 and 5) but none of the changes reached conventional levels of statistical significance.

3.4. Running speed

All groups ran steadily faster over trial blocks with the CDP–CDP group running at approximately one and a half times the speed of the other groups (Fig. 6). This difference was significant for the acquisition trials (Drug × Blocks, Dev × Lin, $F = 6.87$, $df = 2/96$, $P < .001$). The change in drug state between Blocks 13 and 15 did not produce any reliable changes (all F's < 1.0).

4. Discussion

The principal result in the present study is that chlordiazepoxide, an anxiolytic benzodiazepine, impairs nonspatial reference memory in the radial arm maze at a conventional anxiolytic dose. There was no sign of a deficit with reentries

into reward arms — where spatial cues could have been used to the fullest.

These results confirm the implication from Hodges and Green's (1986) study that anxiolytics can impair learning at low doses in a nonspatial as opposed to spatial version of the radial arm maze. However, they also show that Hodges and Green's original data are insufficient to support this conclusion. We obtained a similar loss to theirs of radial arm maze performance on postlearning administration of the drug but showed, through further testing, that the loss was due to a change in drug state not to the presence of the drug as such. Further, the pattern of errors was somewhat different in the state change as compared to the no-state-change case. Similar effects of benzodiazepines have been reported before (McNaughton, 1985).

The 5-mg/kg dose that impaired learning in the present study has previously been reported to impair learning in the water maze but not in the purely spatial version of the radial arm maze. This suggests that the key factor underlying the effects of anxiolytic drugs in all these paradigms is some factor such as complexity of the task, or inhibition of competing responses, and not the factor of space itself. Similar conclusions can be drawn for the effects of septal and hippocampal lesions in spatial tasks. The septal deficit on the radial arm maze disappears with training unless the rats are required to use flexible strategies (Janis et al., 1994). Conversely, the hippocampal deficit disappears if each arm contains a unique visual pattern (Winocur, 1982). Likewise, with one trial per day and experience of only one arm per day, hippocampal system damage improves spatial learning (McDonald and White, 1995).

The results of the probe test are particularly interesting in that the continuously drugged animals (CDP–CDP) showed very little impairment of performance on removal of the cues. This suggests that the modest amount of learning they achieved was independent of the movable visual cues. Equally, the two other groups were brought down, by cue removal, to a level of performance equal to that of the CDP–CDP group. This strongly suggests that the drug was impairing learning about the visual/tactile, i.e. nonspatial, cue of the sandpaper fairly specifically.

It appears then that the conventional radial arm maze is simply weak in its capacity to detect the effects of anxiolytic drugs. Either an increased dose of drug (Gray and Rawlins, 1986) or addition of spatially uncorrelated cues (present results) allows the choice of arms to demonstrate an impairment of performance by anxiolytic drugs. These results fit well with the fact that 5 mg/kg produces a lesser impairment of hippocampal function electrophysiologically than 20 mg/kg (McNaughton and Coop, 1991; McNaughton et al., 1986) and the fact that even a total elimination of hippocampal theta rhythm would not be expected to have as extensive effects as removal of the hippocampus (Gray and McNaughton, 2000). The results are therefore consistent with the idea that anxiolytic drugs act on the septo-hippocampal system and that impairment of hippocampal function need not produce fundamentally spatial deficits even though spatial tasks may often seem the most convenient means of demonstrating impairment of hippocampal function.

Acknowledgments

Chlordiazepoxide was kindly supplied by F. Hoffman-La Roche.

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