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Effects of Chlordiazepoxide and Sodium Valproate in Two Tests of Spatial Behaviour

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WILLNER, P. AND K.-A. BIRBECK. *Effects of chlordiazepoxide and sodium valproate in two tests of spatial behaviour.* PHARMACOL BIOCHEM BEHAV **25**(4) 747–751, 1986.—The effects of chlordiazepoxide (CDP) and sodium valproate (VPA) were studied in rats trained to asymptotic performance on two tests of spatial behaviour, the 8-arm radial maze and the 8-choice arena. The task in the 8-arm maze was to locate a single food pellet at the end of each arm. Both CDP and VPA caused an increase in errors, an increase in performance time, and the utilization of a non-spatial response strategy. The task in the 8-choice arena was to locate a single water bottle from an octagonal array of eight otherwise empty bottles. For one group the goal bottle remained in the same place from trial to trial; for a second group the position of the goal bottle was cued by a black card over the nozzle; for the third group the goal bottle was uncued and moved randomly from trial to trial. VPA had no effect on performance, but CDP impaired performance in all three groups. These patterns of effects suggest that VPA may specifically disrupt working memory, but that the impairment of spatial performance by CDP probably results from a non-specific perceptual or attentional deficit.

Chlordiazepoxide Sodium valproate 8-Arm maze 8-Choice arena Spatial behaviour
Place navigation Cue navigation Working memory Rats

DISORDERS of memory are a well documented side effect of benzodiazepine and other antianxiety drugs [2, 9, 33]. In animals, benzodiazepines do not usually disrupt the performance of well learned tasks [37], but impairments are sometimes reported in tasks which have complex information processing requirements, such as successive or conditional discriminations [3, 4, 14, 30]. If the information processing load of these tasks is in fact the feature that renders them vulnerable to disruption by benzodiazepines, then impairments might also be expected in spatial tasks, which require the animal to synthesize visual information from diverse environmental sources. The classic spatial task is the radial maze, in which the rat locates a reward at the end of each of the arms by using the information provided by distal room cues [22, 23, 25]. Impairment of radial maze performance has indeed been reported with a number of anxiolytic drugs, including ethanol [6], pentobarbital [7] and chlordiazepoxide (CDP) [13].

The results of radial maze experiments tend to be difficult to interpret because in addition to drawing on the animal's ability to process information about spatial arrays, these tasks also require an intact working memory: within a single trial, the animal must continuously monitor which arms have already been visited, and avoid them. The difficulty of distinguishing these two factors is illustrated by studies of the effects of hippocampal lesions and of anticholinergic drugs. Although many studies have demonstrated that both of these interventions severely disrupt radial maze performance, it remains unclear from the radial maze data whether an impairment of spatial information processing or of working memory is primarily responsible [10, 21, 24, 39]. It has also proved difficult to separate these two factors in the effect of CDP in the radial maze [13].

Attempts to distinguish effects on spatial information processing from effects on working memory usually involve modifications of the 8-arm maze procedure, by restricting the number of rewarded arms and/or by adding local, non-spatial (e.g., tactile) cues. An alternative strategy is to turn to different experimental paradigms, in which the contribution of one of these factors is minimized. An example of this approach is an apparatus described by Morris [18,19], which consists of a large tank of water containing an 'island' to which the rat can escape, using a spatial mapping strategy (place navigation) if the platform is sunken and invisible, but stays in the same place from trial to trial, or using a cue-response strategy (cue navigation) if the platform moves from trial to trial, but is visible. We have recently described an appetitive analogue of the Morris maze, the 8-choice arena. In this task, thirsty rats are required to locate a single bottle containing water from an octagonal array of eight otherwise empty bottles, using information provided either by the constant position of the goal bottle in the array (place navigation) or by an associated visual marker (cue navigation) [38].

Because the place navigation tasks in the Morris maze and the 8-choice arena require only a single response on each

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trial, they place minimal demands upon working memory. An additional advantage over the radial maze is that the use of the cue navigation tasks allows an assessment of the contribution of non-specific factors to any impairment of spatial performance. The Morris maze and the 8-choice arena have been used to demonstrate unambiguously that both hippocampal lesions and anticholinergic drugs specifically impair the processing of spatial information [20,38]. In the present study, the 8-choice arena was used to clarify the nature of the effect of CDP on radial 8-arm maze performance.

In addition to their anti-anxiety effects, benzodiazepines also have anticonvulsant properties [5,31]. Benzodiazepines and anticonvulants are both thought to function by potentiating transmission through GABA synapses. In the case of benzodiazepines this occurs through the close association of benzodiazepine and GABA receptors [1,5], while other anticonvulsants are typically GABA receptor agonists or inhibitors of the breakdown of GABA [29]. In general, however, drugs that act to potentiate GABAergic transmission do not share the behavioural actions of benzodiazepines in animal models of anxiety [12, 28, 34, 36]. Sodium valproate (VPA) appears to be an exception. This drug is a putative GABA agonist, increasing brain levels of GABA [27] and potentiating the GABA receptor [15]. In contrast to other anticonvulsants, VPA does consistently show benzodiazepine-like effects in animal models of anxiety [16, 17, 26, 28, 32]. It was therefore of interest to compare the effects of VPA on spatial behaviour with those of the benzodiazepine CDP.

METHOD

Subjects

Sixteen male Lister hooded rats were tested on the 8-arm maze and 24 in the 8-choice arena. The animals (NIMR, Mill Hill, London) weighed approximately 300 g. They were housed singly under conditions of controlled temperature and humidity, on a 12 hour light-dark cycle (09.00–21.00 light). Testing was carried out between 14.00 and 17.00 hr. For the duration of the experiment, animals tested on the 8-arm maze received food for three hours daily, following the behavioural tests, with water freely available in the home cage; for animals tested in the 8-choice arena, food was freely available in the home cage, but access to water was restricted to one hour a day, following the behavioural tests.

Apparatus

The 8-arm maze was constructed from natural wood, and elevated 55 cm from the floor by a central stilt. The maze consisted of a central platform (24 cm diameter) from which radiated 8 arms, each 10 cm wide and 55 cm long. The 15 cm section of the arm closest to the centre was enclosed by walls 7 cm high, and the remainder by 2 cm walls. Each arm contained a 5 cm diameter white plastic food cup.

The 8-choice arena consisted of an octagonal wooden arena (minimum diameter 133 cm) surrounded by 11 cm high walls, and raised 80 cm from the floor. Each corner was straddled by a wall 8 cm wide and 11 cm high, behind which a polythene water bottle was mounted at an angle of 25 degrees to the horizontal, with its spout protruding into the arena at a height of 8 cm. The floor and walls of the arena were painted semigloss white.

Both pieces of apparatus were located within small irregularly shaped rooms, which were evenly lit from above by two fluorescent strip lights. Both rooms contained a variety of visually distinctive cues, including furniture, wall displays, and a partial view of the experimenter, who maintained a constant position.

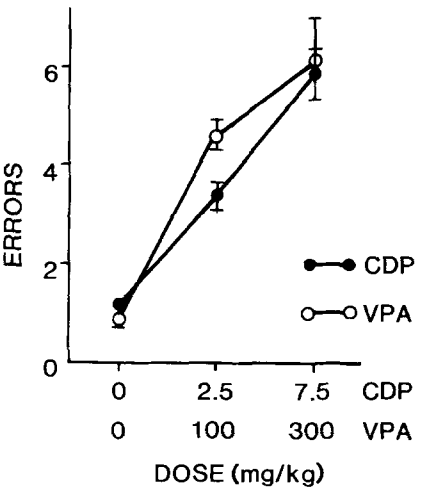

FIG. 1. Errors on the 8-arm maze (arm entries in excess of 8). Scores are means ± standard errors.

Procedure

8-Arm maze. On the first trial in the 8-arm maze, which lasted 20 min, the food cups, each containing four 45 mg food pellets, were placed just inside the arms adjacent to the central platform. The animals then received 6 five min trials (2/day) in which the food cups were gradually moved down the arms away from the centre. During acquisition proper (11 further trials at 1/day) the food cups were at the far end of the arms, each containing a single 45 mg food pellet. The animal was placed in the centre of the maze, and the trial lasted until all 8 pellets had been successfully located. All animals reached asymptotic performance by trial 8 of acquisition.

On three further trials, at two day intervals, one group of animals (n=8) received one of two doses of CDP, or a control injection; a second group (n=8) received one of two doses of VPA, or a control injection. The three treatments were administered to each animal in a random order. On these trials, in addition to recording the time to complete the trial, the time was also noted after the first 8 arm entries.

8-Choice arena. Each animal was first given four pretraining trials in the 8-choice arena, in which all 8 bottles contained water: the animal was placed in the centre of the arena and allowed to move freely until it located the nozzle of a water bottle. It was then allowed to drink for 10 sec before being returned to its home cage; latency to locate the bottle was recorded. Following pretraining, the animals were divided into three matched groups (n=8). On subsequent trials only one of the 8 bottles contained water. For animals in one group (place navigation), the location of the goal bottle was held constant from trial to trial; one of the 8 positions was assigned at random to each of the animals in this group. For a second group (cue navigation), the goal bottle was cued by an 8×11 cm black card placed over the drinking spout and against the wall of the apparatus; the position of the bottle on each trial was determined by reference to a table of random numbers. For the third group (random), the goal bottle was

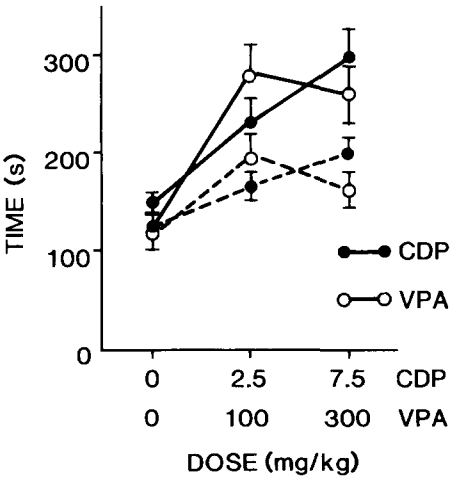

FIG. 2. Time to complete the 8-arm maze (continuous line) and to make the first 8 arm entries (broken line). Scores are means±standard error. For clarity, overlapping error bars have been omitted.

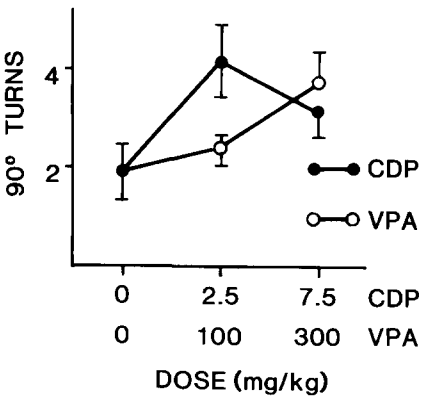

FIG. 3. The number of 90° turns made in the first 9 arm entries on the 8-arm maze. Scores are means±standard error. For clarity, overlapping error bars have been omitted.

uncued, and its position varied randomly from trial to trial. On training trials, the animal was placed in the centre of the arena facing a randomly chosen bottle. It was removed 10 sec after it located the goal bottle and began to drink. A total of 14 training trials were administered over an 8 day period. During the 30–60 minute inter-trial interval, animals were returned to their home cage. Asymptotic performance was reached by trial 10.

After training, the animals were first tested in an unrelated experiment which examined the effects of scopolamine, methylscopolamine and vehicle injections. Performance of the animals during acquisition of the three tasks, and the effects of scopolamine, are described elsewhere [38]. Following an interval of 8 weeks, the animals were reintroduced to the arena for 10 reaquisition trials. Three further trials, at two day intervals, were preceded by an injection of CDP, VPA or saline vehicle. All animals received all three treatments in a counterbalanced order. On these trials, the animal was always placed in the arena facing at 90 degrees to the goal bottle; otherwise, procedures were identical to those described above.

Drugs

Chlordiazepoxide (Roche, Welwyn Garden City) was administered at 2.5 and 7.5 mg/kg for the 8-arm maze and at the intermediate dose of 5 mg/kg for the 8-choice arena. Sodium valproate (Sanofi, Manchester) was administered at 100 and 300 mg/kg for the 8-arm maze and at the intermediate dose of 200 mg/kg for the 8-choice arena. Both drugs were dissolved in physiological saline and injected IP 30 min prior to testing, in a volume of 1 ml/kg.

Analysis

Results were analyzed by analysis of variance, supplemented where appropriate by tests of simple main effects and planned comparisons.

RESULTS

8-Arm Maze

Both drugs caused a dose dependent increase in the number of errors made on the 8-arm maze ($F(2,28)=19.2$ (CDP), 24.8 (VPA), $p<0.001$) (Fig. 1). The time taken to complete the maze was also increased by both drugs ($F(2,28)=13.1$ (CDP), 16.2 (VPA), $p<0.001$) (Fig. 2). A similar increase was apparent in the time taken to make the first 8 arm entries ($F(2,28)=5.8$ (CDP), 7.3 (VPA), $p<0.01$) (Fig. 2). This suggests that the increase in maze completion time in drugged animals results from a slower running speed, rather than being simply a reflection of the greater number of arms visited. In fact, it is likely that measuring the time to make 8 arm entries actually underestimates the slowness of drugged animals: because they make more errors, drugged animals spend proportionally less time consuming food pellets, and more time running.

Inspection of the routes taken around the maze in control conditions failed to reveal any consistent pattern of turning. However, 90 degree turns were very apparent in the records of drugged animals. In order to obtain comparable samples of data for the different conditions, a count was made of the number of 90 degree turns in the first 8 turns (i.e., arm entries 2–9; with only one exception, all animals made at least 9 arm entries on all three trials). A drug-induced increase in the number of 90 degree turns was confirmed, which was significant at the lower dose of CDP, $F(1,28)=6.3$, $p<0.025$, and at the higher dose of VPA, $F(1,28)=4.4$, $p<0.05$ (Fig. 3).

8-Choice Arena

In agreement with our previous observations on this apparatus [38], under control conditions there were no significant differences in performance time between the place and cue navigation groups, $F(1,63)=0.1$, $p>0.1$, but both of these groups performed better than the random group, $F(1,63)=11.3$, 9.4 respectively, $p<0.01$. In contrast to the similarity in the effects of CDP and VPA on the 8-arm maze, in the 8-choice arena CDP impaired performance in all three groups, $F(1,42)=15.3$, $p<0.001$, but VPA had no significant effect in any group, $F(1,42)=0.45$, $p>0.1$ (Fig. 4).

DISCUSSION

From the pattern of effects in the two tests, it is possible to deduce the nature of the behavioural changes caused by the two drugs. An impairment of performance on the 8-arm

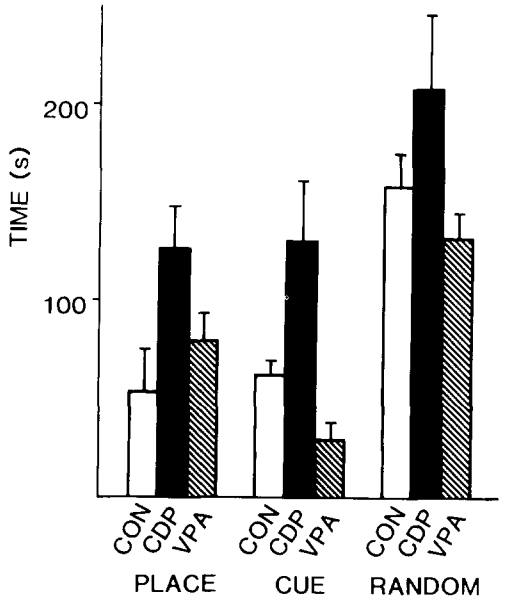

FIG. 4. Effects of CDP (black bars), VPA (hatched bars) and vehicle injections (white bars) on time to locate the goal bottle in the 8-choice arena. Scores are means±standard error. CDP impaired performance in all three groups ($F(1,42)=6.7$, $p<0.01$ (place); 5.9, $p<0.025$ (cue); 3.1, $0.5<p<0.1$ (random)). VPA had no significant effect in any group ($F(1,42)=0.8$, 1.4, 0.8 respectively, $p>0.1$).

maze could result from an inability to process spatial information, from an impairment of working memory, or from a variety of non-specific causes. However, in addition to disrupting 8-arm maze performance, CDP also impaired performance on all three of the 8-choice arena tasks. As these tasks involve only a single response on each trial, they place minimal demands upon working memory. Furthermore, the cue navigation task cannot be solved by using a spatial mapping strategy. Consequently, neither the spatial explanation nor the working memory explanation can parsimoniously account for both sets of data. It is therefore likely that the effects of CDP result from non-specific causes. In passing, it should be noted that this conclusion disagrees with the hypothesis [11] that the behavioural effects of benzodiazepines are functionally equivalent to a (partial) hippocampal lesion. CDP impaired place navigation and cue navigation equally in the 8-choice arena, but hippocampal lesions have been found in the Morris water maze to disrupt place navigation specifically without affecting cue navigation [20].

If CDP disrupts performance non-specifically, it is possible to speculate further on the type of impairment involved: some candidates are disorders of perception, attention, reference memory, motivation or motor systems. Of these, a motivational or motor deficit is unlikely. These factors could explain the slower speed of running after CDP, but could not easily account for the increase in errors or the use of a non-spatial response strategy. Furthermore, the lowest dose of CDP used (2.5 mg/kg) is at the bottom end of the dosage range in the behavioural literature, and impairments in appetitive tasks are commonly encountered only at high doses. This fact would also argue against a memory disorder. By exclusion, a perceptual or attentional explanation of the behavioural deficit seems the most likely. A sensory disorientation is a plausible hypothesis, given that CDP exhibits pronounced state-dependent effects [8].

The same reasoning applied to the effects of VPA leads to very different conclusions. The lack of effect of VPA in the 8-choice arena, and in particular, the absence of any impairment of place navigation, rules out an account of the disruption of 8-arm maze performance in terms either of non-specific impairments or of an inability to process spatial information. By exclusion, an impairment of working memory remains the only plausible explanation. This conclusion is consistent with the results of a study in human volunteers, in which VPA was found primarily to impair mental processing speed, particularly in an intellectually demanding task [35].

The most striking outcome of this study is the close similarity of CDP and VPA in their effects on the 8-arm maze, compared with their extreme dissimilarity in the 8-choice arena. Although only a single dose of each drug was tested in the 8-choice arena, the doses were chosen to be midway between the two doses used in the 8-arm maze, and from the 8-arm maze data appear to be roughly equipotent. The fact that similar patterns of results can be obtained on the 8-arm maze for drugs which clearly operate through different behavioural mechanisms suggests that great caution should be exercised in interpreting the effects of drugs or other manipulations on radial maze performance.

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