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Characterisation of learning and memory deficits following NMDA receptor antagonism

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Summary. The effects of the non-competitive NMDA antagonist dizocilpine in tests of cognitive function have been compared with its effects on motor function in rats. Severe motor impairments were observed at doses above 0.1 mg/kg. Dizocilpine (0.075 mg/kg) had no effect on the acquisition of a spatial discrimination task in a Y-maze, but disrupted reversal learning. Both the acquisition and reversal of a visual discrimination task were impaired following dizocilpine (0.075 mg/kg). Dizocilpine (0.04 mg/kg) also disrupted performance of a five-choice visual reaction time task. It is clear that dizocilpine can impair cognitive function at doses which do not induce pronounced motor dysfunction. The impairment induced by dizocilpine includes a disruption of spatial discrimination learning and a deficit in tasks with sustained attentional demands.

Keywords: Amino acids – Dizocilpine – N-Methyl-D-aspartate – Learning and memory

Introduction

Excitatory amino acids such as L-glutamic acid have been implicated in learning and memory processes for a number of different reasons. Glutamate is the major excitatory neurotransmitter in brain regions such as the hippocampus and cortex, which are known to be involved in learning and memory processing. Moreover the glutamatergic pyramidal neurones in these regions degenerate in dementing illnesses such as Alzheimers disease, and abnormalities in these neurones correlates with cognitive impairment. Activation of NMDA receptors is an important factor in the establishment of long-term potentiation. It is widely accepted that long-term potentiation can be considered as a model of memory storage (Bliss and Collingridge, 1993), and has indeed been interpreted as a mechanism of memory formation.

Studies on the effects of NMDA antagonists on cognitive function in rodents are of paramount importance to the arguments linking excitatory amino acids

with memory processing and long-term potentiation. These studies have encompassed a wide range of NMDA antagonists and an array of learning and memory tasks (reviewed by Izquierdo, 1991; Staubli and Lynch, 1991; McEntee and Crook, 1993), resulting in a consensus that NMDA antagonists interfere with learning and memory function. Despite this broad agreement, a number of difficulties exist when studying the effects of NMDA receptor antagonists. It is clear that these compounds have profound effects on motor function (Tricklebank et al., 1989) and as many tests of cognition in rodents involve a high degree of complex motor skills, this is an obvious complication. In addition, NMDA antagonists may have anxiolytic (Clineschmidt et al., 1982) and analgesic properties.

In the present study we have used the non-competitive NMDA antagonist dizocilpine (MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine) to characterise the involvement of NMDA receptors in learning and memory function. In an attempt to reduce the influence of the psychomotor effects of dizocilpine we have used reversal learning in a Y maze, as in this situation choice accuracy rather than latency is assessed, and reversal can be compared to acquisition in the presence of the drug. To examine the effects of dizocilpine on selective attention we have used a visual reaction time task (Carli et al., 1983).

Methods

Male Lister hooded rats (Harlan Olac, Bicester, U.K.) were used in all experiments. Doses of dizocilpine (Semat Technical (UK) Ltd, St. Albans, U.K.) are expressed as weight of base administered subcutaneously (s.c.) in a volume of 1ml/kg, dissolved in saline. All control treated rats received saline.

Effect of dizocilpine on locomotion

A dose-ranging study was carried out using six rats per dose. Rats were injected s.c. 30 min before being placed in activity boxes with infra red detectors and their activity recorded for the following 10 min.

Ex vivo [^{3}H]-dizocilpine binding following administration of dizocilpine

A dose-ranging study was carried out using six rats per dose. The following doses of dizocilpine were tested, 10, 5, 1, 0.1, 0.01 mg/kg, and saline. Rats were injected s.c. and killed 30 minutes later, frontal cortex and hippocampus were dissected out and [^{3}H]-dizocilpine binding carried out on brain homogenates (5mM Tris HCl pH 7.4).

Spatial and visual discrimination

Two groups of ten rats (250–300 g) were housed in groups of five per cage. These were maintained at 90% of their free-feeding body weight and fed at the end of the test session. They were individually tested in a Y-maze which was made of grey perspex and had three arms of equal size, 60 cm long, 11.5 cm wide and 25 cm high. The start arm had a guillotine door from behind which the rats were held until the start of each trial which commenced when the guillotine door was raised. Correct responses were reinforced by four food pellets (45 mg sucrose reward pellets, Noyes, USA) placed in a food cup at the end of the correct arm. Rats were pretrained for four days so they would become familiar with the maze,

following this, rats were randomly assigned to one of two conditions, saline or dizocilpine (0.075 mg/kg) which was administered, s.c. twenty minutes prior to each test session. Rats were trained for fifteen trials per day, to a criterion of five consecutive correct responses. For saline or dizocilpine treated rats, half were allocated left arm positive and the remainder were allocated right arm positive, the arms remained so until completion of the acquisition phase. On completion of acquisition, reward contingencies were reversed, thus for each rat, the arm which had been positive on acquisition became negative and the reverse applied for the negative arm. The number of trials to criterion, mean number of errors per block of five trials, mean % correct and mean latency to choose an arm were all recorded.

To determine the effect of dizocilpine on acquisition and reversal of a visual discrimination task, black and white floor inserts were used as visual cues and were randomly moved between right and left arms according to a Gellerman schedule, so that no arm was positive for more than two consecutive trials. The experiment was performed using the same schedule as the spatial discrimination task.

Five choice visual reaction time

Operant chambers were used, the rear wall of which contained five illuminated holes which were used as visual stimuli. Rats were trained to discriminate the spatial location of a brief visual stimulus provided by illumination of the lamps at the back of five holes in the chamber, in a random order. The start of each session was signalled by turning on the houselight and the delivery of a free food pellet. The first trial started when the rat pushed the tray flap to retrieve the food pellet. After a fixed interval (inter-trial interval, ITI), the light at the rear of one of the holes was illuminated for a short period (defined as the stimulus duration). Nose-poke responses into the illuminated hole during the stimulus presentation and for a short period after the end of the stimulus presentation (the limited hold) were rewarded with the delivery of a food pellet, and a correct response recorded. Rats were trained to a one second stimulus and five second limited hold. The test schedule required the rats to respond to stimuli of four different durations, 0.25, 0.50, 0.75 and 1.00 seconds. 40 presentations of each stimulus duration were scheduled, presented in a random order, with an equal number of stimuli of a given duration being presented at each of the five holes in each chamber. Performance on this schedule was allowed to reach asymptotic levels and was continued for a further 10 days before drug testing began. Following testing with variable stimulus durations, a further investigation examined the role of variation in the interval between initiation of a trial and stimulus presentation.

Results

Ex vivo inhibition of [^{3}H]-dizocilpine binding

Following subcutaneous administration dizocilpine inhibited [^{3}H]-dizocilpine binding in both frontal cortex and hippocampus (Fig. 1). In both brain regions the ED_{50} was 0.1 mg/kg.

Motor activity and muscle relaxation

Dizocilpine administration produced a distinct behavioural syndrome which included hyperactivity at doses up to 0.1 mg/kg, with slight ataxia (Fig. 2). Higher doses produced a decrease in locomotor activity and severe ataxia was observed. Dizocilpine also produced muscle relaxation, with a maximal effect at 0.25 mg/kg (Fig. 2).

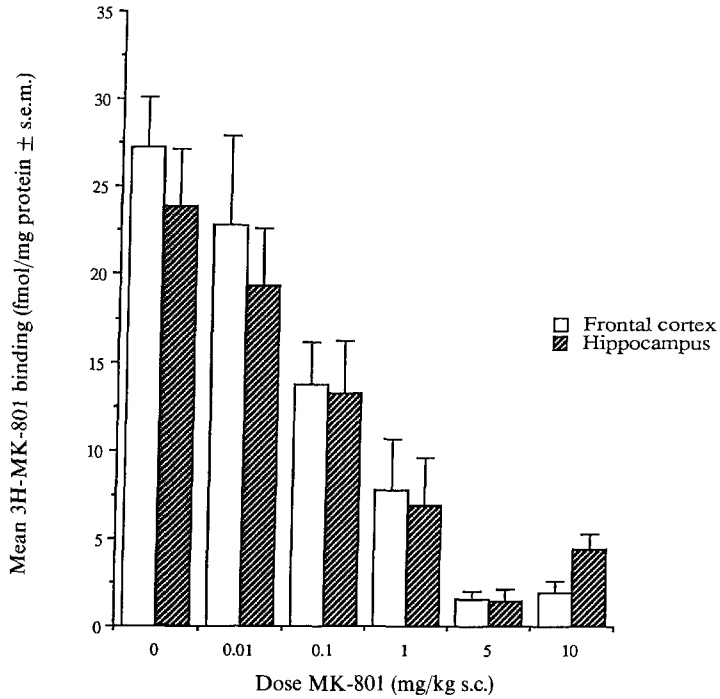

Fig. 1. Ex vivo [^{3}H]-dizocilpine binding in frontal cortex and hippocampus 30 min after subcutaneous administration of various doses of dizocilpine. Values are mean ± s.e.mean, n = 6

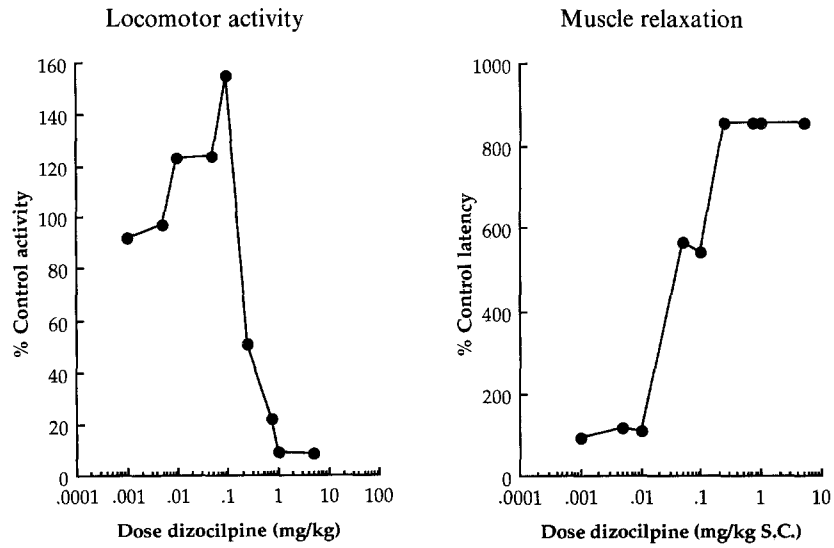

Fig. 2. The effect of increasing does of dizocilpine on locomotor activity and muscle relaxation in rats 30 min post-treatment

Spatial discrimination

Treatment with dizocilpine had no effect on acquisition of the spatial task (Fig. 3a), however, when reversed (Fig. 3b), dizocilpine treated rats took significantly

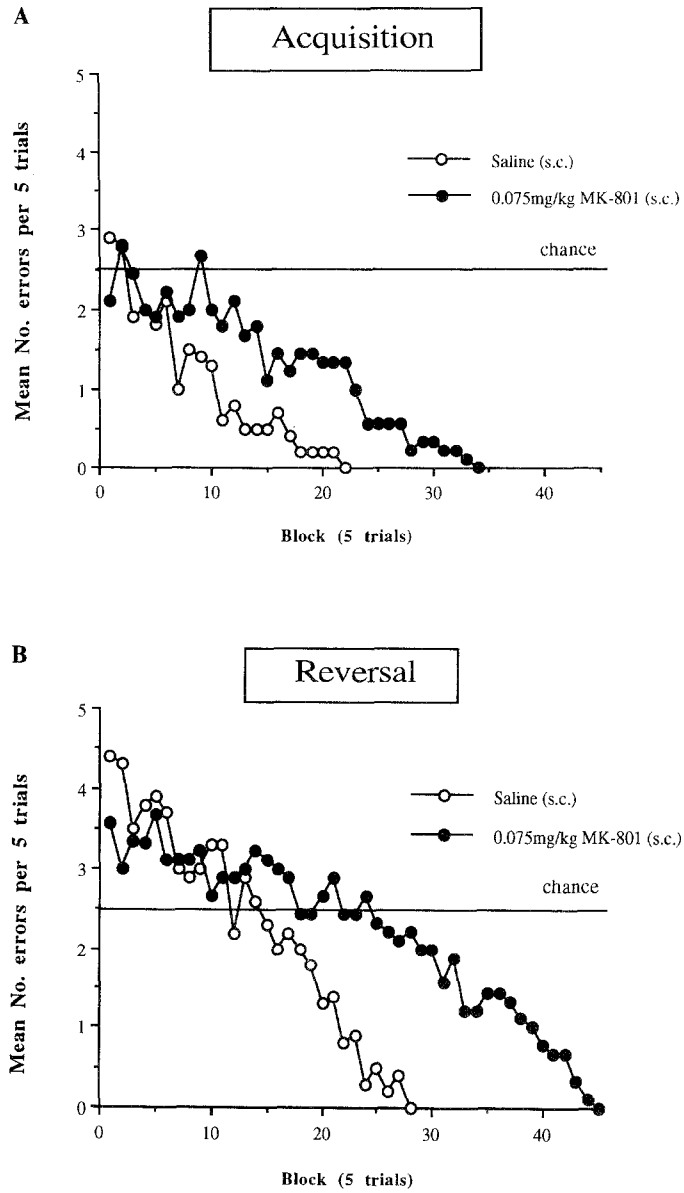

Fig. 3. Effect of 0.075 mg/kg dizocilpine on acquisition (**A**) and reversal (**B**) of a spatial discrimination task in the Y maze

more trials to reach criterion (45.9 ± 7), than saline treated rats (17.9 ± 1.4) ($p < 0.01$).

Visual discrimination

On the visual task dizocilpine produced a significant effect on both acquisition (Fig. 4a) and reversal (Fig. 4b). The effect of dizocilpine was more pronounced on reversal (trials to criterion: saline 107.2 ± 8.7), dizocilpine 172.6 ± 17) ($p < 0.01$), than on acquisition (trials to criterion: saline 37.1 ± 7.8, dizocilpine 84 ± 20.6) ($p < 0.05$).

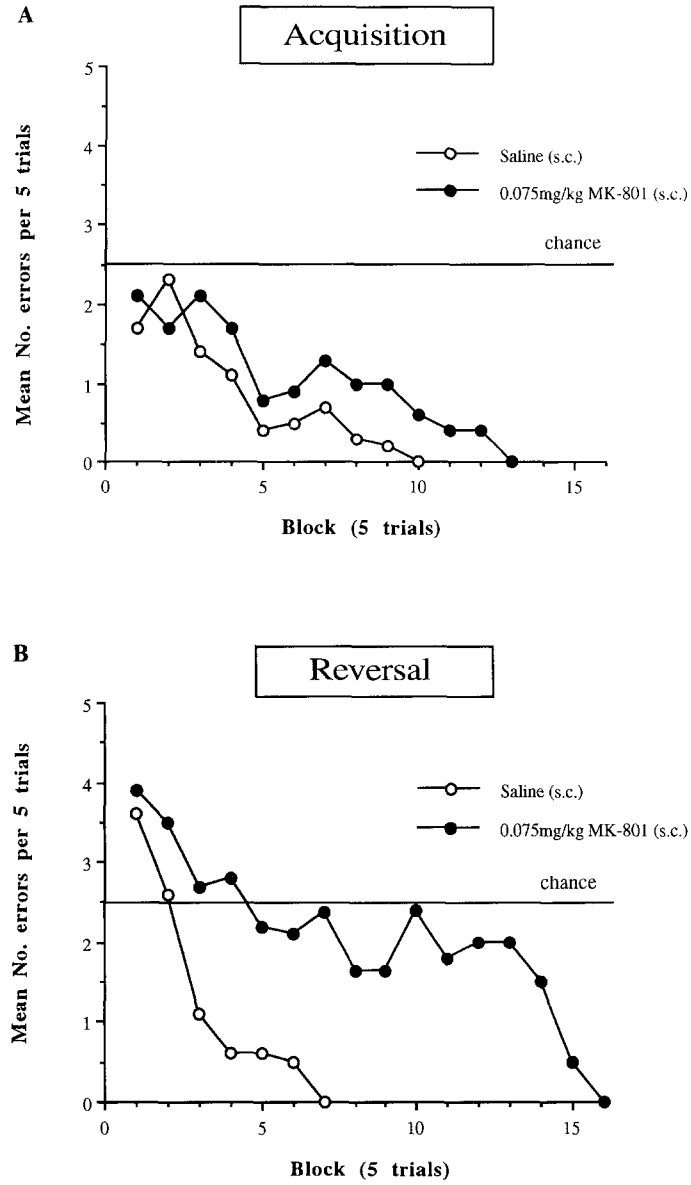

Fig. 4. Effect of 0.075 mg/kg dizocilpine on acquisition (**A**) and reversal (**B**) of a visual discrimination task in the Y maze

Five choice visual reaction time

Dizocilpine did not reduce the number of trials initiated at doses below 0.1 mg/kg (Fig. 5a). Responding during the inter-trial interval (ITI responding) was significantly increased by administration of 0.04 mg/kg and significantly decreased by 0.1 mg/kg dizocilpine (Fig. 5b). Thus, again, this measure suggests that administration of 0.10 mg/kg dizocilpine reduced normal levels of responding. Conversely, administration of 0.04 mg/kg dizocilpine appeared to stimulate overall response rates.

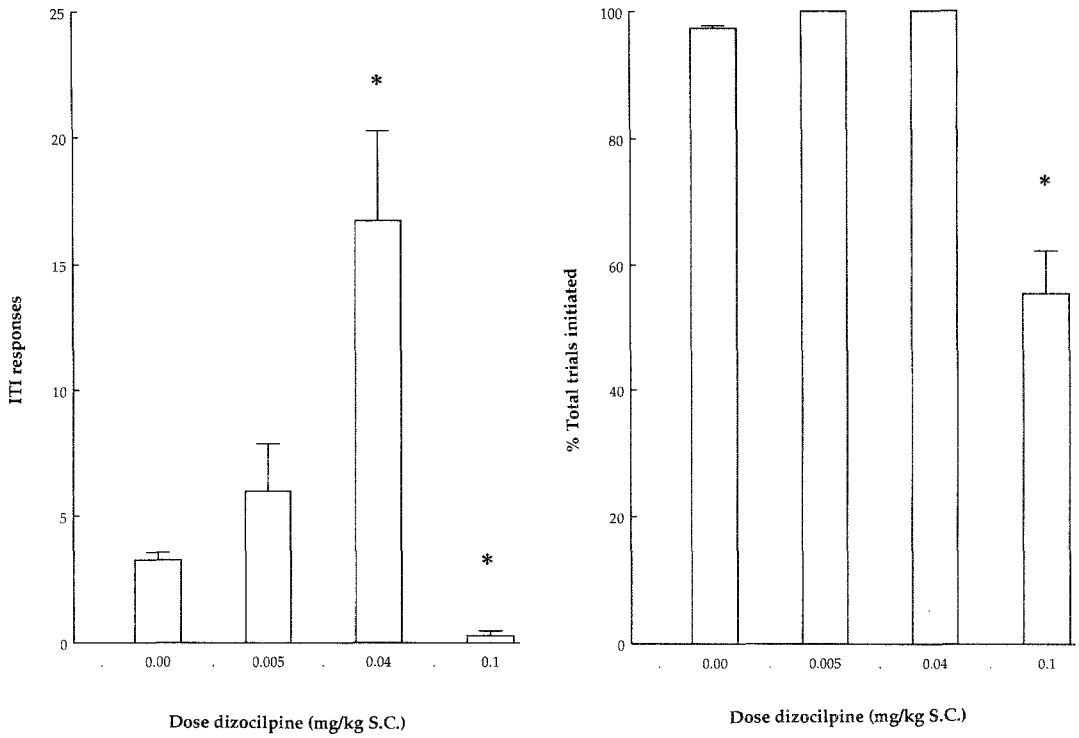

Fig. 5. Effect of increasing doses of dizocilpine on number of trials initiated [right] and inter-trial interval (*ITI*) responses [left] in a five choice reaction time task

Administration of both 0.04 and 0.10 mg/kg disrupted performance of the task, an effect that resulted from both decreased accuracy of responding and an increased failure to detect and respond to the stimuli used (Fig. 6).

Discussion

It is clear from the present study that dizocilpine can produce abnormal locomotor activity at doses which have been used previously to study cognitive impairments. The dose of dizocilpine chosen for studies with the Y maze was below that which produced marked impairments of locomotor activity. At this dose (0.075 mg/kg s.c.) of dizocilpine a significant inhibition of [^{3}H]-dizocilpine binding could be demonstrated in both hippocampus and frontal cortex, thus the effects of this dose of dizocilpine are consistent with blockade of NMDA receptors.

The studies with the Y-maze clearly demonstrated that dizocilpine produced a learning impairment. The lack of impairment on spatial acquisition argues against a global performance deficit, if there was such a performance deficit then one would expect to see an impairment on both acquisition and reversal. When the learning requirement is more demanding i.e. on reversal, a severe learning impairment becomes apparent. This is not a task difficulty effect as reversal is not more difficult than acquisition for controls (trials to criterion: acquisition 15.1 $\pm$ 2.4, reversal 17.9 $\pm$ 1.4). Dizocilpine produces behaviour similar to that induced by amphetamine (Clineschmidt et al., 1982) such as hyperactivity and

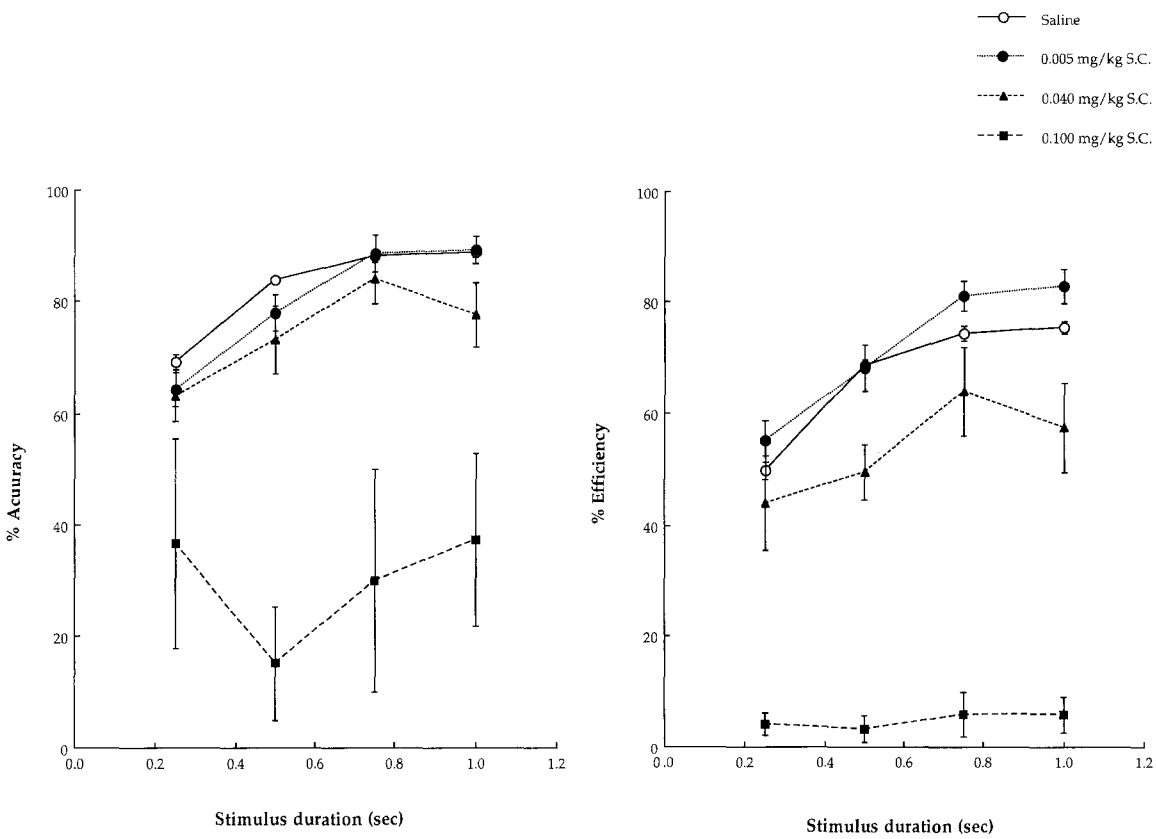

Fig. 6. Effect of dizocilpine on the efficiency (right) and accuracy (left) of responses in a five choice reaction time task

stereotypy as seen in the initial experiment, and by others (Tricklebank et al., 1989). However, the impairment on reversal is not due to rats perserverating at greater than chance. Although this might be expected with amphetamine it should be noted that in some instances amphetamines can facilitate reversal learning in rats. It is clear that dizocilpine treated rats reached chance on the first day of reversal testing and remained at chance for several days, before eventually reaching criterion. Thus they ceased to perform the initially learned response at the same rate as the controls but were unable to acquire the new response as quickly as the controls. In the visual discrimination task, drug treated animals were impaired in both acquisition and reversal. This task may have made extra demands on learning reflected by the fact that for controls in the visual task trials to criterion (37.1 $\pm$ 7.8) were significantly greater than in the spatial task (15.1 $\pm$ 2.4).

Other studies have found impairments in spatial but not visual learning, when studying NMDA antagonists (reviewed by Staubli and Lynch, 1991). However, in our experiments there was an effect on both the spatial and the visual task, although more profound in the latter. These results may be specific to the difficulties of the type of task as the other studies have generally used either the Morris water maze or the radial arm maze.

It is clear that performance of the five choice visual serial reaction time task was disrupted by dizocilpine administration. At high doses (0.1 mg/kg) this disruption reflects a non-specific failure to respond at all, probably reflecting the development of ataxia, pronounced muscle relaxation and hyperactivity. Administration of 0.04 mg/kg dizocilpine had no effect on the number of trials initiated, suggesting that treatment with this dose does not produce non-specific disruption of responding. Indeed, some disinhibition of inter-trial interval responding was seen at this dose. However, whilst no non-specific effects were seen following this treatment, 0.04 mg/kg produced a clear disruption in the overall efficiency of performance in the task. This effect reflected an increase in both errors of commission and errors of omission, suggesting a marked and general deficit in detecting and responding to brief visual stimuli in a task that requires sustained visual attention.

Given that systemic administration of dizocilpine does not appear to disrupt the acquisition of a simple visual discrimination in the Y-maze it would appear that the present findings on the reaction time task reflect a dizocilpine induced deficit in responding to the spatial location of stimuli in a task with sustained attentional demands. Whilst it is possible that such a deficit would explain the effect of dizocilpine on visual discrimination, the effect on spatial discrimination is consistent with a learning deficit.

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