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Chlordiazepoxide-Induced Ataxia, Muscle Relaxation and Sedation in the Rat: Effects of Muscimol, Picrotoxin and Naloxone

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FILE, S. *Chlordiazepoxide-induced ataxia, muscle relaxation and sedation in the rat: Effects of muscimol, picrotoxin and naloxone.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1165–1170, 1982.—Chlordiazepoxide-induced ataxia was potentiated by muscimol (0.5 mg/kg) and antagonised by picrotoxin (2 and 4 mg/kg) and by naloxone (2 and 4 mg/kg). The muscle-relaxant effects of chlordiazepoxide were not significantly altered by any of these three drugs and nor was the chlordiazepoxide-induced decrease in exploratory head-dipping. However, the sedative effects of chlordiazepoxide, measured by a reduction in spontaneous motor activity, were antagonised by picrotoxin.

Chlordiazepoxide Ataxia Muscle relaxation Locomotor activity Exploration

THERE is now evidence that the benzodiazepine binding site in the central nervous system is part of a receptor complex with a GABA receptor site and a chloride ionophore [10,16]. GABA agonists enhance the binding of benzodiazepines [25] and there is electrophysiological evidence that benzodiazepines enhance GABA-mediated inhibition [11]. However, although it has been suggested [13] that all of the pharmacological effects of the benzodiazepines can be explained by this action, the evidence in relation to the behavioural actions is still remarkably sparse.

It is likely that an enhancement of GABAergic function is the main mechanism underlying the anti-convulsant action of benzodiazepines [12] and Schmidt *et al.* [20] suggested that an enhancement of presynaptic inhibition may contribute to the muscle relaxant action of diazepam, but there is little supporting evidence to date. However, Soubrie and Simon [22] found that the GABA antagonist picrotoxin reversed the reduction in grip strength resulting from diazepam (2 mg/kg), and the motor inco-ordination produced by diazepam (4 mg/kg), as measured by the number of falls from a horizontal bar.

It is not clear whether there is GABA mediation of the sedative effects of benzodiazepines. Ongini *et al.* [17] found no correlation between the GABA-mediated changes in cerebellar cGMP and benzodiazepine-induced sedation in mice and picrotoxin did not reverse the diazepam-induced hypoactivity of rats in an open field [22], although this measure may reflect exploration as well as the general level of motor activity and hence it may not be a good measure of pure sedative effects. The opiate antagonist naloxone has been reported to antagonise the anti-conflict actions of benzodiazepines in some, but not all, test situations [1, 4, 8, 23], diazepam-induced feeding [2, 4, 24] and the excitatory effects of chlordiazepoxide on hypothalamic self-stimulation [14]. The effects of naloxone have not been tested on benzodiazepine-induced sedation, measured by a decrease in spontaneous motor activity, but both naloxone and picrotoxin were able to antagonise the loss of righting reflexes induced by chlordiazepoxide [1].

It was the purpose of the present experiments to determine whether benzodiazepine-induced ataxia, muscle relaxation and sedation could be enhanced by the GABA agonist muscimol or antagonised by the GABA antagonist picrotoxin or the opiate antagonist naloxone. Picrotoxin was chosen rather than bicuculline because the latter is so rapidly metabolised it is unsuitable for IP administration and behavioral experiments. Parenterally administered picrotoxin has been found to antagonise the effects of GABA electrophoretically applied to presynaptic terminals of the cuneate nucleus [9]. Although muscimol and picrotoxin have GABA agonist and antagonist actions, respectively, it should be pointed out that they do act at different receptor sites [21].

Ataxia was measured by the latency to fall off an inclined plane and muscle relaxation was measured by the impaired ability of rats to heave their hind-legs onto a horizontal wire from which they were suspended by their forepaws [3]. Sedation was measured in a holeboard, which permits independent measurement of directed exploration, measured by head-dipping, and of locomotor activity [8].

METHOD

Animals

Male hooded Lister rats (Olac Ltd., Bicester), approximately 350 g, were housed in groups of 6 with food and

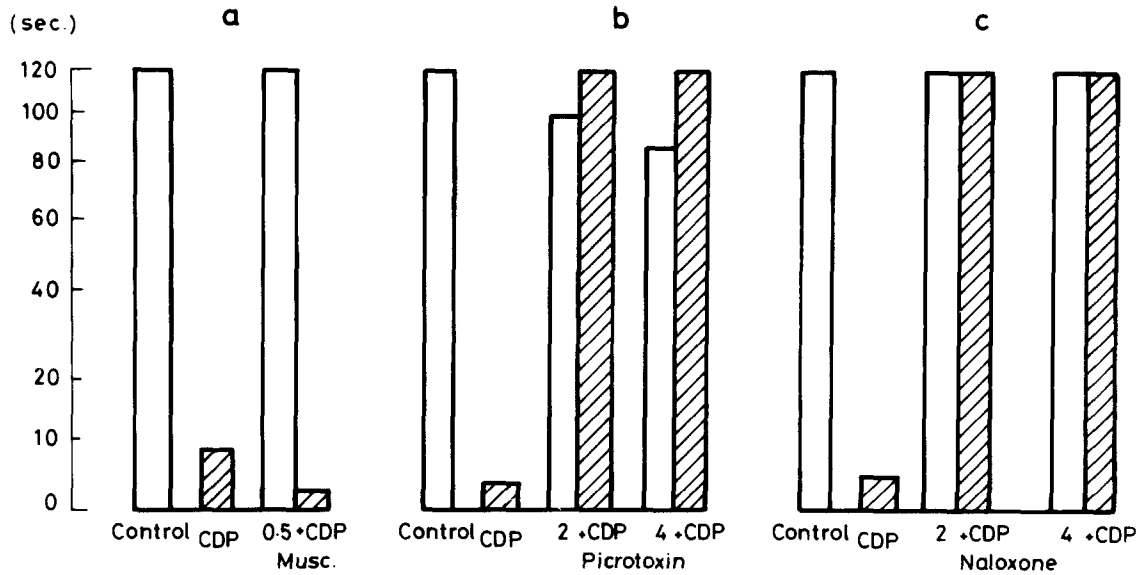

FIG. 2. Median Latency to slide off inclined plane. (a) Rats injected with water or muscimol (0.5 mg/kg), either alone (clear columns) or in combination with chlordiazepoxide (10 mg/kg, hatched columns). (b) Rats injected with water or picrotoxin (2 and 4 mg/kg), either alone (clear columns) or in combination with chlordiazepoxide (10 mg/kg, hatched columns). (c) Rats injected with water or naloxone (2 and 4 mg/kg) alone (clear columns) or in combination with chlordiazepoxide (10 mg/kg, hatched columns).

water freely available, in a light:dark cycle of 11:13 hr with lights on at 0600 hr.

Apparatus

Holeboard. This was a wooden box 60×60×36 cm tall, in the floor of which were four equally spaced holes, 3.8 cm in diameter. Head-dipping was measured by infra-red cells placed under the holes and locomotor activity and rearing were measured by infra-red cells in the walls of the box 4.5 cm and 11 cm from the floor, respectively.

Inclined Plane. This was a wooden surface, 60 × 60 cm, placed at an angle of 40° to the horizontal.

Horizontal wire. A 40 cm long wire, 2 mm in diameter, was suspended 46 cm from the ground.

Drugs

Chlordiazepoxide hydrochloride (Roche Products Ltd.) was dissolved in distilled water to a concentration of 5 mg/ml and injected intraperitoneally 30 min before the start of behavioural testing.

Muscimol (Fluka AG) was dissolved in distilled water to a concentration of 0.25 mg/ml and injected IP 30 min before testing began.

Picrotoxin (Sigma) was dissolved in distilled water to concentrations of 1 and 2 mg/ml and injected IP 30 min before test.

Naloxone (Endo Laboratories) was dissolved in saline to concentrations of 1 and 2 mg/ml and injected intraperitoneally 10 min before the start of testing.

Procedure

The experiment was conducted in three phases. In the first phase, 31 rats were randomly allocated among the following 4 drug groups: vehicle controls; muscimol (0.5 mg/kg); chlordiazepoxide (10 mg/kg); chlordiazepoxide (10 mg/kg) plus muscimol (0.5 mg/kg). In the second, 51 rats were randomly allocated among the following 6 drug groups: vehicle controls; picrotoxin (2 and 4 mg/kg); chlordiazepoxide (10 mg/kg); chlordiazepoxide (10 mg/kg) plus picrotoxin (2 and 4 mg/kg). In the third phase, 64 rats were randomly allocated among the following 6 groups: vehicle controls; naloxone (2 and 4 mg/kg); chlordiazepoxide (10 mg/kg); chlordiazepoxide (10 mg/kg) plus naloxone (2 and 4 mg/kg).

Testing took place between 0730 and 1200 hr. In each phase rats were subjected to the three test situations in the following order:

Holeboard test. Rats were placed singly in the centre of the holeboard and given a 10 min trial during which the number of head-dips made, the time spent head-dipping, locomotor activity and rears were scored. At the end of each trial any fecal boluses were removed and the floor of the box was wiped clean. Immediately after the holeboard test the rats were placed in the next test.

Inclined plane. Rats were placed singly on this plane and the latency to fall off was recorded. The trial was terminated after 120 sec. Immediately after this test the rats were placed on the horizontal wire.

Horizontal wire. Rats were suspended from this wire by their forepaws and their ability to heave one or both hindpaws onto the wire was recorded. This test was terminated after 120 sec.

Statistics

The scores from the holeboard were analysed by two-way analyses of variance with chlordiazepoxide as one factor and drug (muscimol, picrotoxin or naloxone) as the second. Antagonism of a chlordiazepoxide effect would be shown by a chlordiazepoxide × drug interaction.

The latency scores from the inclined plane were analysed by Mann-Whitney U-tests, since they were not normally dis-

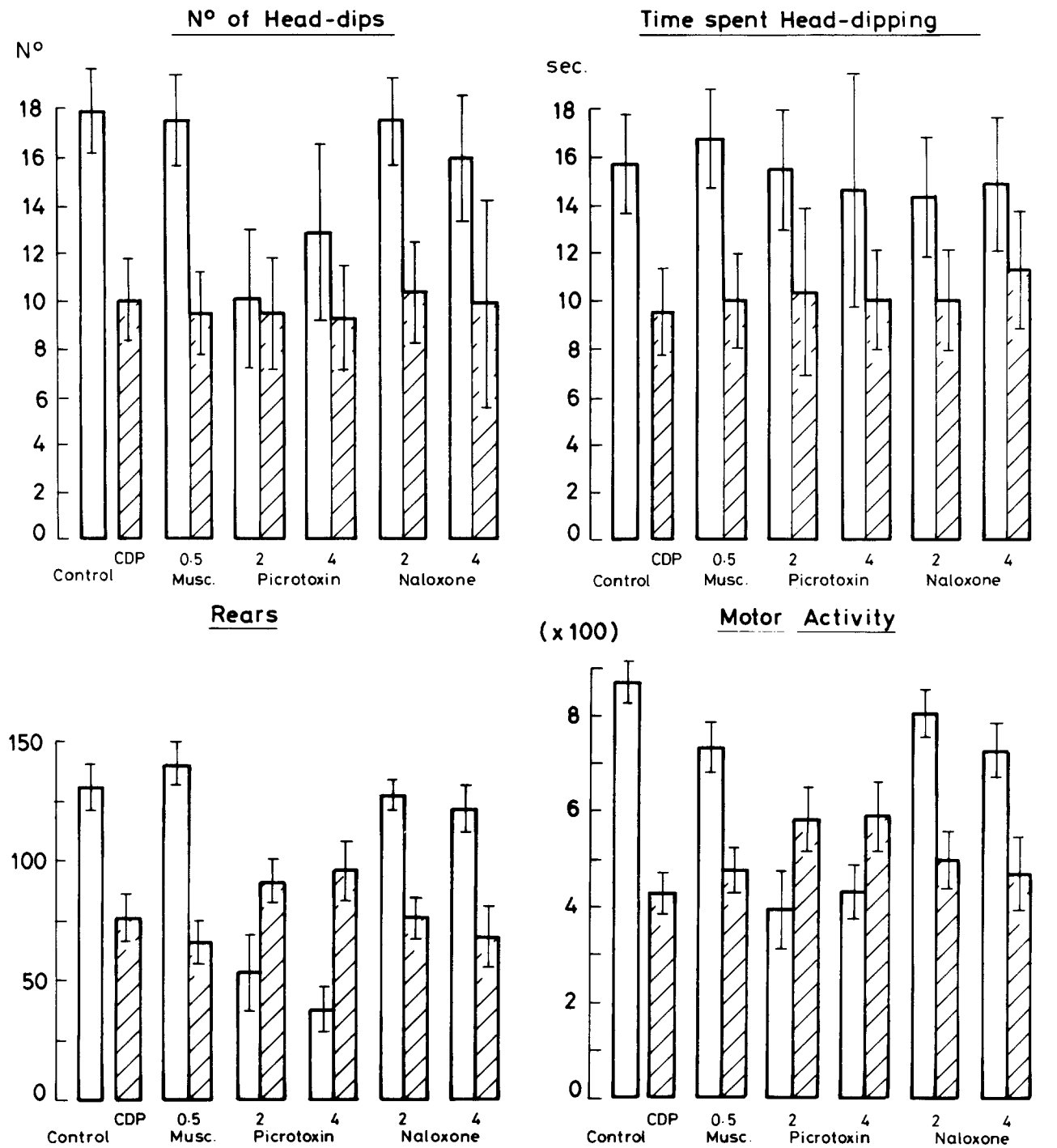

FIG. 1. (a) Mean (±SEM) number of head-dips and time spent head-dipping for rats injected with water (control), muscimol (0.5 mg/kg), picrotoxin (2 and 4 mg/kg) or naloxone (2 and 4 mg/kg). These drugs were either given alone (clear columns) or in combination with chlordiazepoxide (10 mg/kg, hatched columns). (b) Mean (±SEM) rears and motor activity scores of rats injected with water (control), muscimol (0.5 mg/kg), picrotoxin (2 and 4 mg/kg), or naloxone (2 and 4 mg/kg). These drugs were either administered alone (clear columns) or in combination with chlordiazepoxide (10 mg/kg, hatched columns).

TABLE 1

A SUMMARY OF THE QUALITATIVE EFFECTS OF CHLORDIAZEPOXIDE ALONE AND IN COMBINATION WITH MUSCIMOL, PICROTOXIN AND NALOXONE IN THE HOLEBOARD, INCLINED PLANE AND HORIZONTAL WIRE TESTS

	Holeboard		Inclined Plane	Horizontal Wire
	Head-dipping	Motor Activity		
Chlordiazepoxide	↓	↓	↓	↓
Muscimol	No effect	No effect	No effect	↓
CDP + muscimol	No potentiation	No potentiation	*Potentiation*	↓
Picrotoxin	No effect	↓	No effect	No effect
CDP + Picrotoxin	No antagonism	*Antagonism*	*Antagonism*	No antagonism
Naloxone	No effect	No effect	No effect	No effect
CDP + Naloxone	No antagonism	No antagonism	*Antagonism*	No antagonism

tributed. The scores for the number of rats successfully heaving their hindpaws onto the horizontal wire were analysed by χ^2 tests.

RESULTS

Holeboard Test

There were no significant differences between the control scores in the three phases of the experiment, or between the three chlordiazepoxide groups. Therefore, for illustrative purposes only, in Fig. 1 the 3 control groups have been combined, as have the 3 chlordiazepoxide groups; however, for each phase of the experiment the analyses of variance were performed only with the relevant control and chlordiazepoxide groups.

From Fig. 1 it can be seen that chlordiazepoxide (10 mg/kg) significantly reduced all 4 measures in the holeboard. Muscimol alone had no effect on head-dipping ($Fs<1.0$) and did not potentiate the chlordiazepoxide-induced reductions in head-dipping (chlordiazepoxide (CDP) effects $F(1,27)=13.01$ and 6.71, $p<0.002$ and $p<0.02$ for number of head-dips and time spent head-dipping, respectively; CDP × muscimol interactions $Fs<1.0$). Muscimol was also without effect on rearing and motor activity ($Fs<1.0$) and did not potentiate the chlordiazepoxide-induced reductions (CDP effects $F(1,27)=23.1$ and 33.3, respectively, $p<0.0001$; CDP × muscimol interactions $Fs<1.0$).

Picrotoxin reduced the number of head-dips, but this failed to reach significance, $F(2,46)=2.6$, $p=0.09$, and the time spent head-dipping was not reduced. Further, picrotoxin did not antagonise the chlordiazepoxide-induced reduction in head-dipping (CDP × picrotoxin interactions $F(2,46)=1.99$ and 2.28). Picrotoxin significantly reduced the number of rears made and locomotor activity, $F(2,46)=4.47$ and 3.20, $p<.02$ and $p<.05$, respectively, and inspite of these reductions was also able to antagonise the chlordiazepoxide effects (CDP × picrotoxin interactions $F(2,46)\times 16.2$ and 11.7, respectively, $p<0.0001$).

Naloxone was without significant effects on any of the measures in the holeboard ($Fs<1.0$ for head-dipping and rears, $F(2,56)=1.95$ for motor activity) and was unable to antagonise any of the chlordiazepoxide-induced reductions (CDP effects $F(1,56)=6.44$, 5.82, 29.5 and 11.0 for number of head-dips, time spent head-dipping, rears and motor activity, respectively, $ps<0.02$, 0.05, 0.001, 0.002; CDP × naloxone interactions $Fs<1.5$).

Thus none of the 3 drugs tested significantly modified the chlordiazepoxide-induced reductions in exploratory head-dipping, but picrotoxin antagonised the chlordiazepoxide-induced decrease in rearing and locomotor activity, in spite of having sedative actions itself.

Inclined Plane

Figure 2 shows the median latencies to slide off the inclined plane for the various drug groups tested. In all groups the scores ranged from a latency of <5 sec to the maximum score of 120 sec.

Chlordiazepoxide produced significant ataxia, as measured by the latency to fall off the inclined plane ($U=11$, $p<0.05$). Muscimol alone did not cause ataxia, but it did potentiate the ataxic effects of chlordiazepoxide (CDP vs CDP × muscimol, $U=10.5$, $p<0.03$).

In the second phase of the experiment, chlordiazepoxide again produced significant ataxia ($U=24$, $p<0.05$), and this was significantly antagonised by both doses of picrotoxin ($U=17$ and 19, $ps<0.025$ and 0.05). Picrotoxin alone was without significant effect.

In the third phase the chlordiazepoxide-induced ataxia ($U=20$, $p<0.01$) was significantly antagonised by both doses of naloxone ($U=14$ and 30, $ps<0.002$ and .05), but naloxone alone was without effect.

Horizontal Wire

In each phase of the experiment 70% of the control rats successfully heaved up both hind paws, whereas only 20% of the chlordiazepoxide-treated rats did ($\chi^2=5.05$, $p<0.05$). Muscimol also impaired performance in this test (only 25% were successful) and none of the rats treated with both muscimol and chlordiazepoxide were successful. Picrotoxin alone slightly impaired performance (40% were successful), but this difference did not reach significance, and picrotoxin was unable to antagonise the chlordiazepoxide impairment. Similarly, naloxone alone impaired performance in this test (40% were successful) and was unable to antagonise, and even enhanced, the chlordiazepoxide effect (10% success in the CDP + naloxone group).

A summary of the qualitative effects of chlordiazepoxide alone and in combination with muscimol, picrotoxin and

naloxone in the holeboard, inclined plane and horizontal wire tests, together with the effects of muscimol, picrotoxin and naloxone given alone can be found in Table 1. Only significant changes are included.

DISCUSSION

The results from the present experiment would support a GABAergic mechanism mediating the ataxic effects of chlordiazepoxide: these were enhanced by muscimol and blocked by picrotoxin. However, the chlordiazepoxide-induced ataxia was also reversed by naloxone. It is possible that this was due to the GABA antagonist properties of naloxone [5], but in general these are seen only with high doses. The interactions between benzodiazepines and morphine are complex and seem to involve non-opiate transmitter systems. The benzodiazepine reduction of morphine's antinociceptive effects had been linked to GABA systems [15], and the benzodiazepine enhancement of morphine-induced hyperactivity in mice has been linked to catecholamines [19]. Both diazepam and muscimol, after peripheral administration, reduced striatal met-enkephalin levels and both bicuculline and naloxone reversed these diazepam effects; in contrast diazepam elevated met-enkephalin levels in the hypothalamus, and this effect was not antagonised by naloxone or bicuculline [6]. The results from the present experiment suggest that there might be two possible mechanisms underlying benzodiazepine-induced ataxia, one via GABAergic paths and the other involving opiate receptors.

In contrast to the results from the inclined plane, neither picrotoxin nor naloxone was able significantly to antagonise the chlordiazepoxide-induced muscle relaxant effects, as measured in the horizontal wire test and, if anything, when naloxone was combined with chlordiazepoxide performance was even worse. A similar effect was seen with the combination of muscimol and chlordiazepoxide, but because of the low performance of the rats treated with chlordiazepoxide alone the significance of this possible potentiation could not be assessed. The mechanisms underlying the muscle relaxant effects of the benzodiazepines remain unknown, but diazepam's effects in the horizontal wire test can be antagonised by caffeine [18].

Muscimol was completely without effects in the holeboard test and did not potentiate any of the chlordiazepoxide effects. Because of its poor penetration into the CNS, the failure to find significant effects after peripheral administration of muscimol must be viewed with caution. However, it did seem effective in potentiating ataxia and so this dose and route of administration clearly had some behavioural effects, although these could have been due to a metabolite and therefore not necessarily attributable to a GABA agonist effect. Naloxone was also without significant effect in the holeboard, either alone or in combination with chlordiazepoxide. In this experiment, naloxone was given 10 min before test, because of its rapid action and short half-life; in previous experiments, with an injection-test interval of 30 mins naloxone has been found to reduce head-dipping and to antagonise the decrease in head-dipping produced by ACTH and by morphine [7].

Picrotoxin was also without significant effect on exploratory head-dipping, alone or in combination with chlordiazepoxide; so none of the drugs administered in this study was able to modify the effects of chlordiazepoxide on exploratory behaviour. However, picrotoxin was able significantly to antagonise the chlordiazepoxide reductions in rearing and locomotor activity, in spite of reducing these measures when given alone. It is perhaps surprising that picrotoxin is sedative since it is a convulsant, but such effects have been noted before [22]. A role of GABA in the sedative effects of benzodiazepines, measured by the reduction of locomotor activity and by the loss of righting reflexes [1] is therefore not precluded. The inability of picrotoxin to antagonise the effects of benzodiazepines on exploration, measured by head-dipping, or in the open field [22] emphasises the importance of distinguishing between measures of simple locomotor activity and those of directed exploration and suggests that tests where motor activity cannot be clearly separated from exploration must be interpreted with caution.

The results of the present experiment do not support the notion that GABAergic mechanisms mediate all of the behavioural effects of the benzodiazepines. In the same animals, picrotoxin was ineffective at reversing the muscle relaxant effects or the decrease in exploratory head-dipping, whilst clearly antagonising the ataxia and decrease in locomotor activity. Neurotransmitters other than GABA must mediate at least some of the behavioural effects of benzodiazepines, and the naloxone reversibility of ataxia suggests an interaction with opiate systems.

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