EFFECTS OF SOME 5-HYDROXYTRYPTAMINE AND RELATED LIGANDS IN ANXIETY MODELS.

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SUMMARY

Drugs acting at 5-HT receptors were evaluated on three animal models of anxiety.

On the elevated X-maze test the majority of 5-HT1 agonists were found to be anxiogenic. However, ipsapirone was anxiolytic and buspirone and gepirone were inactive. The 5-HT2 agonist DOI and the 5-HT2 antagonist ritanserin were anxiolytic while ICI 169,369, a 5-HT2 antagonist was inactive. All 5-HT3 antagonists tested were inactive in this test, while the indirect serotoninomimetics zimeldine and fenfluramine were anxiogenic. Neither beta-adrenoceptor agonists nor antagonists had reproducible effects on anxiety in this model. Combined beta-1/beta-2 adrenoceptor antagonists reversed the anxiogenic effects of 8-OH-DPAT while selective beta-1 or beta-2 antagonists did not.

On the social interaction model the 5-HT1 agonists 8-OH-DPAT, RU 24969 and 5-MeODMT were anxiogenic and ipsapirone was anxiolytic. The 5-HT2 agonist DOI and the beta-adrenoceptor- and 5-HT- antagonist pindolol were anxiolytic, while the 5-HT2 and 5-HT3 antagonists were inactive.

In the marble burying test, the 5-HT uptake inhibitors zimeldine, fluvoxamine, indalpine and citalopram, the 5-HT1B/5-HT1C agonists mCPP and TFMPP and the 5-HT2/5-HT1C agonist DOI reduced marble burying without affecting locomotor activity. 5-HT1A agonists and the 5-HT2 and 5-HT3 antagonists were without effect.

Lesions of the dorsal raphe nucleus reversed the anxiogenic effects of 8-OH-DPAT in the X-maze model.

The implication of these results for the understanding of the pharmacology of 5-HT in anxiety is discussed.

KEY WORDS: 5-HT receptors, X-maze, social interaction, marble burying, anxiety.

Dedicated to my parents

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LIST OF ABBREVIATIONS.

The following non-standard abbreviations were used in this work.

ANOVA	Analysis of variance
Beta-CCE	ethyl-beta-carboline-3-carboxylate
CNS	Central nervous system
DOI	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane
DRN	Dorsal raphe nucleus
5,7-DHT	5,7-hydroxytryptamine
GABA	Gamma aminobutyric acid
5-HIAA	5-hydroxy-indole acetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
i.c.v.	Intra-cerebroventricular
i.p.	Intra-peritoneal
Ki	Equilibrium dissociation constant
MRN	Median raphe nucleus
mCPP	1-(3-chlorophenyl)piperazine
5-MeODMT	5-Methoxy-N,N-dimethyltryptamine
mg/kg	milligrams per kilogram body weight
PCPA	para-chlorophenylalanine
1-PP	1-(2-pyrimidyl)piperazine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
SAP	Stretch Attend Posture
s.e.m.	standard error of the mean
s.c.	subcutaneous
TFMPP	1-(m-trifluoromethylphenyl)piperazine

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1. Introductory

Anxiety is one of the most common problems encountered in contemporary medical practice. For instance, a National Institute of Mental Health (NIMH) survey estimated that 8.3% of the adult human population of the United States had suffered from anxiety disorders in the previous 6 months (Rickels and Schweizer 1987). The prevalence of the condition is also reflected by the fact that in 1975 there were 100 million prescriptions in the U.S. for benzodiazepines, the current treatment of choice for anxiety (Griffiths and Sannerud 1987, Snyder 1986). The prescription rate has fallen since because of the side effects of these drugs (Ibid). On acute administration they produce excessive CNS depression, impairment of learning, memory and performance and potentiate the effects of alcohol and other centrally acting agents (Taylor and Tinklenberg 1987), while on chronic administration they produce physical dependence leading to withdrawal symptoms and rebound anxiety on ceasing drug treatment (Griffiths and Sannerud 1987, Rickels and Schweizer 1987, Snyder 1986). In addition, only 65-75 % of anxiety patients treated with benzodiazepines obtain moderate to marked improvement in their condition (Rickels and Schweizer 1987). For these reasons there is a lot of effort being devoted by the pharmaceutical industry to develop alternatives. Among the lines of approach, the one receiving the most contemporary interest are agents acting via 5-HT receptors.

1.2. 5-Hydroxytryptamine pathways in the brain.

5-hydroxytryptamine (5-HT) also known as serotonin was discovered in the blood in 1948 and subsequently found to be a neurotransmitter. 5-HT containing neurones in the

brain have however only been mapped relatively recently. It is now known that 5-HT containing neurones occur in nine clusters of cells [B1-B9 by the classification of Dahlstrom and Fuxe (1964)] lying in or near the midline or raphe regions of the pons and upper brain stem. There are also some cells in the area postrema, the caudal locus coeruleus and around the interpenduncular nucleus (Azmitia 1978, Dahlstrom and Fuxe 1964). The more caudal groups [B1 - B3] project largely to the medulla and pons. The more rostral 5-HT cell groups (raphe dorsalis, medianus and centralis superior or B7 - B9) are thought to provide the extensive 5-HT innervation of the telencephalon and diencephalon. The B8 group (median raphe) provides a major component of the 5-HT innervation of the limbic system while B7 (dorsal raphe), which contains the greatest number of 5-HT cell bodies, projects to the neostriatum, cerebral and cerebellar cortices and thalamus (Breese 1975).

Lesion of the dorsal raphe produced a selective decrease in 5-HT levels in the striatal region while lesion of the median raphe produced selective reduction of 5-HT in the hippocampal and septal areas (Geyer et al 1976). It was therefore suggested that there were at least two distinct 5-HT systems. The mesostriatal pathway originates in the dorsal raphe nucleus and projects to the striatum, thalamus and some cortical (primarily dopaminergic) regions. The mesolimbic pathway originates in the median raphe nucleus and innervates such limbic structures as the septal nuclei, hippocampus, cingulate and entorhinal cortices and mammilary bodies (primarily noradrenergic regions) (Geyer et al 1976).

1.3. 5-HT receptors subtypes.

1.3.1 Historical perspective.

As early as 1957, Gaddum and Picarelli found two 5-HT receptor subtypes in the guinea-pig ileum: one, mediating smooth muscle contraction and antagonised by dibenzyline (phenoxabenzamine), was designated the "D" receptor while the other, mediating depolarisation of the cholinergic nerves and antagonised by morphine, was called the "M" receptor. This classification was not entirely satisfactory as neither morphine nor dibenzyline was a specific receptor antagonist, but not much progress was made until the application of radioligand binding techniques to this problem in the late 1970's.

1.3.2 Radioligand binding data.

On the basis of the radioligand binding data, Peroutka and Snyder (1979) identified two 5-HT receptor subtypes: the 5-HT1 receptor sites which were labelled with high affinity by [3H]5-HT and other 5-HT receptor agonists and the 5-HT2 receptor sites labelled by [3H]spiperone and other 5-HT receptor antagonists. Later, Pedigo et al (1981) established that 5-HT1 receptor sites were not homogeneous and proposed two sub-types: the 5-HT1A site with a higher affinity for spiperone, and 5-HT1B those with lower affinity for spiperone. Pazos et al (1984) later proposed a further subtype the 5-HT1C receptor labelled by [3H]mesulergine in addition to [3H]5-HT.

Another receptor sub-type, the 5-HT1D site has been reported in the brain of man, pig and calf (Hoyer et al 1988).

It has recently been suggested that there are two subtypes of 5-HT2 binding sites, a high affinity 5-HT2A subtype and a low affinity 5-HT2B site. [3H]-ketanserin labels both sites, while [77Br]-R-(-)DOB and [125I]-DOI label the 5-HT2A site (Peroutka et al 1989).

A different binding site has also been located in the brain which binds selectively to [3H]-quarternised ICS 205-930 and [3H]-GR 65630 and other 5-HT3 agents and corresponds to 5-HT3 receptor sites (Kilpatrick et al 1987, Watling 1988, Dumuis et al 1988).

1.3.3 Functional studies

Studies show that the binding affinities of a variety of 5-HT antagonists for the 5-HT2 site correlate with their potencies at the "D" receptor in functional tests on vascular and intestinal smooth muscle (Engel et al 1985).

Thus the cortical 5-HT2 binding sites are probably identical with the "D" receptors described by Gaddum and Picarelli (1957). Functional correlates for the 5-HT2 receptors have been obtained: stimulation elicits neuronal depolarisation, smooth muscle contraction and platelet aggregation all of which are blocked by the 5-HT2 antagonist ketanserin (Leysen 1985).

The 5-HT1 receptor site has not been so well characterised largely because of the absence of selective antagonists. Methysergide and methiothepin have antagonist effects but are not selective; methysergide is a partial agonist while methiothepin is a potent antagonist at 5-HT2 receptors (Bradley et al 1986). Because of inadequate characterisation it has been proposed that effects that appear to be subserved by the 5-HT1 sites should be ascribed to the "5-HT1-like" receptor (Bradley et al 1986).

Using the available agents in a variety of behavioural studies it has nevertheless been possible to distinguish between the effects of different 5-HT1 receptor subtypes and between these and those of 5-HT2 receptor (see below).

The presynaptic 5-HT cell body autoreceptor is of 5-HT1A subtype (Dourish et al 1986b) and has been located in human frontal cortex and hippocampus (Hoyer et al 1986a). The terminal autoreceptor is associated with the 5-HT1B subtype in the rat cortex (Middlemiss 1984, Engel et al 1986) and hippocampus (Maura et al 1986) but is apparently absent in the human brain (Hoyer et al 1986a). 5-HT2 receptors are abundant in the hippocampus and frontal cortex in both animals and humans (Hoyer et al 1986b).

Recent work (Hoyer 1988, Hoyer et al 1989) suggests that the 5-HT1C receptors are most likely to be a subtype of the 5-HT2 receptor rather than of 5-HT1 receptors. For instance, radiolabelled mesulergine, LSD and 1-methyl-LSD label both 5-HT1C and 5-HT2 sites. In addition, 5-HT2 and 5-HT1C receptors share the same second messenger system, stimulation of phosphoinositol metabolism (Sanders-Bush and Conn 1987). Many antagonists such as ritanserin and LY 53857 are potent at both 5-HT1C and 5-HT2 receptors; the same high activity at 5-HT1C receptors was seen with the agonist, 1-(2,5-Dimethoxy-4- iodophenyl)-2- aminopropane (DOI) (Hoyer et al 1989) which was previously considered to be selective for 5-HT2 receptors (Glennon 1987).

Nevertheless there are some differences between 5-HT1C and 5-HT2 receptors (Hoyer et al 1989). Gaddum's "M" receptors do not correspond to 5-HT1 or 5-HT2 binding sites and have been designated the 5HT3 receptors (Bradley et al 1986, Richardson and Engel 1986). They are antagonised by cocaine, MDL 72222, ICS 205-930 ((Bradley et al 1986, Richardson and Engel 1986) as well as BRL 24924 (Fake et al 1987) and GR 38032F (Brittain et al 1987). Functional studies in the peripheral nervous system have also indicated that 5-HT3 receptors are heterogenous with 3

possible subtypes (Richardson and Engel 1986).

1.3.4 5-HT receptors and second messenger systems.
5-HT receptors are coupled to several major effector systems: adenylate cyclase; phospholipase C mediated phosphoinositide (PI) hydrolysis; and ion channels (K+ and Ca2+) (Roth and Chuang 1987).

5-HT1A receptors are linked to adenylate cyclase and are coupled either positively in some tissues and negatively in others depending on the pre-existing state of activation (Conn and Sanders-Bush 1987, Sanders-Bush and Conn 1987). 5-HT1A receptors are negatively coupled to the adenylate cyclase system in the calf and guinea pig hippocampus (Bockaert et al 1987, Schoeffter and Hoyer 1988).

Both 5-HT1B receptors (Bouhelal et al 1988) and 5-HT1D receptors (Hoyer and Schoeffter 1988) are negatively coupled to the adenylate cyclase system.

5-HT1C and 5-HT2 receptors are linked to phosphoinositide hydrolysis for signal transduction with stimulation leading to activation of phospholipase C, accumulation of inositol phosphates and mobilization of intracellular Ca2+ (Conn and Sanders-Bush 1987).

The 5-HT3 receptors are positively coupled with adenyl cyclase (Dumuis et al 1988) and increase cyclic GMP levels (Reiser and Hamprecht 1989). These receptors have also been reported to be ligand-gated to ion channels (Hartig 1989).

Three 5-HT receptor subtypes, 5-HT1C, 5-HT1A, and 5-HT2 have been cloned (Hartig 1989). All three are single subunit proteins and members of the G protein superfamily (Hartig 1989).

Table 1.1: Drug affinities for 5-HT receptor subtypes. (Ki values, nanomolar.)

Drug	5-HT1A	5-HT1B	5-HT1C	5-HT2	alpha-2
8-OH-DPAT	0.9	4980	7240	4870	224
Ipsapirone	2.9	52000	9000	10000	1740
Buspirone	10	68000	110000	2100	23200
RU 24969	2.5	0.4	398	590	2320
5-MeODMT	7.0	50		625	1400
TFMPP	148	10		88	887
mCPP	263	0.5		118	436
Ritanserin	1660		2.5	0.29	56
Ketanserin	4680	10000	98	0.63	370
Pindolol	19	7	53700	>10000	
Methysergide	25	520	62	2.6	
Cyproheptadine	110	840	2600	2.0	
DOI	2355	1261	30	0.7	
LSD	0.43	6.6	3.8	0.54	
Pirenperone	1660		60	0.25	3.3

From Glennon 1987; Peroutka 1987; Titeler et al 1988; Leysen, unpublished data.

1.4 Behavioural effects of 5-HT.

1.4.1 The 5-HT syndrome.

Compounds that increase central 5-HT levels or stimulate central 5-HT receptors induce dramatic changes of behaviour in the rat. This change, called the 5-HT behavioural syndrome is characterised by reciprocal forepaw treading, flat body posture with abducted hindlimbs, head weaving, hyperactivity, resting tremor and straub tail (Tricklebank 1985). Compounds which can induce the syndrome include the 5-HT precursors, L-tryptophan, 5-hydroxytryptamine (5-HTP), the 5-HT releasers fenfluramine and p-chloroamphetamine and the 5-HT agonists 5-Methoxy-N,N- dimethyltryptamine (5-MeODMT), 8-hydroxy-2- (di-n-propylamino) tetralin (8-OH-DPAT), quipazine and lysergide (Tricklebank 1985) and BAY R 1531 (Glaser et al 1987). The syndrome is also modulated by catecholamines.

Reciprocal forepaw treading appears to be mediated by 5-HT1A receptors as it is induced by the predominantly 5-HT1A agonist 8-OH-DPAT (Middlemiss and Fozard 1983) and blocked by spiperone (which has 5-HT1A antagonist activity) but not by ketanserin, a 5-HT2 antagonist (Tricklebank 1985). The behaviour is blocked by (-)pindolol but this appears to involve antagonism at 5-HT1 receptors rather than ß-receptor blockade since betaxolol and ICI 118,551, a ß-1 and a ß-2 antagonist respectively, which lack 5-HT effects are without effect (Tricklebank 1985). There is also a 5-HT2 component as ritanserin, a 5-HT2 antagonist, blocks forepaw treading induced by the non-selective agonist quipazine (Tricklebank 1985).

The hyperlocomotion component appears to be mediated by the 5-HT1 receptors as it is produced by the selective 5-HT1A agonist 8-OH-DPAT and is not blocked by 5-HT2 antagonists (Tricklebank 1985). Headshakes on the other hand appear to

be mediated by 5-HT2 receptors: they are induced by the non-selective agonists 5-MeODMT and quipazine but not by the more selective 5-HT1 agonists 8-OH-DPAT and RU 24969 (Tricklebank 1985) and are inhibited by low doses of the 5-HT2 antagonist ritanserin (Goodwin and Green 1985).

1.4.2 Drug discrimination studies.

In addition to classical pharmacological experiments, drug discrimination studies have also been useful in studying the effects of 5-HT receptor subtypes. For example, in discrimination tests the 5-HT1A agonists ipsapirone, buspirone and 8-OH-DPAT substitute for each other but not with the non-selective agonists 5-MeODMT, and quipazine, or with RU 24969, lysergic acid diethylamide (LSD) and 1-(m-trifluoromethylphenyl)- piperazine (TFMPP) (Glennon 1987, Cunningham et al 1987, Stolerman et al 1987).

1.4.3 Physiological roles of 5-HT in the CNS.

5-HT has been implicated in many behavioural functions and in the aetiology of various psychiatric illnesses. These include sleep regulation (Jouvet 1973, Idzikowski et al 1986), pain perception (Evans 1961, Roberts 1984), feeding (Blundell 1984), sexual behaviour (Gessa and Tagliamonte 1979), depression (van Praag and Korf 1971, Abrams 1978, Aprison et al 1978), anxiety (Geller and Blum 1970, Wise et al 1972) and migraine (Glover and Sandler 1989, Saxena and Ferrari 1989).

1.5 Central adrenergic receptors.

Radioligand binding techniques have identified two subtypes of alpha and ß adrenoceptors (Lands et al 1967, Maguire et al 1977, Tanaka and Starke 1980) in the CNS. Of the ß-adrenoceptors, the ß-1 subtype is predominant except in the cerebellum, where ß-2-adrenoceptors are predominant (Minneman et al 1979, Janowsky and Sulser 1987). Both are

located postsynaptically (Ibid). Alpha-2 adrenoceptors are localised both pre- and postsynaptically, whereas alpha-1 adrenoceptors appear to be exclusively located postsynaptically (Ibid).

1.5.1 Interaction of 5-HT and ß-adrenergic receptors.
(-)Propranolol, pindolol and other non-selective ß-adrenoceptor antagonists have a high affinity for brain 5-HT receptors (Middlemiss et al 1977, Green et al 1983, Nahorski and Willcocks 1983). The ß-1 selective antagonists such as practolol and metoprolol and the ß-2 selective antagonists like ICI 118,551 as well as (+)propranolol (which has no ß-adrenoceptor activity), have very low affinity for 5-HT receptors (Ibid).

Propranolol blocks the 5-HT1A-mediated inhibition of firing of dorsal raphe neurones (Sprouse and Aghajanian 1986). (-)Propranolol and the other non-selective ß-1 and ß-2 adrenoceptor antagonists inhibit 5-HT-induced hyperactivity, hypothermia and behavioural syndrome in rats (Goodwin and Green 1985). The selective ß-1 selective antagonists like metoprolol and ß-2 selective antagonists like butoxamine were ineffective (Costain and Green 1978, Goodwin and Green 1985).

Clenbuterol, dobutamine and other ß-adrenoceptor agonists on the other hand stimulate central 5-HT turnover (Waldmeier 1981) and enhance 5-HT mediated hyperactivity, headtwitch (Cowen et al 1982, Ortmann et al 1981, Handley and Singh 1984), hypothermia (Green et al 1986) and tremor (Hallberg 1986). This potentiation appears to be mediated by ß-adrenoceptor mechanisms rather than by direct effects on 5-HT receptors since these compounds do not bind to 5-HT receptors (Green et al 1983) and their modulation of 5-HT effects is blocked by ß-1 and ß-2 selective antagonists

(Green et al 1986, Hallberg 1986) which neither bind to 5-HT receptors (Middlemiss et al 1977, Green et al 1983, Nahorski and Willcocks 1983) nor affect 5-HT mediated behaviour.

1.5.2 Interaction of 5-HT and alpha-adrenergic receptors. The 5-HT1A agonist 8-OH-DPAT has appreciable affinity for alpha-2-adrenoceptors (see Table 1.1) and has been shown to have alpha-2-adrenoceptor antagonist properties both in electrophysiological studies (Crist and Surprenant 1987) and in drug discrimination studies (Winter 1988). It is therefore likely that some of the actions attributed to its agonist effects at 5-HT receptors may be due to alpha-2-adrenoceptor blockade.

However, 8-OH-DPAT also facilitates noradrenaline release hence indirectly producing postsynaptic alpha-2-adrenoceptor agonist effects such as mydriasis (Heal et al 1989).

In addition, the alpha-2-adrenoceptor antagonists yohimbine, rauwolscine and idazoxan bind to 5-HT1A receptors {pKi Yohimbine = 7.3, Idazoxan = 7.4, rauwolscine = 7.5} (Armah 1989).

Ipsapirone, buspirone and gepirone have a common metabolite 1-(2-pyrimidyl)piperazine (1-PP) which has alpha-2-adrenoceptor antagonist effects (Bianchi and Garratini 1988, Bianchi et al 1988). This compound has been reported to have anxiolytic effects in punished conflict (Gower and Tricklebank 1988). Buspirone's anticonflict activity is increased by alpha-2-adrenoceptor antagonists idazoxan and yohimbine and decreased by the alpha-2-adrenoceptor agonist clonidine, at a dose which had no effects on punished responding (Gower and Tricklebank

anxiolytic effects of these agents.

1.6 Psychology of anxiety.

Anxiety is a normal emotion and in its mild forms stimulates productive thinking and motivates activity geared at economic survival and is an adaptation for survival (Snyder 1986). However in its pathological forms it renders the sufferer unable to function adequately (Ibid).

Acute anxiety has been defined as an emotional state characterised by subjective feelings of dread, apprehension, utter distress and a feeling of impending catastrophe and/or impending disaster (Schweitzer and Adams 1979, Snyder 1986). In addition to psychological content, acute anxiety is often accompanied by physiological signs such as palpitations, tremulousness, hyperventilation, faintness and dizziness (Ibid).

The Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-III) has divided anxiety into several subtypes: generalised anxiety disorder, panic disorder, phobic disorder, obssessive-compulsive disorder and posttraumatic stress disorder (American Psychiatric Association 1980, Fyer et al 1987). Each of these categories of anxiety represents a different clinical entity with different aetiology and pharmacotherapy. Of these anxiety subtypes only generalised anxiety disorders respond to benzodiazepines and other classical anxiolytics (Rickels and Schweizer 1987).

Based on a analysis of the behavioural effects of anti-anxiety drugs, Gray (1982) has proposed a comprehensive model of the neuropsychology of anxiety. He suggests that anxiety is a central state consisting of activity in a

behavioural inhibition system (BIS) in the brain which is activated by stimuli that warn of punishment or non-reward (secondary aversive stimuli), novel stimuli and innate fear stimuli. Activation of the BIS results in inhibition of ongoing behaviour and increase in level of arousal and attention to the environment. Anxiolytics reduce the activity of the behavioural inhibition system releasing the behaviour suppressed by the system. He also suggests that anxiolytics act by reducing the activity of ascending noradrenergic and serotonergic pathways to the septo-hippocampal system, noradrenergic pathways to the hypothalamus and dopaminergic pathways to the prefrontal cortex (Gray 1982).

1.7 Animal models of anxiety.

The biological role of fear and anxiety as an aid to survival suggests that the emotion is also present in lower animals and has a similar content as in humans (Gray 1982, Cloninger 1987). Nevertheless there are some aspects of human anxiety animal models cannot emulate.

In humans, anxiety is diagnosed principally on the basis of its cognitive content with the associated behavioural changes being of secondary importance. In animals this cognitive aspect is inaccessible hence assessment of anxiety is restricted to measuring behavioural changes. Animal models assess behaviour that in humans would be associated with feelings of anxiety but there is no certainty that such behaviour is specific to anxiety in the test animal (Kahn et al 1988). Whether assessing anxiety from behavioural changes only results in quantitative and/or qualitative differences between animal models and human anxiety can only be speculated.

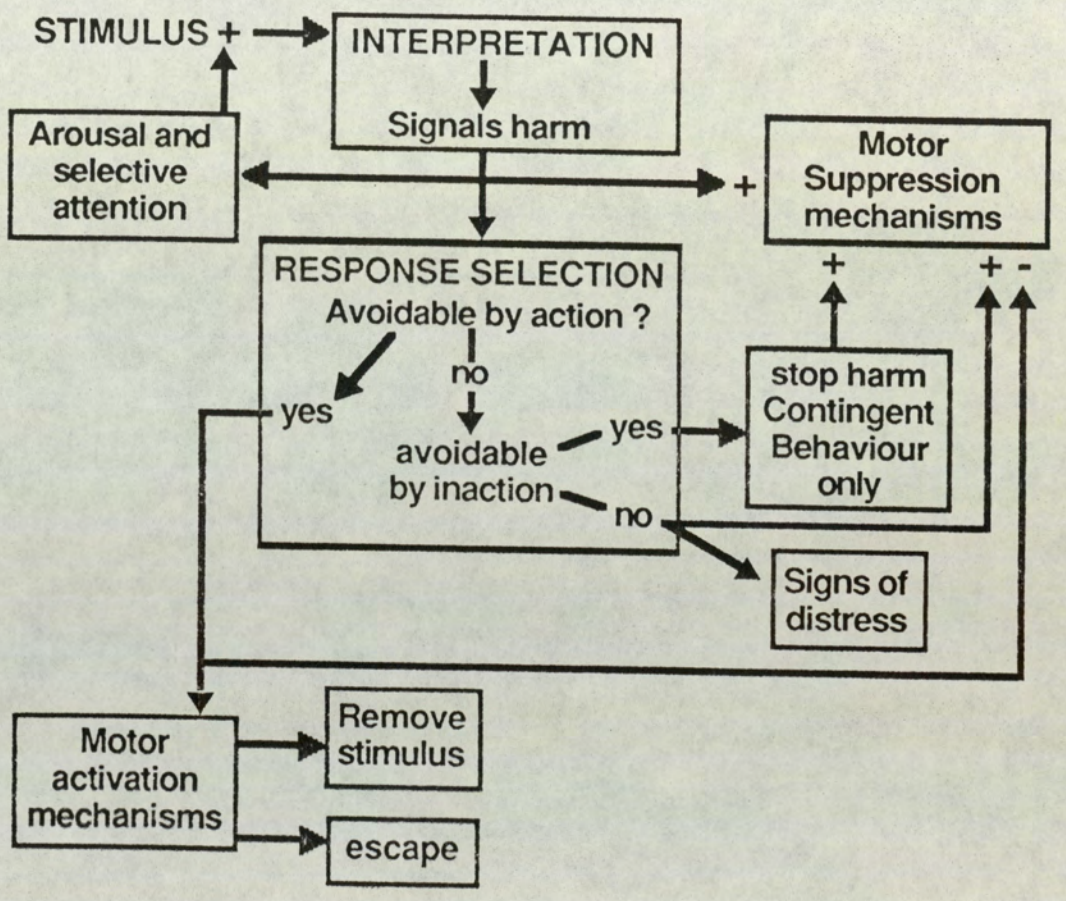
In humans, anxiety has been suggested to be a product of

the complex interaction of individual personality traits and the environment, generally over a long period of time i.e. humans learn to be anxious (Cloninger 1987). Even though some forms of anxiety such as a phobic experience are apparently acute, the tendency/proneness to experience such anxiety lasts for months if not years. By comparison, all animal models are acute states and are restricted to narrowly defined experimental conditions. As already mentioned, not all types of anxiety respond to benzodiazepine treatment. In developing animal models of anxiety, sensitivity to the benzodiazepines is generally considered a necessary aspect of their pharmacological validation (File 1986). This means that anxiety models are oriented only to conditions of anxiety responsive to the benzodiazepines.

Basically, an animal model of anxiety has a threatening stimulus which could either be a secondary aversive stimulus or an innate fear stimulus (Gray 1982). Once this stimulus is interpreted as signaling harm, the animal responds with an appropriate change of behaviour. This behavioural change would be directed at reducing the occurrence of harm either by withholding a behaviour (behavioural inhibition) if harm is best avoided by inaction, or by emitting a behaviour if by so doing the stimulus can be removed or avoided (Handley 1989, see Fig 1.1).

Animal models of anxiety can be classified in many ways. The classification used here is based on whether the threatening stimulus provoking the behavioural change is a secondary aversive stimulus or an innate fear signal and if the response taken involves emitting or withholding a behaviour or merely increased arousal and attention or even signs of distress (Handley 1989, see Table 1.2).

Fig 1.1 Features of anxiety models.



From Handley (1989).

Table 1.2 Classification of anxiety models.

	THREATENING STIMULI	
1. BEHAVIOUR WITHHELD	SECONDARY AVERSIVE	INNATE FEAR
Primary drives	Punished lever press for food Punished drinking	Hyponeophagia
Curiosity	Punished exploration Electrified object (shock probe)	Light/dark box Elevated X-maze Social interaction Staircase model
2. BEHAVIOUR EMITTED	Conditioned defensive burying	Object burying Defensive aggression Aversive brain stimulation
3. INCREASED AROUSAL AND ATTENTION	Potentiated startle	
4. SIGNS OF DISTRESS		Rat pup model Primate test

Adapted from Handley (1989).

1.7.1 Methods using secondary aversive stimuli.

1.7.1.1 Punished responding (Geller-Seifter) test:

In this method (Geller and Seifter 1960) a rat is trained to press levers on two different alternating schedules. On one it initially receives a high level of reinforcement, on the other, a variable interval schedule, food is delivered rather infrequently. Once the response is established the high level reinforcement schedule is modified so that each reinforcement is accompanied by footshock. This is the conflict schedule. Benzodiazepines and other anxiolytics increase punished responding without producing corresponding increases of unpunished responding. The test detects both acute and chronic effects of benzodiazepines (Sepinwall and Cook 1978).

1.7.1.2 Conditioned emotional response.

In this test rats are trained to press a lever in return for food on a fixed interval schedule. Once the schedule is established, a tone which is followed by an unavoidable shock, is presented at regular intervals every few minutes. This procedure eventually results in a disruption of operant responding during the preshock tone. This disruption has been attributed to anxiety (Estes and Skinner 1941) but the effect of anxiolytics on this test show it to be unreliable as a model of anxiety (Millenson and Leslie 1974).

1.7.1.3 Punished drinking.

In this test, thirsty rats receive a shock every 20th lick at a water spout. Conflict is created between thirst (24-hour water deprivation) and the drinking-contigent shock. Anxiolytics increase punished drinking (Vogel et al 1971). This is a widely used test since it does not require training of animals.

1.7.1.4 Punished exploration (four plate test).
The test apparatus has four electrifiable plates on the floor. Locomotor activity in rats is reduced when they receive shocks on transversing the plates, an effect that is attenuated by anxiolytics (Boissier et al 1968).

1.7.1.5 Potentiated Startle.
Rats startle to sudden noises. The startle response is enhanced when the noise is preceded by light, if the light has previously been paired with a electric shock. This enhanced startle response is reduced by benzodiazepines (Davis 1979).

1.7.1.6 Shock probe
Rats housed with an electrified prod explored it and on getting an electric shock after touching it refrained from approaching it subsequently. Benzodiazepines increased the number of times the animals approached and touched the prod (Meert 1985).

1.7.2 Methods using innate fear signals.
1.7.2.1 Social interaction.
This model involves measuring the time spent in active social interaction by pairs of rats unfamiliar to each other. The test is performed in familiar or novel surroundings. Anxiolytics increase the duration of social interaction (File and Hyde 1978, File 1980).

1.7.2.2 Elevated X-maze.
This method uses an elevated X-shaped maze with 2 open and 2 enclosed arms. A conflict is created between the curiosity drive (entry into the exposed open arms) and the caution drive (entry into the more secure enclosed arms). Anxiolytics increase the open/total arm entry ratio (Handley and Mithani 1984) and the time spent in the open

arms (Briley et al 1985). The test has been successfully transferred to mice (Lister 1987).

1.7.2.3 Light/dark discrimination test.
This method compares the activity of mice or rats in a two compartment box, one compartment being brightly lit and the other dark. The number of transitions (Crawley and Goodwin 1980) or the difference in locomotor activity and rearings (Costall et al 1987, Carli and Samanin 1988) between the compartments is used as the measure of anxiety.

1.7.2.4 Other exploratory models.
Anxiolytics the increase exploration of an open field (Christmas and Maxwell 1970), the Y-maze (Marriot and Spenser 1965) and the holeboard (Nolan and Parkes 1973).

1.7.2.5 Object burying.
Rodents bury noxious objects and innocuous objects that are housed with them. A subset of this test uses secondary aversive stimuli (conditioned defensive burying), with the animals being housed with objects they learn to develop aversion, to such as pepper sauce and electrified prods (Treit et al 1981, Wilkie et al 1979). Anxiolytics such as the benzodiazepines and the barbiturates reduce the burying of electrified prods by rats (Treit et al 1981) and of glass marbles by mice (Broekkamp et al 1986). On the basis of these findings reduction of object burying has been proposed as an anxiety model (Ibid.).

1.7.2.6 Fear of novel food (hyponeophagia).
Rats exhibit hyponeophagia, avoiding novel food or familiar food in novel surroundings. Benzodiazepines increase both the consumption of unfamiliar food (Poschel 1971) and of familiar food in novel surroundings (Shephard and

Broadhurst 1982). This effect was attributed to their anxiolytic effects and reversal of hyponeophagia was therefore proposed as a model of anxiety. However increased feeding could also be due to the appetite-stimulant effect of these drugs (Cooper 1980) rather than their anxiolytic effects.

1.7.2.7 Stretch-attend posture (SAP).

This is an investigatory forward prolongation of the body in a novel environment. The anxiolytics diazepam, clobazam and pentobarbitone reduce SAP (Kaesermann 1988). However there is evidence that the test is non-selective as doses that reduce SAP also increase immobility (Pollard and Howard 1988).

1.7.2.8 Staircase model

This test involves measuring the number of stairs climbed by rodents in a 3 minute period with the number of rears in the same period being used to check for sedative effects. Benzodiazepines increase the number of stairs climbed without reducing the number of rears (Thiebot et al 1973, Steru et al 1987). The test was found to be non-selective as it detects morphine as anxiolytic and fails to detect the anxiolytic effects of buspirone and alprazolam or the anxiogenic effects of pentylenetetrazol and FG 7142 (Pollard and Howard (1986).

1.7.2.9 Intraspecific aggression.

Intraspecific aggression can be induced by isolation or electric shock. Mice housed singly for three weeks or more readily attack group housed intruders. The latency and incidence of attack are reduced by benzodiazepines and other anxiolytic compounds (DaVanzo et al 1987). Electric shock to the feet induces fighting in rats and attenuation of this shock-induced aggression is occasionally used to

screen compounds for their anti-aggression and possibly anxiolytic potential (e.g. Dourish 1987).

1.7.2.10 Primate distress test.
This test involves scoring anxiety motivated responses in marmosets or cynomolgus monkeys as they are approached and threatened by the experimenter. Benzodiazepines decrease these responses (Piper et al 1989, Costall et al 1988).

1.7.2.11 Rat pup stress test.
This test is based on measuring the ultrasonic vocalisations induced by separation and physical manipulation in 10-13 day old rat pups. Benzodiazepines decrease these vocalisations (Gardner 1985).
1.7.2.12 Antagonism of interoceptive cues of anxiogenic compounds.

Antagonism of discriminative cues of anxiogenic drugs has been proposed as a model of anxiety (Lal and Fielding 1984). Pentylenetetrazol is the commonly used anxiogenic agent and its interoceptive stimulus has been found to be antagonised by the benzodiazepines but not by non anxiolytic anticonvulsants (Lal and Fielding 1984) indicating a specificity to the anxiolytic aspects of benzodiazepine pharmacological effects. This approach has been criticized for merely testing the subjective cues of the test drug rather than anxiety per se (Johnston and File 1988).

1.7.2.13 Aversive brain stimulation.
Electrodes placed in various nuclei in the brain such as the raphe nuclei and the periaqueductal gray (PAG) and passing current through them produce flight responses which are attenuated by anxiolytics (Graeff and Silviera-Filho 1978).

1.7.3 Validation of animal models

Animal models of anxiety are generally validated by their ability to detect the anxiolytic effects of the benzodiazepines. From the different results obtained with non benzodiazepine anxiolytics with most of these tests it seems as if, almost by definition, they test for the effects of benzodiazepines rather than anxiety. With the availability of non-benzodiazepine anxiolytics such as buspirone, it is probably no longer necessary to use benzodiazepines for pharmacological validation of animal models. Despite these reservations, none of the newer agents is close to superceding the benzodiazepines as the mainstay anxiolytics in clinical use. Buspirone, unlike the benzodiazepines has to be used chronically to produce anxiolytic effects (Wettstein 1988), and so would be unsuitable as a standard for detecting acute effects of anxiolytics.

Methods involving punished behaviour can be criticized since increases in punished responding could be due to indifference to pain and therefore the threat of pain rather than reduction in anxiety. However morphine does not produce consistent anxiolytic effects in these test despite its analgesic effects (Sepinwall and Cook 1978).

Tests involving food intake (Geller-Seifter, punished drinking and hyponeophagia) could be compromised if the test drugs have effects on appetite. Benzodiazepines (Cooper 1980) and most 5-HT modulating drugs (Blundell 1984) modulate appetite and this may confound the conclusions to be drawn from these models.

Tests involving operant responding may in turn be compromised by drug effects on responding being dependent

on the ongoing responding rate (Sepinwall and Cook 1978).

1.8 Involvement of 5-HT in anxiety

Benzodiazepines decrease the turnover of catecholamines and indoleamines in the brain (Stein et al 1977). The effects on catecholamines show rapid tolerance and have been suggested to correspond to the sedative effects while the effects on 5-HT persist and appear to correspond to the anxiolytic effects of these drugs (Stein et al 1977). The lack of tolerance to the decrease in 5-HT turnover was correlated to the lack of tolerance of the anxiolytic effects of the benzodiazepines (Goldberg et al 1967). This appears to suggest that 5-HT may modulate benzodiazepine anxiolytic effects and that reduction in brain 5-HT function might reduce anxiety and vice versa. However there is evidence that benzodiazepine modulation of 5-HT also shows tolerance. Acute but not chronic diazepam reduced L-tryptophan induced increases in plasma prolactin and growth hormone without itself altering baseline levels of the two hormones (Nutt and Cowen 1987).

Benzodiazepines reduce 5-HT synthesis and turnover (Koe 1979) and the release of 5-HT from serotonin neurones (Soubrie et al 1983). This inhibition of 5-HT release appears to be mediated by the raphe nuclei as it was prevented by superfusion of the dorsal raphe by the benzodiazepine antagonist Ro 15-1788 (Soubrie et al 1983).

Benzodiazepines reduce neuronal activity in the dorsal raphe of encephale isole rats and conscious cats but this effect only occurs at doses well above those needed to produce anxiolytic effects (Trulson et al 1982). This appears to suggest that the benzodiazepine inhibition of of raphe neurones is associated with their CNS depressant effects rather than their anxiolytic effects. This

conclusion is supported by the finding that unlike the benzodiazepines, CL 218872, a ligand of benzodiazepine binding sites which has anxiolytic but not muscle relaxant effects, does not inhibit 5-HT-mediated hypothermia (Lippa et al 1979b).

There is also other evidence suggesting that 5-HT mediates increases in impulsivity by benzodiazepines rather than their anxiolytic effects (Thiebot 1986). Benzodiazepines decreased the ability of rats to wait for a large but delayed food reward in a T-maze in preference to a small and readily available reward while 5-HT reuptake inhibitors had the reverse effect (Thiebot 1986).

1.8.1 Lesions of 5-HT pathways.

1.8.1.1 Use of a 5-HT depleter.

Para-chlorophenyalanine (PCPA) depletes brain 5-HT by inhibiting the enzyme tryptophan hydroxylase.

PCPA was anxiolytic on the Geller-Seifter model (Tye et al 1979, Geller and Blum 1970); punished drinking (Petersen and Lassen 1981); ultrasonic vocalisation (Gardner 1985; social interaction (File and Hyde 1977); and on the elevated X-maze (Critchley and Handley 1987). PCPA however produced an increase in potentiated startle (Davis et al 1986).

1.8.1.2 Neurotoxins.

The 5-HT neurotoxins 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) injected intra-ventricularly (i.c.v.) in the rat produce anxiolytic effects in the Geller-Seifter model (Tye et al 1977); in the punished drinking model (Lippa et al 1979); in the rat pup ultrasonic vocalisation model (Gardner 1985); and in the social interaction model when injected in the dorsal

raphe (File et al 1979) but not when injected in the amygdala (File and Deakin 1980). 5,7-DHT lesions produce an increase in potentiated startle (Davis et al 1986).

1.8.2 Stimulation of 5-HT neurones.
Electric stimulation of the median raphe nucleus produced behavioural inhibition characterised by crouching defaecation, piloerection and teeth chattering (Graeff and Silviera-Filho 1978); chemical stimulation of the dorsal raphe suppressed punished drinking and unpunished drinking in the Geller-Seifter model (Stein et al 1977); while 5-HT injected i.c.v. also produced behavioural depression (Gardner 1985), and depressed potentiated startle when administered into the forebrain and elevated startle when administered in the spinal cord (Davis et al 1986). Intraventricular 5-HT reduced the punishment releasing effect of oxazepam (Wise et al 1972).

These results are difficult to interpret and presumably depend on whether i.c.v. 5-HT acts on autoreceptors, pre- or post- synaptic receptors. Stimulation of ascending 5-HT pathways resulted in inhibition of ongoing behaviour rather than specific effects on anxiety (Gardner 1985).

1.8.3 Serotoninomimetics.
On the basis of the above these would be expected to be anxiogenic. The results obtained have not been consistent.

The 5-HT precursor 5-hydroxytryptamine (5-HTP) was anxiogenic in the Geller-Seifter model (Nagayama et al 1981), and in the potentiated startle test (Davis et al 1986) and had a biphasic effect: anxiolytic effects at low doses and anxiogenic at high doses in the X-maze (Soderpalm et al 1988) and in punished drinking (Hjorth et al 1987). In humans the compound was anxiolytic (Kahn and

Westenberg 1985).

The non-selective 5-HT agonist 5-MeODMT was anxiogenic in the elevated X-maze (Critchley and Handley 1986), in potentiated startle (Davis et al 1986); inactive in punished responding (Shephard et al 1982, Critchley 1988), and anxiolytic in the isolation-induced aggression test in mice (Olivier et al 1989).

Quipazine, a non-selective 5-HT agonist decreased both punished and unpunished responding (Commissaris and Rech 1982), while eltoprazine a mixed 5-HT1 agonist reduced isolation-induced aggression in mice (Olivier et al 1989). The 5-HT uptake inhibitor fluvoxamine reduced isolation -induced aggression in mice (Olivier et al 1989).

The 5-HT1A partial agonist 8-OH-DPAT has been reported to be inactive in the X-maze test (File et al 1987), in the four-plate test and CER (Dourish 1987), in punished drinking (Dourish 1987, Moser et al 1988) and in the Geller-Seifter test (Critchley 1988); or anxiogenic in the X-maze (Critchley and Handley 1986, 1987, Dourish 1987, Moser 1987),in the Vogel conflict test (Moser et al 1989), potentiated startle (Davis et al 1986) and periaqueductal gray stimulation (Jenck et al 1989); and anxiolytic in Vogel conflict (Engel et al 1984), in isolation-induced aggression test in mice (McMillen et al 1988, Olivier et al 1989) and in X-maze (Soderpalm et al 1988).

Buspirone has been reported to have no effect on anxiety in the X-maze test (Critchley and Handley 1987, Johnston and File 1988, Pellow et al 1987), in the four plate test (Krahling et al 1987, Dourish 1987) and in the CER tests (Dourish 1987), the social interaction test (File 1985), Vogel conflict (Gardner 1986, Moser et al 1988), marble

burying (Broekkamp and Jenck 1987): and anxiolytic in punished responding (Geller and Hartman 1982) and Vogel conflict tests (Dourish 1987, Moser et al 1988) light/dark two compartment test in mice (Costall et al 1988) and in rats (Carli et al 1989, Merlo Pich and Samanin 1986), potentiated startle (Kehne et al 1988), X-maze test (Soderpalm et al 1988) and in isolation-induced aggression test in mice (McMillen et al 1988, Olivier et al 1989); and anxiogenic (Dourish 1987, Moser 1987) in the X-maze test. Buspirone is anxiolytic in humans (Goldberg and Finnerty 1979, Taylor et al 1985).

Ipsapirone has been reported to be anxiolytic in the Vogel conflict test (Traber et al 1984, Boaventura et al 1987) X-maze test (Critchley and Handley 1987, Soderpalm et al 1988), four plate test (Boaventura et al 1987), staircase (Boaventura et al 1987), conditioned defensive burying (Fernandez-Guasti 1987), ultrasonic vocalisation (Mos and Olivier 1987) and in isolation-induced aggression test in mice (McMillen et al 1988, Olivier et al 1989); but inactive in X-maze (Johnston and File 1988, Chopin and Briley 1987), shock probe test (Meert 1985), potentiated startle (Davis et al 1988), four-plate and CER tests (Dourish 1987) and punished responding (Deacon and Gardner 1986).

Gepirone has been reported to be anxiolytic in the Vogel conflict, punished responding and the shock-induced aggression tests of anxiety (Dourish 1987), X-maze (Soderpalm et al 1988) and in the isolation-induced aggression in mice (McMillen et al 1988) and in humans (Cott et al 1988).

The 5-HT1B agonist RU 24969 was anxiogenic in the X-maze test (Critchley and Handley 1986) and inactive in punished

responding (Shephard et al 1982, Critchley 1988, Gardner 1986).

The 5-HT1B and 5-HT1C receptor agonist mCPP (1-(3-chlorophenyl) piperazine) has been reported to be anxiogenic in the X-maze test (Chopin and Briley 1987) in the social interaction test (Kennett et al 1989), and anxiolytic in potentiated startle (Davis et al 1986), isolation-induced aggression (McMillen et al 1988) and periaqueductal gray stimulation (Jenck et al 1989).

Clinical experience with serotoninomimetics is also ambiguous: the 5-HT1B/C agonist mCPP is anxiogenic (Murphy et al 1989); while neither the 5-HT releaser fenfluramine nor the agonists quipazine and MK-212 (1-(6-chloro-2-pyrazino) piperazine) elicit anxiety in humans (Gardner 1985).

On the other hand the serotoninomimetics zimeldine and clomipramine (Gardner 1985) and fluoxetine and 5-HTP (Liebowitz et al 1986) are used clinically to treat panic anxiety disorders. In addition to being inconsistent with the results on animal models these clinical applications suggest that panic attacks are responsive to enhancement of 5-HT pathways or more likely, since these agents are effective only on chronic usage, 5-HT receptor down regulation.

The inconsistent results with these compounds are difficult to explain. That the compounds have different selectivity for 5-HT receptor subtypes may account for some of the differences but even the selective 5-HT1A agonists 8-OH-DPAT, buspirone and ipsapirone all which reduce firing of 5-HT neurones in the dorsal raphe (Sprouse and Aghajanian 1985, 1987) produce different

results in animal models. That they are partial agonists at the 5-HT1A receptor (De Vivo and Maayani 1986) may be part of the explanation. Even more puzzling is why the same compound should exhibit different results in different models and even in the same model in different laboratories. This question is addressed in Chapter 10. Suffice it to say here that some of the factors involved include that different models may measure different components of anxiety, variation in basal 5-HT tone in the test animals may affect the actions of partial agonists and it is possible that dorsal and median raphe nuclei may have different functions with respect to anxiety (Carli and Samanin 1988, Chopin and Briley 1987, Costall et 1988, Traber and Glaser 1987).

1.8.4 5-HT antagonists.

These would be expected to produce anxiolytic effects by reducing the activity of endogenous 5-HT. The picture however is far from consistent. Some workers have found methysergide, cyproheptadine and cinanserin to increase punished responding while others have found them inactive (Gardner 1985). The situation with the newer and more specific antagonists is not much clearer.

The 5-HT2 antagonist ritanserin has been variously reported to be anxiolytic in the light/dark box (Colpaert et al 1985) and in the X-maze (Critchley and Handley 1987), or inactive in the X-maze (File et al 1987), in social interaction (Gardner 1986), in punished responding (Critchley 1988, Gardner 1986) and in Vogel conflict (Colpaert et al 1985). Other 5-HT2 receptor antagonists ketanserin and seganserin have been reported to be anxiolytic in the X-maze test (Critchley and Handley 1987) and inactive in punished responding (Critchley 1988). Two 5-HT2 antagonists, ritanserin (Arriaga et al 1984) and

pirenperone (Ansseau et al 1983) have been reported to show modest anxiolytic effects in humans.

MDL 73005EF, a 5-HT ligand with predominantly antagonist effects has been reported to be anxiolytic in X-maze and punished drinking (Moser et al 1988).

The 5-HT3 receptor antagonist, GR 38032F has been reported to be anxiolytic in the light/dark discrimination test (Costall et al 1987, Tyers et al 1987), the social interaction model (Costall et al 1987, Piper et al 1989), primate aggression model (Costall et al 1987, Piper et al 1989), passive avoidance test (Papp and Przegalinski 1989); and inactive in the X-maze (Piper et al 1988, Johnston and File 1988), and social interaction tests (Johnston and File 1988), Vogel conflict (Jones et al 1987, Piper et al 1989), ultrasonic vocalisation (Molewijk et al 1987), marble burying (Broekkamp and Jenck 1987), four plate test (Molewijk et al 1987) and light/dark discrimination test (Molewijk et al 1987, Morinan 1989).

Other 5-HT3 antagonists, MDL 72222 and ICS 205-930, have also been shown to be anxiolytic in the light/dark discrimination, social interaction and primate aggression tests (Tyers et al 1987).

1.9 ß-adrenergic drugs in anxiety.

Propranolol and other ß-adrenoceptor antagonists are effective in treating anxiety in humans especially where it is characterised by a major somatic component (Granville-Grossman and Turner 1966, Kelly 1980, Noyes 1985, Peet 1984). Stressful conditions like public speaking, performing surgery and pre-examination stress are particularly responsive to ß-adrenoceptor antagonists (Kelly 1980). The effects are peripherally mediated and

are unlikely to have central component since practolol which has very poor penetration into the brain is effective (Bonn et al 1972). Metoprolol, a selective ß-1 antagonist (Ablad et al 1975) is also effective in anxiety treatment (Chaturvedi 1985). These effects are probably unrelated to 5-HT receptor blockade since both practolol and metoprolol have very low affinity for 5-HT receptors (Middlemiss et al 1977).

In animal models, ß-adrenoceptor antagonists produce inconsistent effects on anxiety, being generally inactive or only weakly anxiolytic (Durel et al 1986, Sepinwall and Cook 1978).

Adrenaline and other ß-adrenoceptor agonists can produce experimental anxiety in humans, an effect that has been ascribed to their peripheral effects (Pitts and Allen 1982, Gorman et al 1987).

Whether the effects of ß-adrenoceptor agonists and antagonists on anxiety involve interaction with 5-HT receptors or are mediated solely by ß-adrenoceptors is not documented.

1.10 Choice of animal models

A ideal animal model should be able to detect anxiolytic agents without false positives or false negatives. To be useful in studying mechanisms of anxiety it should also be able to detect anxiogenic agents. The results should be consistently reproducible and not be overly sensitive to minor changes in testing conditions. Ease of testing and lack of need to train animals would be a bonus.

The X-maze model and the social interaction tests were chosen for this project because neither requires that

animals undergo training, and both were suitable for studying 5-HT ligands since neither uses the consummatory drive nor painful stimulus both of which are modulated by 5-HT (Blundell 1984, Dourish et al 1986b, Evans 1961, Roberts 1984) and also because at the time this project started there was not much published work involving their use with 5-HT ligands. The Geller-Seifter test has previously been considered de rigour in testing for anxiety. However in this laboratory (Critchley 1988) the test was was found to be insensitive to the effects of the 5-HT ligands, 8-OH-DPAT, ritanserin and seganserin.

The object burying test has been proposed as a model for obsessive compulsive disorders (Broekkamp and Jenck 1987). Burying of innocuous objects like glass marbles has not been validated as an anxiety model and this test was adopted in this work and an attempt to assess its applicability as an anxiety model was made.

Before a model could be used it was necessary to establish that it was sensitive enough, in the conditions of prevailing in our laboratory, to detect the effects of a standard anxiolytic and an anxiogenic compound. Diazepam was chosen as the standard anxiolytic and the ß-carboline, ß-CCE as the prototype anxiogenic compound. Diazepam is in widespread clinical use as an anxiolytic while ß-CCE has been reported to be anxiogenic in primates (Hommer et al 1987) and in rodents (Pellow and File 1984).

1.11 Aims of the project.

(1) To set up several animal models of anxiety in the laboratory and then employ them to study the involvement of 5-HT in anxiety by establishing the effects of agonists and antagonists varying in selectivity for 5-HT receptor subtypes.

(2) To study the interaction of central ß-adrenergic and 5-HT systems in animal models of anxiety.

CHAPTER 2

EXPERIMENTAL METHODS.

2.1 Animals and animal husbandry.

The experiments reported in this work used either male and female MF1 mice or male hooded PVG rats. The mice were bred in the animal house unit at Aston University from a stock originally obtained from Bantin and Kingman Ltd, Hull. The rats were supplied by Bantin and Kingman Ltd with the exception of one batch used for the social interaction test with GR 38032F which was obtained from Glaxo Ltd., Ware. All animals were kept in the animal housing unit at an ambient temperature of 22 ± 2 degrees C. and under a 12 hour light/dark cycle (light 0800 - 2000 hours). They were maintained on a conventional 41B cube diet from Pilsbury Ltd, Birmingham with tap water ad libitum.

2.2 Experimental conditions.

Rats for use in the X-maze test were housed in groups of six in a quiet room for a least one week before use. Rats used for the social interaction test were housed in pairs for a least a week prior to experiments. Rats used in the lesion experiments were housed singly for a week to allow recovery from surgery. Mice were housed in groups of ten in the experimental room for a least a week before use. All experiments were, unless otherwise stated, performed between 1000 and 1800 hours in rooms either in their housing rooms or in adjacent rooms at the same temperature as they were housed in.

2.3. Drug administration techniques.

Drugs were administered in rats either intraperitoneally (i.p.) or orally. Intraperitoneal injections were performed by inserting the needle into the abdominal wall towards the diaphragm. Care was taken not to penetrate too deeply so as not to damage the internal organs. Where more than one

injections was made on the same animal care was taken to avoid using the same injection site. The injection volume was 1 ml/kg except where otherwise stated. The injection vehicle was normal saline (0.9 NaCl mg/kg) unless otherwise stated. Control animals received the injection vehicle.

In addition to i.p. administration, ritanserin was also administered orally. Oral administration was performed in either of two ways. Acute oral administration was by gavage using stomach tubes in rats fasted overnight. Chronic administration was performed by suspending 100 mg ritanserin in a litre of hot 10mM tartaric acid in saline and adding a few drops Tween 80. The pH of this suspension was raised to 5.5 with dilute sodium hydroxide. The suspension was changed every day. Since oral ritanserin even a high dose (10 mg/kg) did not produce anxiolytic effects in the X-maze model (see Chapter 4), for the chronic study the experimental animals were treated intraperitoneally with an anxiolytic dose of the drug for 3 days while they adapted to drinking ritanserin suspension (or vehicle) instead of drinking water.

Mice were either injected intraperitoneally or subcutaneously (s.c.). Intraperitoneal injections were performed as in the rat. Subcutaneous injections were made into the loose skin on the back of the neck. The injection volume was 10 ml/kg.

2.4. Behavioural assessment.

2.4.1 Elevated X-maze.

The apparatus was that developed in this laboratory (Handley & Mithani 1984) from Montgomery (1955). It consists of an X-shaped wooden maze with two open and closed arms each 35 cm long and 10 cm wide with the closed arms having 10 high walls without a ceiling and raised 70

cm from the floor. There was a wire grid on the floor surface to provide a better grip. Male PVG rats (180-280g) were housed in groups of six and handled by the experimenter for 3 days prior to testing. On the test day they were injected intraperitoneally with the test drugs and 30 mins later placed an enclosed arm of the elevated X-maze. The test was performed at a light intensity of 125 lux. In one case while testing the effects of differing light intensity, 440 lux was used. The number of entries in open and enclosed arms of the maze was recorded for 10 min by an observer watching via a closed circuit TV system (Ferguson Camcorder and Amstrad Televideo), sitting either at least 1.5 metres away or in different room.

2.4.2 Social interaction.

The method was essentially that described by File and Hyde (1978) and modified by Gardner and Guy (1984) in order to detect the acute effects of the benzodiazepines. Male PVG rats (170-190g) were housed in pairs for at least 5 days. On two consecutive days before testing, each pair was familiarized with the apparatus for 5 minutes. On the test day 2 rats from different and not adjacent cages and of bodyweight within 10g of each other were injected intraperitoneally with the same drug and 20 min later (10 min for 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT) placed into a wooden box (58 x 58 x 46 cm) with an open roof in low lighting conditions for 5 minutes. Each rat met its test partner only in test conditions. The following measures were recorded by 2 observers via a television monitor: duration of social interaction, number of rears, and number of walks greater than 1 body length of continuous ambulation. The box was cleaned between tests with a cloth soaked in a dilute disinfectant to remove the odour of the previous animals. The rats were then returned to their original pairs and reused once after a one week

interval with an unfamiliar partner. Both observers scored independently and mean readings were used. Alternatively when only a single observer was available, the videotapes of the experiment were scored a few days later by the same observer without reference to the previous results. In either case where inter-observer variations were greater than 10% the results were discarded. Increase in the duration of social interaction indicates a reduction in anxiety while a reduction indicates increase in anxiety. The experiments were performed at a light intensity of 125 lux. In addition to these conditions GR 38032F was also tested on rats that had not been familiarised with the test arena and at a high light intensity (250 lux). In another experiment with GR 38032F, sniffing the hindquarters was excluded from the duration of social interaction. Because of the large numbers of animals needed per dose (the test uses a pair of rats for each data point), the test was used only with doses of drugs that had been found effective in the X-maze test [Chapter 4 and Critchley and Handley (1986, 1987)].

2.4.3 Marble burying.

The method was adapted from Broekkamp et al (1986). Female MF1 mice (23-35g) were placed individually in polypropylene cages (42 x 24 x 12 cm) containing 20 glass marbles of diameter 1.5 cm evenly spaced on 5 cm deep sawdust without food or water. The ceiling was the metal grid cage cover placed upside down to give the maximum clearance from the sawdust surface. The number of marbles at least two thirds buried was counted 30 min later.

Swimming-induced grooming was used by Broekkamp et al (1986) as a measure of sedation but this was considered inappropriate since the behaviour is affected by some of the agents used e.g DOI (Chapter 4).

Motor activity was assessed separately by 5 min exposure to a circular runway placed on an Animex activity recorder.

2.4.4 Measurement of locomotor activity.
Locomotor activity was measured with an Animex activity meter type SE (LKB Farad, Sweden). This consists of 6 inductance coils forming part of a resonant circuit. Movement over any of the coils results in a change in current within the circuits which is then amplified and registered.

Two concentric cylinders were placed on top to create a circular runway passing over 4 of these coils. The inner cylinder was solid and the outer was wire meshed lined with polyfilm to stop mice from climbing out.

The apparatus was placed in a quiet room. The activity meter was tuned to 40 microamperes and the sensitivity was set at 25 microamperes. This sensitivity had previously been shown to detect locomotion, but not small movements such as tremor and grooming (Thomas 1975).

2.4.5 Gross behavioural assessment of DOI.
This was done in mice using a modified form of the scoring scheme described by Irwin (1968). Three mice were placed in the cage for 10 minutes to allow for initial exploration of the novel environment. One mouse then received saline s.c. and the other two varying doses of DOI.

Behavioural ratings were made over 10 minute periods, beginning 5 minutes after injection and subsequently every 20 minutes until there was no difference between the mice. The method of scoring was standardised by using a 0 - 6

scale, a score of 0 being allocated for absence of effect, 6 for maximal effects, 2 or 4 for progressively increasing effect and odd numbers where the degree of effect could not be allocated otherwise. Each mouse was compared to the mean of the control mice. The ratings were made using a standardised procedure, observations on unrestrained mice being rated first, followed by testing which involved approaching the mice and observations which required some degree of handling, as follows:

2.4.5.1 Observations on unrestricted animals.
The following were scored by degree when present: straub tail, exophthalamos/ptosis, head twitches, convulsions, writhing, tremor, abnormal gait, limb splay, piloerection, lachrymation, salivation, vasodilatation or cyanosis, vocalisation and diarrhoea. Increases in total motor activity, respiration rate and depth, grooming and stereotyped activity, the nature of which was also noted, and the intensity and incidence of abnormal posturing were also scored.

2.4.5.2 Observations which required minimal handling.
The following were scored as increases or decreases over controls:

fearfulness - the freezing or withdrawal on approach of a pen towards the mouse's head.

alertness - assessed simultaneously as amount of interest.

startle response - response to a puff of air directed at the mouse's head

touch response - escape response to application of pressure to the flanks with the finger and thumb.

tail pinch - reaction to application of pressure to a point 1 cm from the base of the forceps.

flexor/reflexor - limb withdrawal on pinching the toe of the hind limb with forceps.

pinna reflex - degree of twitch response to stimulation of either external auditory meatus with a fine wire

2.4.5.3. Observations which required removal of the mice from the box.

The degree of pupil dilation and hypothermia were assessed when holding the animal in a scruff grip. The following were holding as increases or decreases over controls:

passivity - point at which the mouse struggled to escape when held first by the scruff, then the hind limb, then the fore limb.

grip strength - ability to hang from a wire grid

body position - the height of the ventral abdomen above a raised bar.

The animals were also assessed for catalepsy by placing the forepaws on a 4 cm high bar; and for loss of righting reflex when flipped over to land in the box from a height of 20 cm.

2.4.6 Studies on pinna reflex.

A fine wire was placed in each ear in the test mice 20, 40 and 60 min after drug administration. The pinna reflex was deemed to be different if this action was no followed by a rapid flick of the head. For this test female mice were housed in groups of 3 and were unrestrained.

2.5 Stereotaxic Lesioning.

Lesions of the dorsal raphe nucleus were performed on male PVG rats weights 290 +/- 20g. Rats were injected with desipramine 10 mg/kg and atropine 1 mg/kg intraperitoneally and 40 minutes later anaesthetised with pentobarbitone sodium 40 mg/kg followed by diazepam 5 mg/kg and then 0.25 lignocaine 0.1% injected subcutaneously into the operation site. The rats were positioned in the stereotaxic frame such that the upper incisor bar was 5.0 mm above the interaural line according to Pellegrino et al (1979) and the skull exposed. A hole was drilled in the skull and a 30 gauge cannula lowered to the following co-ordinates:-

-5.7mm from the bregma
0.0mm lateral from the midline
6.0mm below the dura.

The above co-ordinates correspond (according to the rat brain atlas of Pellegrino et al 1979) to the point prior to differentiation of the dorsal raphe nucleus into two distinct bodies. Lesions were performed by injecting 4 micrograms of 5,7-dihydroxytryptamine (5,7-DHT) in 1.0 microlitres ascorbic acid (0.2%) in saline over a period of 1 minute. The injection cannula was left in place for a further minute to allow diffusion from the tip. The skin was then sutured and the animal allowed to recover.

Prior to the lesions being performed, indian ink was injected at the above co-ordinates and the brains subsequently dissected and the relevant histology performed to determine the exact location of the dye. Once the histological sections indicated the dye to be in the correct place then the lesions were performed on the test animals. Control animals receive sham-lesions, undergoing the same procedure with vehicle injection. After surgery the animals were housed singly until the wound healed and were allowed at least 14 days recovery before being used for further behavioural experiments. Behavioural

experiments were performed two to four weeks after the lesions.

2.6 Brain Dissection.

Brain dissections were performed by the method of Glowinski and Iversen (1966). Rats were killed by decapitation, their brains carefully removed and placed on an ice-cooled glass slide. Seven regions were separated out. The striatum and hippocampus were used in the lesion studies.

The rhombencephalon was first separated from the rest of the brain. This region was further divided by a transverse section in the cerebellum and the pons/medulla region. A transverse section was then made at the level of the optic chiasma, which separated the cerebrum into two parts, B (parietal and C (frontal). The hypothalamus was then dissected out of part B by taking the anterior commissure as a horizontal reference and the line between the posterior hypothalamus and mammillary bodies as the caudal limit. The striatum was then dissected out with the external walls of the lateral ventricles as the internal limit and the corpus collosum as the external limit. The frontal parts of the striatum were also dissected out from part C. The midbrain was gently separated from the remaining part of the brain and the hippocampus was carefully removed. The remainder of parts B and C were then combined to form the cortex.

2.7 Biochemical Studies.

Biochemical estimation of 5-hydroxytryptamine was carried out by the method of Curzon and Green (1970) as modified by Gould (1979). Each brain was homogenised (Citenco Ltd.) in 3ml of ice-cold acidified n-butanol. The homogenate was then shaken for 5 minutes on an automatic shaker (Griffin Ltd.) allowed to stand in a freezer for 5 minutes to

maintain the cold temperature and then centrifuged at 2,500 rpm (1000g) for 5 minutes in a bench centrifuge (MSE Ltd.).

2.5 ml of the supernatant from the acidified n-butanol homogenate was added to 5ml n-heptane and 0.4ml cysteine hydrochloride (2% w/v) in 0.1M hydrochloric acid. This mixture was shaken and centrifuged as above. The supernatant organic layer was aspirated off together with any tissue disc that might have formed and the aqueous layer was used for the assay.

0.2 ml of the aqueous phase was added to 0.60 ml of o-phthalaldehyde solution (OPT) and 0.05 ml 0.2% w/v cysteine hydrochloride solution. The tube contents were shaken and heated in a water bath at 80 degrees C for 20 minutes. The solutions were then removed, allowed to cool and the fluorescence was read on an Aminco Bowman Spectophotofluorimeter at excitation and emission wavelengths of 360 and 470 nm respectively. The excitation and emission slits were 3.0 mm.

The amount of 5-HT were determined by running known standards and reagent blanks through the assay. A stock solution of 5-HT creatinine sulphate complex (1 mg/ml) in 0.2% cysteine/HCl was used and aliquots frozen. Standard solutions for the assay were prepared using serial dilutions from these aliquots. Before actual assays were performed the extraction values were monitored to determine recovery levels and the linearity of the assay was checked with standards. Actual recovery values were expressed as a percentage assuming partition coefficients between aqueous and organic layers to be 100%.

The recovery value found was 86% +/- 4 N=6
Amounts or 5-HT in the various brain samples were expressed as ng/g of wet brain tissue.

2.8 List of drugs used.

DRUG	SOURCE
Adrenaline	BDH
BAY R 1531 6-methoxy-4-(di-n-propylamino) -1,3,4,5-tetrahydrobenz(c,d)indole	Troponwerke
Buspirone	Bristol Myers
BRL 24924 (+)-(endo)-4-amino-5-chloro-2-meth-oxy-N(1-azabicyclo(3,3,1) non-4-ylbenzamide	Beecham
Carbidopa	MSD Ltd.
Citalopram	Duphar
Clenbuterol	Dr. Karl Thomas Gmbh.
Cyproheptadine	MSD Ltd.
Desipramine	Geigy
Diazepam (Valium inj)	Roche Products
DOI (1-(2,5-Dimethoxy-4-iodophenyl) -2-aminopropane)	Research Biochem.
Dobutamine	Eli Lilly & Co.
5,7-dihydroxytryptamine	Sigma Ltd.
Ethyl-beta-carboline-3-carboxylate (beta-CCE)	Roche Products
Fenfluramine	Sigma
Fluvoxamine	Duphar
Gepirone	Bristol Myers

GR 38032F (1,2,3,9-tetrahydro-9-methyl-3[(C2-methyl-1H-imidazol-1-yl) methyl]-4H-carbazol-4-one.	Glaxo
8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT).	Research Biochem.
5-methoxy-3(tetrahydro-pyridin-4-yl)1H-indole (RU 24969)	Roussel Uclaf
5-methoxy-N,N-dimethyl-tryptamine (5MeODMT)	Sigma Ltd.
ICI 118,551 erythro-DL-1-(7-methylindan-4-yloxy) -3-isopropylamino-2-butan-2-ol.	ICI Ltd
ICI 169,369 2-(2-dimethylaminoethylthio)-3-phenylquinoline	ICI Ltd
ICS 205-930 (3-alpha-tropanyl)-1H-indole-3-carboxylic acid ester	Sandoz Ltd
Idazoxan	Reckitt & Colman
Indalpine	Roussel Uclaf
Ipsapirone	Troponwerke
Ketanserin	Janssen

Metoprolol	Geigy
Methysergide	Sandoz Ltd.
mCPP (1-(3-chlorophenyl)piperazine	Research Biochem.
MDL 72222 (tropan-3-yl)3,5-dichlorobenzoate	Merrell Dow
Pentobarbitone sodium	May & Baker
Pindolol	Sigma Ltd.
Practolol	ICI Ltd.
Propranolol	ICI Ltd.
DL-p-chlorophenylanine methyl ester (pCPA)	Sigma Ltd.
5-Hydroxytryptamine (5-HTP)	Sigma Ltd.
Ritanserin	Janssen
Sotalol	Bristol-Myers
Terbutaline	Astra
TFMPP 1-(3-trifluoromethylphenyl) piperazine	Research Biochem.
Timolol	MSD Ltd.
Xylazine	Sigma
Yohimbine	Sigma
Zimeldine	Astra

The drugs were dissolved in normal saline (0.9 % sodium chloride with the following exceptions.
Diazepam - the commercial product (Valium) was diluted in saline immediately before use.
Cyproheptadine - dissolved in saline by adding a minimum amount of 1N HCl.
Beta-CCE - dissolved in saline by adding a minimum amount of 1N HCl.
Ketanserin - dissolved in saline by adding a minimum amount of 1N HCl.
Ritanserin - suspended in saline by adding 2 drops of Tween 80.

2.9 List of reagents.

n-butanol (analar)	BDH
cysteine hydrochloride	Sigma
Decon-90	Fisons
ethanol (absolute)	BDH
n-heptane (analar)	BDH
hydrochloric acid (analar)(HCl)	BDH
5-hydroxytryptamine sulphate creatine complex	Sigma
nitric acid (analar)	BDH
o-phthalaldehyde (OPT)	Sigma
tartaric acid	Sigma

(i) Acidified butanol - butanol containing 0.85 ml concentrated HCl per litre.
(ii) Cysteine hydrochloride - dissolved at 0.2 % and 2 % in 1N HCl. Frozen or freshly prepared.
(iii) o-phthalaldehyde reagent (OPT) - o-phthalaldehyde (4% w/v) dissolved in concentrated hydrochloric acid.
(iv) 5-hydroxytryptamine sulphate creatinine complex in 0.2 % cysteine in HCl at 1 mg/ml base equivalent.

2.10 Statistical methods

The raw data was analysed using the Student t-test relative to concurrently run vehicle controls or in those tests involving drug interactions, by a two-way Analysis of variance (ANOVA) followed by Tukeys test for unconfounded means.

CHAPTER 3

ANALYSIS OF RAT BEHAVIOUR IN THE ELEVATED X-MAZE MODEL OF ANXIETY.

INTRODUCTION

Since the elevated X-maze was suggested as a model of anxiety (Handley and Mithani 1984a) it has become widely adopted as a test model in many laboratories (Pellow et al 1985, Dourish 1987, Costall et al 1989, Moser 1987). The method has been well validated and shown to be a satisfactory model of anxiety (Pellow et al 1985).

However, results obtained from different laboratories on this model have differed with respect to certain drugs. For instance, one group found ritanserin and ipsapirone to be anxiolytic (Critchley and Handley 1986) while another (Johnston et al 1987) found both compounds inactive and yet another found ipsapirone to be anxiogenic (Moser 1987).

This project sought to conduct an analysis of the behaviour of the rat when on the X-maze and from this to establish what factors are critical for optimal use of this model. The effect of factors like lighting conditions on X-maze behaviour was also investigated. The only previous examination of the effects of light in this model had found that whether the light intensity in the open arms was equal to or higher than that in the enclosed arms did not affect the behaviour of rats in the X-maze (File 1985). There has been no previous study on the effect of changing the light intensity in the whole testing environment in this model. However it is known from other models of anxiety that light intensity has a strong bearing on anxiety: mice find a brightly lit compartment aversive (Costall et al 1988) and the duration of social interaction in rats is reduced in brightly lit testing conditions (File 1980). The hypothesis was that differing lighting conditions in the test environment could explain

some of the differences observed in different laboratories.

As used in different laboratories there are differences in maze design: for example the plus maze used by the File group has enclosed arms 30 cm high compared with 10 cm for the X-maze used in our laboratories. This project also attempted to establish the effects such design differences may have on behaviour of animals in the X-maze.

The model was first validated by establishing that it was possible to detect anxiolytic effects of diazepam and the anxiogenic effects of beta-CCE.

The effect of behaviour of rats in the X-maze was also evaluated after an anxiogenic dose of RU 24969 and an anxiolytic dose of ipsapirone (see Chapter 4). These drugs were chosen for the behavioural analysis, together with 8-OH-DPAT in the experiment involving different orientation of the X-maze because different results while using them have been obtained in different laboratories. Johnston et al (1988) found all three compounds to be without effect in the elevated X-maze while Critchley and Handley (1986, 1987) found 8-OH-DPAT and RU 24969 anxiogenic and ipsapirone anxiolytic in the X-maze. It was hoped the results obtained in the behavioural analysis could explain these differences.

RESULTS.

3.1 Behaviour of rats in X-maze after vehicle treatment.

Rats were always placed in the X-maze facing the same enclosed arm. Most of the rats spent a very short time looking around before entering that arm. However a small minority - the number varied from batch to batch - would hesitate before entering the arm they were facing, turn around and enter either the other enclosed arm or one of the open arms. A rat was considered to have entered an arm if all four limbs entered the arm.

3.1.1 Behaviour in enclosed arms.

In the enclosed arms rats engage in most of the components of their normal behavioural repertoire. The bulk of the time was divided almost equally between home cage activity (such as standing or lying still and grooming) and exploratory activity (ambulation, rearing and sniffing) (Table 3.1 and Fig 3.1). On reaching the end of the enclosed arm the rats paused and peered into the central square and the open arm before entering the central square. There was a considerable amount of rearing and peering over the walls of the enclosed arms. Control rats spent 73 % of the time in the enclosed arms.

3.1.2 Behaviour in central square.

Over half the time in the central square was spent peering into both open and enclosed arms before making a choice of arm to enter (Table 3.1 and Fig 3.1). Peering activity was defined as turning the head in the direction of an arm and included partial entry into an arm with the head, forelimbs and part of the torso but excluding entry with all four limbs. Most of the peers into the enclosed arms, but only a small fraction of those into the open arms, were followed by entry into the arm. The rest of the time was spent frozen in indecision (Table 3.1 and Fig 3.1).

Control rats spent about 14 % of the time in the central square.

3.1.3 Behaviour in open arms.

Almost all the time in the open arm was spent in exploratory activity (Table 3.1 and Fig 3.1). Most of this activity comprised of walking and sniffing. There was very little rearing (Table 3.1 and Fig 3.2). When in the open arm they were very sensitive to sudden changes in the sound level such as knocks on the walls and reacted by rushing into an enclosed arm. For this reason it was necessary to work in very quiet conditions. There was a tendency for the rats to come from an open arm straight to an enclosed arm without a pause in between. There was very little grooming and other home cage activity (Table 3.1 and Fig 3.1). Control rats spent 13 % of the time in the open arm.

Most of the rearing and grooming activity occurred in the enclosed arms, some in the central square and much less in the open arms (Table 3.1 and Fig 3.2). Most locomotion (assessed in units of half arms) occurred in the enclosed arms (Table 3.1).

3.2. Effect of standard anxiolytic and anxiogenic drugs.

3.2.1 Effect of diazepam.

Diazepam 1 and 2 mg/kg significantly increased the open/total arm entry ratio without affecting the total number of arm entries (Table 3.2 and Fig 3.3).

3.2.2 Effect of beta-CCE.

Beta-CCE at 1 and 2 mg/kg significantly reduced the open/total arm entry ratio without changing the total number of arm entries. (Table 3.2 and Fig 3.4).

3.3 Effect of different heights of enclosed arms.
The ratio of open arm entries to total entries was significantly increased when the rats were tested in the X-maze with enclosed arms 30 cm high as compared to the 10 cm high walls. There was a slight increase in the number of total arm entries as well as a slight increase in the number of open arm entries (Table 3.3).

There was a significant increase in the time spent in the central square by rats in the X-maze with 30 cm high walls. The duration spent in the open arms was increased though not significantly while the duration in the enclosed arms was correspondingly decreased (Table 3.4).

3.4 Effects of different orientation of the enclosed arms.
There was no significant difference in the open/total arm entry ratio or in the total number of arm entries or in the time spent in either the open or enclosed arms whether similar arms were opposite each other or at right angles to each other (Table 3.5).

However, when 8-OH-DPAT treated rats were tested instead of controls there was a distinct difference between the two configurations. With enclosed arms at right angles to each other the number of total entries were significantly increased but the compounds anxiogenic effects (Chapter 4) were not detectable although there was a slight decrease in the open/total arm entry ratio (Table 3.5 and Fig 3.5). When the enclosed arms were opposite each other there was no significant change in total arm entries but the open total arm entry ratio was significantly reduced (Table 3.5 and Fig 3.5).

3.5 Defaecation in the X-maze: the effects of putative anxiolytics.

Diazepam at 2.0 mg/kg, DOI at 0.1 mg/kg and ritanserin at 1.0 mg/kg, all at anxiolytic doses, had no effects on the number of faecal boli produced by rats in 10 minutes in the X-maze (Table 3.6 and Fig 3.6).

3.6 Effects of RU 24969 on behavioural changes in the X-maze.

Compared with vehicle controls, RU 24969 at 1 mg/kg produced a significant change in the behaviour of rats in the elevated X-maze. Drug-treated rats tended to restrict most of their movements to the enclosed arms and made very few entries into the open arms. They were also reluctant to venture out of an enclosed arm once they were in it. They would walk to the open end of the enclosed arm, peer into the central square and turn back into the enclosed arm without exiting. There was a significant decrease in the number of entries into the open and enclosed arms and the number of total entries. (Table 3.7).

There was a significant reduction in the time spent in the open arms and in the central square and an increase in the time spent in the enclosed arms. The amount of locomotion (in units of half arms) in the open arms was substantially reduced while the locomotion in the enclosed arms was significantly increased. There was no change in overall locomotor activity (Table 3.7).

There were no rears in the open arm and in the central square and the number in the open arms was sharply reduced. The number of grooming bouts was sharply reduced and the few that did occur were all in the enclosed arm. The amount of peering was sharply reduced (Table 3.7).

Relative to vehicle treated animals there was a

significant increase in the time spent in home cage behaviour in the enclosed arms and significant decreases in the time spent in home cage behaviour in the open arms and in exploratory activity in both the open and enclosed arms (Table 3.7 and Figs. 3.7 and 3.8).

3.7 Effect of ipsapirone on rat behaviour in the X-maze.
Relative to vehicle controls, Ipsapirone at 1 mg/kg produced a significant increase in the number of open arm entries but did not affect the number of enclosed arm entries or total entries (Table 3.8).

There was an increase in the time spent in the open arms (about 50 %) accompanied by a slight increase in the time spent in the central square and a decrease in the time spent in the enclosed arms. There was a significant increase in the amount of locomotion in the open arms (almost 200 %) and a non-significant increase in amount of total locomotion (Table 3.8).

There was no significant change in the number of rears, grooming bouts or peerings into the arms (Table 3.8). Relative to saline treated animals there was no change in the time spent in home cage behaviour or in exploratory behaviour (Table 3.8 and Figs. 3.7 and 3.8).

3.8 Effects of altering lighting.
X-maze behaviour was also tested in two different lighting conditions: the normal laboratory environment (125 lux); and with additional lighting (440 lux). There was no difference in either the open/total entry ratio or in the total number arm entries (Table 3.9).

3.9 Comments on variations in baseline levels.
There was substantial variation in baseline exploratory

activity of rats from different batches of rats. For most rats, between 25-35% of total arm entries were into open arms. However, some batches of rats entered the open arms as frequently as they entered the enclosed arms while yet others hardly entered the open arms. It was not possible to isolate the factors responsible for this. Generally such animals, where the open/total arm ratio was lower than 20% or higher than 40%, were unresponsive to both anxiolytic or anxiogenic drugs. A high level of extraneous sounds tended to make animals unresponsive to drugs. There were however groups of animals whose unresponsiveness to drugs could not be attributed to such factors. Whenever drugs that consistently gave anxiolytic or anxiogenic effects were undetectable with a particular group of rats, results obtained with that group of animals were discarded. There was a phase when the anxiogenic effects of 8-OH-DPAT were difficult to detect and the dose necessary to produce anxiogenic effects had to be increased (Chapter 4).

DISCUSSION.

Two different measures of anxiety are generally used in the X-maze model of anxiety: the open/total arm entry ratio (Handley and Mithani 1984a, Critchley and Handley 1987) and the comparison of time spent in the open and enclosed arms (Pellow et al 1985).

From the results in Tables 3.1 and 3.7 the time spent in the respective arms is a merely a reflection of the number of arm entries and either measure would be an adequate indicator of the animals behaviour in the X-maze. However, the time measure fails to consider that the activity in the enclosed arms and in the open arms is quite different: in the open arms there is very little home cage behaviour and the rats spent virtually all the time exploring the arm, whereas in the enclosed arms the time is evenly divided between both types of behaviour; and also that part of the time in the X-maze is spent in the central square rather than in the arms. In addition, the time measure alone does not record stimulant or sedative effects of the test drugs. Therefore in this work the ratio of open to total arm entries was used as the indicator of anxiety. Anxiolytic agents increase the ratio and anxiogenic drugs have the opposite effect. The number of total entries served to indicate if the test drug had sedative or motor stimulant effects. In particular, decrease of total entries could either indicate high anxiety or gross sedative effects in which case the open/total arm entry ratio would be a misleading measure of anxiety.

It is noteworthy that there was 20 times as much as rearing in the enclosed arms as in the open arms. This is not merely a reflection of the rats spending more time in

the enclosed arms as the difference in the time spent in the enclosed arms vis-a-vis the open arms was only seven fold. The higher rearing rate could indicate rats feeling more secure in the enclosed arms. This pattern is similar to that seen in the light/dark discrimination test where the number of rears in the dark compartment is out of proportion to the time spent there (Costall et al 1988).

In most cases, after coming from an enclosed arm, vehicle-treated rats paused in the central square and spent some considerable time peering into the other three arms before entering into the other arms. On coming from the open arms, the tendency was for the rats to walk straight into an enclosed arm without a pause in between. This indicates that rats find the open arms aversive and consider the enclosed arms a safe haven.

Diazepam at 1 and 2 mg/kg increased the open/total arm entry ratio without affecting the total number of arm entries. This by the above definition constitutes anxiolytic activity. Beta-CCE at 1 and 2 mg/kg reduced the open/total arm entry ratio without changing the total number of arm entries. This indicates anxiogenic activity. This shows that in the conditions prevailing in our laboratory standard anxiolytic and anxiogenic drugs are detectable in this anxiety model.

The orientation of the arms of the X-maze does not appear to be of much importance in so far as control animals are concerned. Whether the open arms are perpendicular to or opposite each other does not affect the entry ratio in control animals. The results with 8-OH-DPAT however indicate that arm orientation does affect sensitivity to drugs, with similar arms opposite each other being the more sensitive configuration.

The height of the enclosed arms however is important, when very high, the enclosed arms themselves acquire greater aversive properties. This is probably because the rats can no longer peer over the top to see the surrounding from the enclosed arm, and they find it claustrophobic.

The failure of anxiolytic agents to modify defaecation shows that at least on this model bowel movements are not associated with the animals' anxiety state. This is in contradiction to the findings of Meert (1985) who found anxiolytics reduced defaecation in the open field and proposed reduction in defaecation as a model of anxiety. However the animals used by Meert (1985) had been pre-selected for high defaecation rates.

The reduction in open entries seen with RU 24969 was accompanied by a reduction in the open/total arm entry ratio (Chapter 4) which was consistent with its anxiogenic effects (Chapter 4, Critchley and Handley 1987). The changes in time in the open and enclosed arm are again consistent with the reduced open/total arm entry ratio of an anxiogenic drug. The reduction in the time spent in the central square is striking. After the first few arm entries the rats stopped staying in the central square "dithering" about what arm to enter and entered the enclosed arms just as a matter of course. The reduction in time spent in the open arms was consistent with the reduced entries into those arms.

The increase in locomotion in the enclosed arms belies the decrease in the enclosed arm entries. This is because rats would walk to the end of the enclosed arm, peer into the central square and turn back into the enclosed arm without exiting. Normally, reduction in enclosed arm entries

indicates sedative effects but in this case there was no reduction in locomotor activity.

The reduction in the number of rears is unlikely to be due to effects on anxiety as the flat body posture which would be accompanied by reduced rearing was evident in most of the rats injected with RU 24969.

The reduction in number of peers into the open arms was because after the first few entries the rats ran from one enclosed arm to the other without pausing in the central square.

The baseline exploratory activity of the test animals is an important indicator as to whether anxiolytic and anxiogenic compounds were detectable. The influence of baseline activity on the sensitivity of an anxiety model has also been reported in the light/dark discrimination test. It was found that the baseline exploratory activity predicted the anxiolytic responsiveness to diazepam in mice (Crawley and Davies 1982). The X-maze anxiety model is therefore not unique in being very sensitive to minor changes in testing environment for reproducible results and this is reflected in the differences in results obtained in different laboratories.

The fact that use of very different lighting conditions did not change the behaviour of rats in the X-maze indicates that the different results obtained in different laboratories are unlikely to be due to different lighting levels in testing environments. The higher lighting conditions used in the test (440 lux) is so much brighter than their normal housing conditions (125 lux) and normal room lighting that behavioural changes should have been evident. It is unlikely the difference in the lighting

conditions in the various laboratories could be as great as this.

Table 3.1 : Behaviour of control rats in the elevated X-maze.

Mean (s.e.m.) N=6

Behaviour	Open	Centre	Enclosed
Entries (number)	3.6(0.5)	-	9.6(0.9)**
Time (seconds)	77(13)	87(15)	436(8)**
Rears (number)	1.2(1.2)	2.8(.8)	27(2.8)**
Grooms (number)	0.2(.2)	-	1.2(.6)**
Locomotion (half arms)	6.8(1.8)	-	29(2.6)**
Peers into (number)	15.0(2.4)	-	0.8(0.5)**
Peering from (sec)	0.2(0.2)	47(8)	36(12)**
Home cage activity(sec)	14(6)	38(15)	182(24)**
Exploration (sec)	63(13)	2.0(1.3)	218(18)**

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to values for the open arms.

Table 3.2: Effect of diazepam and beta-CCE on arm entries in the X-maze.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values] N = 6

Drug/dose (mg/kg)	Open	Total	Open/total
Diazepam			
1	8.8(0.8) [8.2(0.9)]	22.2(1.7) [25.0(2.6)]	0.41(.15)* [0.33(.02)]
2	5.0(0.7)* [3.8(0.5)]	11.8(1.4) [13.3(1.0)]	0.42(.02)** [0.28(.02)]
Beta-CCE			
1	3.0(0.5)* [5.2(0.6)]	13.2(1.0) [15.8(1.6)]	0.22(.03* [0.34(.01)]
2	3.3(0.3) [5.2(0.6)]	14.2(0.8) [15.8(1.6)]	0.23(.01)* [0.34(.01)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls

Table 3.3: Effects on height of enclosed arms on number of X-maze entries.

Mean number of arm entries in 10 minutes (s.e.m) N = 6

	Open	Total	Open/total
10 cm walls	6.2(1.5)	17.7(3.2)	0.33(.03)
30 cm walls	10.2(1.4)	23.3(2.3)	0.43(.03)*

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls

Table 3.4: Effect of height of enclosed arm on time spent in various parts of X-maze.

Mean time in seconds (s.e.m) N = 6

	Open	Enclosed	Centre
10 cm walls	113(35)	381(34)	106(18)
30 cm walls	149(20)	301(21)	151(21)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls

Table 3.5: Effect of changing the orientation of X-maze arms on arm entries.

Mean number of arm entries in 10 minutes (s.e.m) N = 6

Drugs (mg/kg)	Open	Total	Open/total
Enclosed arms at right angles.			
8-OH-DPAT .2	7.2(1.5)	26.7(3.1)*	0.26(.02)
Saline	[5.4(0.7)]	[18.2(1.2)]	[0.30(.04)]
Enclosed arms opposite each other.			
8-OH-DPAT .2	4.4(1.7)	16.7(3.8)	0.23(.06)*
Saline	[5.2(0.9)]	[15.5(4.3)]	[0.34(.03)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 3.6: Effect of anxiolytic drugs on defaecation in X-maze.

Measured during 10 minutes of X-maze exploration (s.e.m)

[vehicle control values] N = 6

Drug/dose	Open/total ratio	Faecal boli
Diazepam 2.0	0.42(.02)**	4.5(1.3)
	[0.28(.02)]	[3.7(1.5)]
DOI 0.1	0.39(.01)*	4.2(1.4)
	[0.31(.03)]	[3.2(1.0)]
Ritanserin 1	0.44(.04)**	2.5(0.9)
	[0.31(.03)]	[3.2(1.0)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 3.7: Effect of RU 24969 on rat behaviour in the various parts of the X-maze.

[vehicle control values] Mean (s.e.m) N = 6.

Behaviour	Open	Centre	Enclosed
Entries (number)	0.4(0.2)** [3.6(0.5)]	- -	4.6(1.4)* [9.6(0.9)]
Time (seconds)	2.4(1.0)* [77(13)]	33(8)* [87(15)]	584(26)* [436(8)]
Locomotion (Half arms)	0.4(0.2)** [6.8(1.8)]	- -	36(6)* [29(3)]
Rears (number)	0* [1.2(1.2)]	0* [2.8(0.8)]	7.6(2.7)* [26.6(2.8]
Grooms (number)	0* [0.2(0.2)]	0 0	0.4(0.4)* [1.2(0.6)]
Peers (number)	9.2(0.8)* [15(2.4)]	- -	0* [0.8(0.5)]
Peering from (sec)	0 [0.2(0.2)]	18(7)* [47(8)]	30(7) [36(12)]
Home cage activity(sec)	0.4(.2)** [14(6)]	15(6)* [38(15)]	423(38)* [182(24)]
Exploration (sec)	2.2(1)** [63(13)]	0* [2(1.3)]	131(19)* [218(18)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls. - = not applicable.

Table 3.8: Effect of ipsapirone on rat behaviour in the various parts of the X-maze.

[vehicle control values] Mean (s.e.m) N = 6.

Behaviour	Open	Centre	Enclosed
Entries (number)	5.2(0.6)* [3.6(0.5)]	- -	11.2(0.5) [9.6(0.9)]
Time (seconds)	107(22)* [77(13)]	100(7) [87(15)]	394(21) [436(8)]
Locomotion (Half arms)	12.2(1.2)* [6.8(1.8)]	- -	34(2) [29(3)]
Rears	0.4(0.2) [1.2(1.2]	4.8(0.3) [2.8(0.8]	26.0(1.8) [26.6(2.8]
Grooms	0 [0.2(0.2)]	0 [0]	0.4(0.2) [1.2(0.6)]
Peers into (number)	13(0.8) [15(2.4)]	- -	0.4(0.2) [0.8(0.5)]
Peering from (sec)	1.4(1.4) [0.2(0.2)]	75(12) [47(8)]	37(17) [36(12)]
Home cage activity(sec)	23(7)* [14(6)]	25(14) [38(15)]	151(41) [182(24)]
Exploration (sec)	79(23) [63(13)]	0.2(0.2) [2.0(1.3)]	236(30) [218(18)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls. - = not applicable.

Table 3.9: Effect of different lighting levels on rat behaviour in X-maze.

Mean (s.e.m)	Arm entries		
Lighting	Open	Total	Open/total
Low	5.0(0.7)	14.0(0.4)	0.35(.02)
High	6.2(1.0)	16.6(1.7)	0.36(.02)

Low light - 125 lux.

High light - 440 lux.

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$, **= $p<0.01$ relative to vehicle controls.

FIG 3.1 BEHAVIOUR OF VEHICLE-TREATED MICE IN X-MAZE.

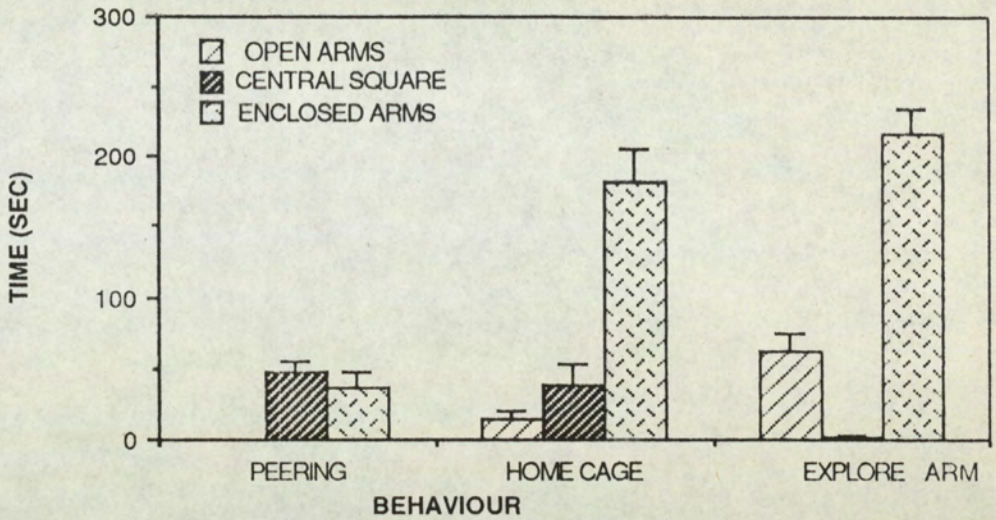
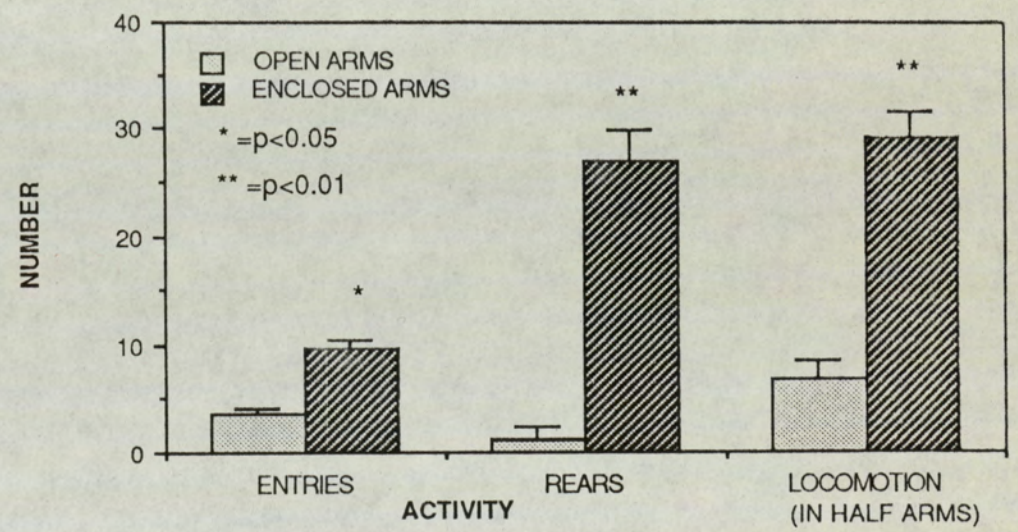


FIG 3.2 DIFFERENCES IN X-MAZE ARMS (VEHICLE TREATED RATS)



The duration and frequency of each type of behaviour in different parts of the X-maze was measured over a 10 min. period. Statistical comparisons were made on raw data using a t-test.

FIG 3.3 EFFECT OF DIAZEPAM ON X-MAZE BEHAVIOUR.

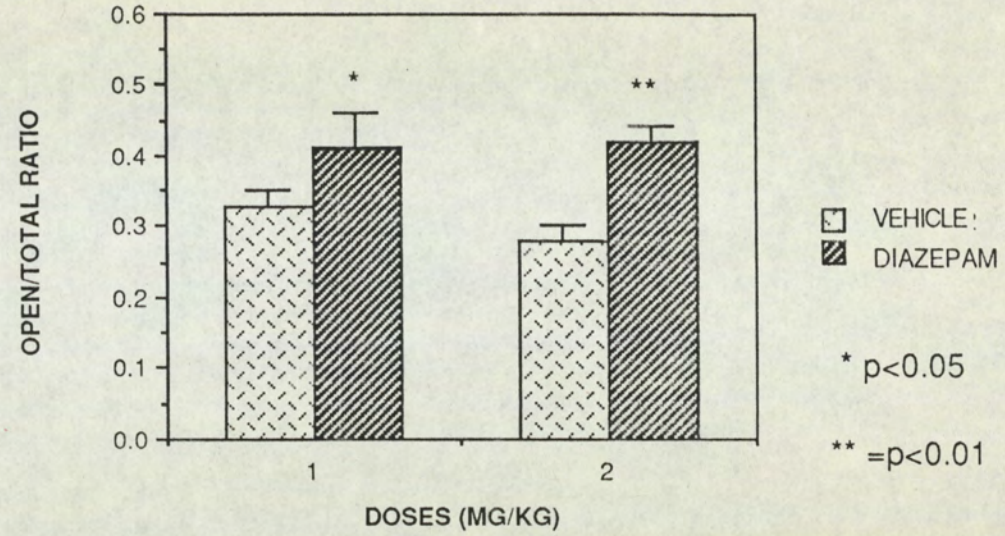
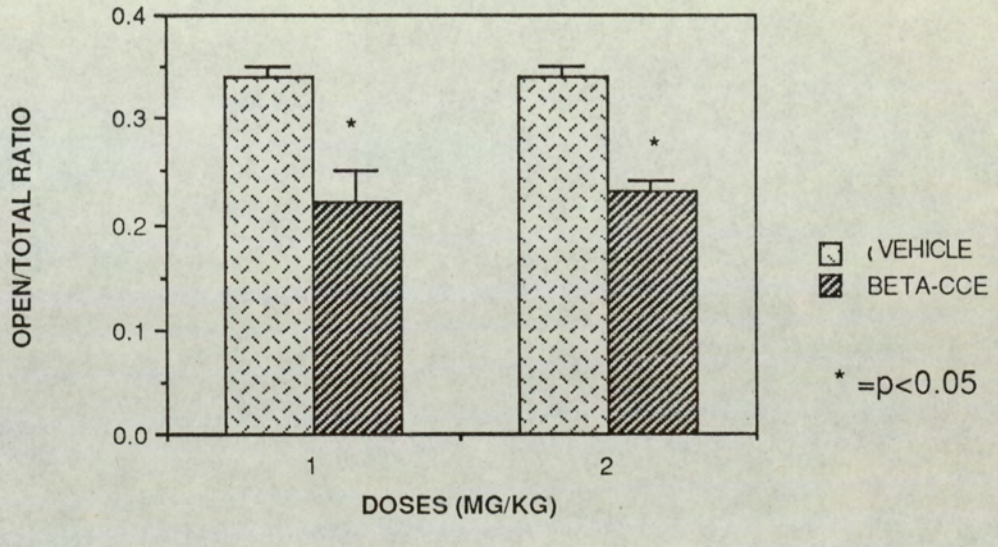


FIG 3.4 EFFECT OF BETA-CCE ON X-MAZE BEHAVIOUR.



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 3.5 EFFECT OF CHANGING ORIENTATION OF X-MAZE ARMS.

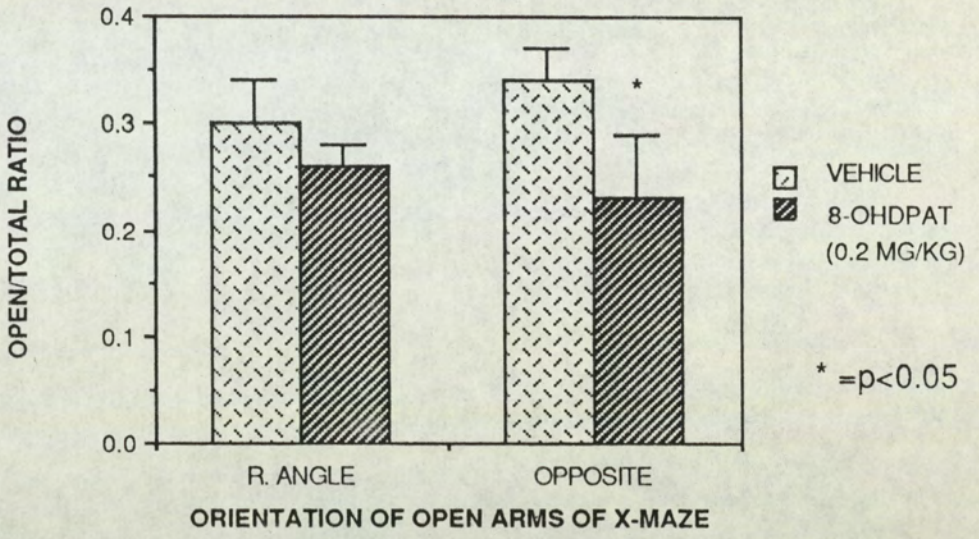
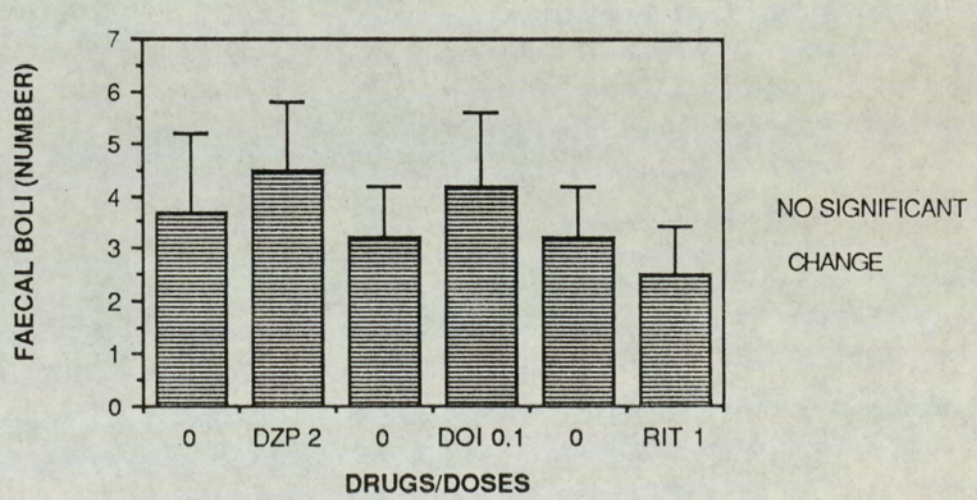


FIG. 3.6 EFFECT OF PUTATIVE ANXIOLYTICS ON DEFAECATION.



Effects on the open/total entry ratio or number of faecal boli during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 3.7 DRUG EFFECTS ON BEHAVIOUR IN ENCLOSED ARMS.

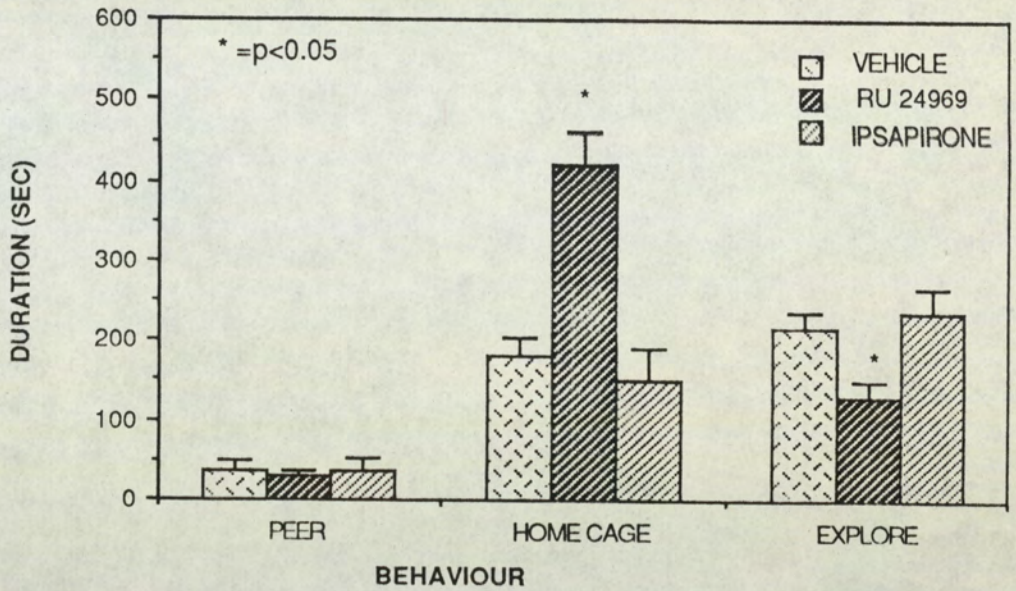
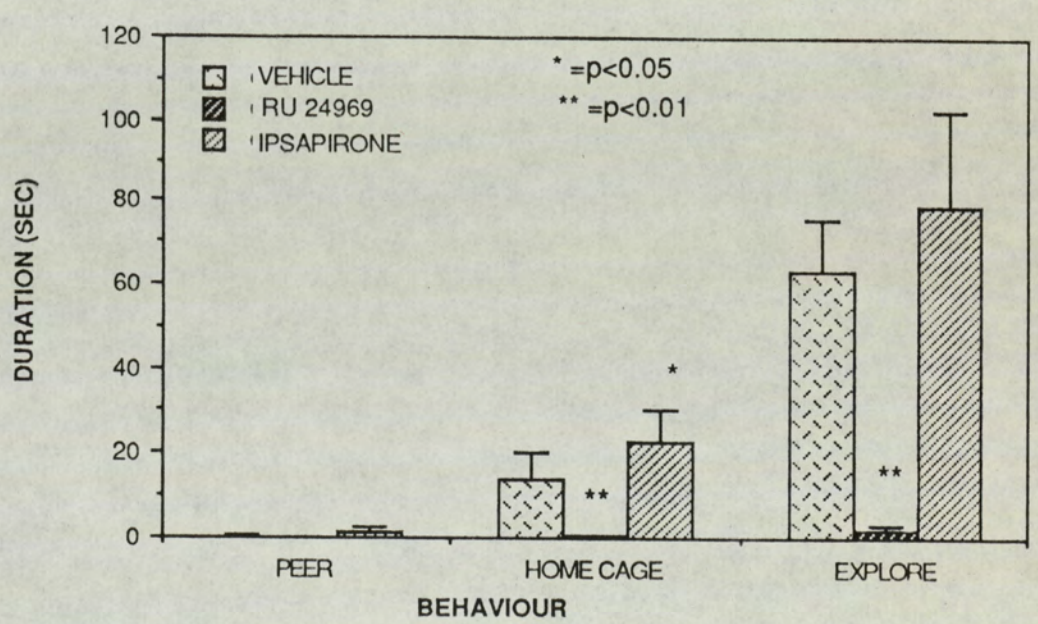


FIG 3.8 EFFECTS OF DRUGS ON BEHAVIOR IN OPEN ARMS



Each type of behaviour was measured over a 10 min. period as the rats explored the X-maze. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

CHAPTER 4

EFFECTS OF 5-HT LIGANDS ON THE ELEVATED X-MAZE.

INTRODUCTION

The 5-HT partial agonists 8-OH-DPAT, buspirone, gepirone and ipsapirone have been reported variously as having anxiolytic and anxiogenic effects (Dourish 1987, Johnston et al 1988, Moser 1987, see Introduction). Even using the same model there are contrasting results from different laboratories. For instance, in the elevated X-maze model both 8-OH-DPAT and ipsapirone were either inactive (Johnston and File 1988) or anxiogenic (Dourish 1987, Moser 1987). Under the conditions of our laboratory, ipsapirone and ritanserin were anxiolytic and 8-OH-DPAT and RU 24969 were anxiogenic while buspirone was inactive (Critchley and Handley 1986, 1987).

This project sought to establish the effects of those 5-HT ligands whose effects had not previously been reported on the X-maze model. These included the 5-HT1A agonists BAY R 1531 (Glaser et al 1987) and gepirone; the 5-HT1B/1C agonists mCPP and TFMPP; the 5-HT2 agonist DOI (1-(2,5-Dimethoxy-4-iodophenyl)-2- aminopropane); the 5-HT2 antagonist ICI 169,369; the 5-HT3 antagonists GR 38032F, MDL 72222, ICS 205-930 and BRL 24924; the 5-HT reuptake inhibitor zimeldine and the 5-HT releaser fenfluramine.

One of the compounds used here, BAY R 1531, has the autoradiographical characteristics and behavioural effects of a selective and potent 5-HT1A agonist (Glaser et al 1987). Like 8-OH-DPAT, BAY R 1531 displaced triated 5-HT and ipsapirone from calf hippocampal membranes and produced the 5-HT syndrome, generalised with 5-MeODMT and shortened ejaculation latencies in male rats (Glaser et al 1987). There are no reports about its effects on anxiety.

In addition, it also sought to establish the pharmacological mechanisms of the effects of the compounds previously reported. This included establishing if: (a) the anxiolytic effects of ritanserin were mediated by histamine-1 receptors for which the compound has high affinity (Leysen, unpublished data) using mepyramine as a prototype histamine (H1) receptor antagonist; (b) whether the effects of 8-OH-DPAT were due to the alpha-2 adrenoceptor antagonist (Crist and Suprenant 1987) or agonist effects (Heal et al 1989, Marsden and Martin 1986); (c) whether these alpha-2 adrenoceptor effects of 8-OH-DPAT translate into measurable behavioural effects; (d) and whether the anxiogenic effect of RU 24969 was mediated by 5-HT1A or 5-HT1B receptors. This involved establishing if the anxiogenic effects of RU 24969 could be antagonised by the 5-HT1A agonist ipsapirone. In such cases the only dose(s) of these drugs that were used were those that had been shown to have potent anxiolytic or anxiogenic effects in this laboratory (Critchley and Handley 1987, Critchley 1988). Antagonism studies were also performed with ipsapirone to establish if the effects of BAY R 1531 in the X-maze involved 5-HT1A receptors and with ritanserin to establish the receptors involved in the effects of fenfluramine.

Until recently, there have been no agonists with selectivity for 5-HT2 as opposed to 5-HT1 receptors. Glennon (1987) and Titeler et al (1988) reported that DOI is one such agent. Other than drug discrimination studies in animals there were no other reports of the effects of the compound on other aspects of an animal behavioural repertoire. An assessment of gross behavioural effects of DOI on the lines of Irwin (1968) was therefore conducted followed by studies of its effects on the X-maze.

There was also a study to establish the effects of chronic administration of the putative anxiolytic compound ritanserin. Since even a high oral dose (10 mg/kg), acutely did not produce effects on this anxiety model, for the chronic study the experimental animals were treated i.p. with an anxiolytic dose (1 mg/kg) of the drug for 3 days while they were getting used to drinking the ritanserin-tartaric acid suspension which replaced their drinking water.

The pinna reflex is a rapid flick of the head produced by stimulation of the auditory meatus of a rodent (Irwin 1968). The pinna reflex is inhibited by many centrally active compounds but most of them do so at sedative doses. Ability to inhibit the pinna reflex in the absence of severe sedation is strongly, though not exclusively, indicative of central alpha-2 adrenoceptor agonist activity (Handley 1984). Conversely, the inhibition of the pinna reflex by alpha-2 agonists was blocked by agents with antagonist effects at alpha-2 adrenoceptors (Brown 1980, Handley 1984). The pinna reflex was used in this work in an attempt to demonstrate behavioural evidence for the alpha-2 agonist (Heal et al 1989, Marsden and Martin 1986) and antagonist (Crist and Suprenant 1987) effects reported for 8-OH-DPAT. Xylazine was used as a prototype alpha-2 agonist (Colpaert and Raeymaekers 1986) and yohimbine as the alpha-2 antagonist.

RESULTS

4.1 Effects of 5-HT1A agonists.

8-OH-DPAT at 0.05 to 0.2 mg/kg decreased the open/total arm entry ratio without consistently affecting the total number of arm entries (Table 4.1 and Fig 4.1). There were no signs of the 5-HT syndrome at 0.05 mg/kg but at 0.1 and 0.2 mg/kg signs of the syndrome, especially flat body posture were prominent.

There was a phase, about one year in duration, when the anxiogenic effects of 8-OH-DPAT were difficult to detect and 0.05 and 0.1 mg/kg could not consistently produce anxiogenic effects and 0.2 mg/kg was the dose used in most of this work to produce anxiogenic effects of 8-OH-DPAT.

Gepirone at 0.1 to 5 mg/kg had no effects on the open/total arm entry ratio or the total number of arm entries (Table 4.1 and Fig 4.2). At the doses used there were no signs of the 5-HT syndrome.

BAY R 1531 at 0.3 to 1.2 mg/kg decreased the open/total arm entry ratio without consistently affecting the total number of arm entries (Table 4.1 and Fig 4.3). At 0.6 and 1.2 mg/kg there was pronounced flat body posture, vocalising, shivering, and uniquely walking or sliding down the arms of the X-maze.

Buspirone at 1.0 mg/kg both acutely and after 5 day sub-chronic pre-treatment had no effects on the open/total arm entry ratio. There was a slight though insignificant decrease in the total number of arm entries with the acute treatment (Table 4.1 and Fig 4.4).

Ipsapirone at 1.0 mg/kg significantly increased the open/total arm entry ratio without affecting the total number of arm entries (Table 4.1 and Fig 4.4). This effect was not always observed, and sometimes the compound was without effect on this test.

4.2 Effects of 5-HT1B/1C agonists.

TFMPP at 0.25 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.2). At 1 mg/kg there was a significant reduction in the open/total arm entry ratio without effects on the total number of arm entries (Table 4.2). At 5 mg/kg TFMPP reduced the open/total arm entry ratio and the total number of arm entries (Table 4.2 and Fig 4.5).

MCPP at 0.1 and 0.3 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.2). At 1 mg/kg there was a significant reduction in the open/total arm entry ratio and the total number of arm entries (Table 4.2 and Fig 4.6).

RU 24969 at 0.5 to 2.0 mg/kg produced a significant reduction in the open/total arm entry ratio without consistent effects on the total number of arm entries (Table 4.2 and Fig 4.7). In some rats, RU 24969 produced a sharp fall in the total number of arm entries with rats walking from the closed end of the enclosed arms to the open end, peering at the central square and turning back into the arm. In such cases the amount of locomotion within individual enclosed arms was greatly increased. However other rats under the same drug treatment made very many enclosed arm entries, rapidly crossing from one enclosed arm to the other without even pausing to look into the open arms.

4.3 Effects of the 5-HT2 agonist DOI.

DOI at 0.1, 0.5 and 1.0 mg/kg produced a significant increase in the open/total arm entry ratio (Table 4.3 and Fig 4.8). There was no change in the total number of arm entries (Table 4.3).

In the DOI treated animals an appreciable number of wet dog shakes was observed as the rats explored the X-maze (Table 4.4 and Fig 4.9). These were restricted to the enclosed arms or the central square. No wet dog shakes occurred in the open arms.

4.4 Effects of 5-HT2 antagonists.

Ritanserin at 1.0 mg/kg increased the open/total arm entry ratio without effecting the total number of arm entries (Fig 4.10).Ritanserin at 10 mg/kg orally had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 4.5).

ICI 169,369 at 0.3 to 3.0 mg/kg produced no effects on either the open/total arm entry ratios or the total number of arm entries (Table 4.5 and Fig 4.11).

4.5 Effects of 5-HT3 antagonists.

ICS 205-930 at 0.01 and 0.1 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.6 and Fig 4.12.1).

BRL 24924 at 0.01 and 0.1 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.6 and Fig 4.12.1).

GR 308032F at 0.01 and 0.1 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.6 and Fig 4.12.2).

MDL 72222 at 0.01 and 1.0 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.6 and Fig 4.12.2).

4.6 Effect of fenfluramine.

Fenfluramine at 1 mg/kg reduced the open/total arm entry ratio and the total number of arm entries but neither reduction was statistically significant. At 2.5 and 5 mg/kg fenfluramine produced a significant decrease in the open/total arm entry ratio and the total number of arm entries (Table 4.7 and Fig 4.13). At 5 mg/kg there was appreciable flat body posture, fearfulness and vocalisation on handling.

4.7 Effects of zimeldine.

Zimeldine at 0.3 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries. At 3 mg/kg however, there was significant reduction in the open/total arm entry ratio without effects on the total number of arm entries (Table 4.7 and Fig 4.14). Zimeldine at 10 mg/kg significantly reduced both the open/total arm entry ratio and total number of arm entries.

4.8 Effects of mepyramine

Mepyramine at 1 to 10 mg/kg did not affect either the open/total arm entry ratio or the total number of arm entries (Table 4.8 and Fig 4.15).

4.9 Drug interactions.

4.9.1 Ipsapirone and RU 24969.

Ipsapirone at 1 mg/kg did not change either the open/total arm entry ratio or the total number of arm entries (Table 4.9 and Fig 4.16).

RU 24969 at 1 mg/kg significantly reduced both the open/total arm entry ratio and the total number of arm entries (Table 4.9 and Fig 4.16). However, total locomotor activity was increased because there was a lot of ambulation from one end of the enclosed arm to the other without the animal leaving to explore any other arm.

Ipsapirone at 1 mg/kg administered 10 minutes before RU 24969 reduced the RU 24969 reduction in the open/total arm entry ratio without changing the total number of arm entries.

4.9.2 Ipsapirone and BAY R 1531

BAY R 1531 at 0.1 mg/kg produced a significant reduction in the open/total arm entry ratio and the total number of arm entries (Table 4.9 and Fig 4.17). Ipsapirone at 1 mg/kg did not change either the open/total arm entry ratio or the total number of arm entries (Table 4.9 and Fig 4.17).

Ipsapirone at 1 mg/kg administered 10 minutes before BAY R 1531 produced a slight but significant reversal of the BAY R 1531 induced reduction in the open/total arm entry ratio. The reduction in the total number of arm entries was partially reversed (Table 4.9 and Fig 4.17).

4.9.3 Gepirone and 8-OH-DPAT.

Gepirone at 1.0 and 2.5 mg/kg did not change either the open/total arm entry ratio or the total number of arm entries. 8-OH-DPAT at 0.2 mg/kg reduced the open/total arm entry ratio without changing the total number of arm entries (Table 4.10 and Fig. 4.18).

Gepirone at 1.0 and 2.5 mg/kg administered 20 minutes before 8-OH-DPAT at 0.2 mg/kg produced a slight potentiation of the latter compound's reduction of the open/total arm entry ratio (Table 4.10 and Fig. 4.18). The combination did not change the total number of arm entries.

4.9.4 Idazoxan and 8-OH-DPAT.

Idazoxan 1 mg/kg produced a non-significant reduction in the open/total arm entry ratio and a significant reduction in the total number of arm entries. 8-OH-DPAT at 0.2 mg/kg reduced the open/total arm entry ratio without affecting the total number of arm entries (Table 4.10 and Fig. 4.19).

Idazoxan at 1 mg/kg administered 20 minutes before 8-OH-DPAT 0.2 mg/kg produced an additive effect on the 8-OH-DPAT anxiogenic effect with the open/total arm entry ratio being further reduced. The drug combination also produced a reduction in the total number of arm entries (Table 4.10 and Fig. 4.19).

4.9.5 Ritanserin and DOI.

Ritanserin at 1 mg/kg significantly increased the open/total arm entry ratio without significantly changing

the total number of arm entries (Table 4.11 and Fig. 4.20). DOI at 0.1 mg/kg significantly increased both the open/total arm entry ratio and the total number of arm entries (Table 4.11).

Injection of ritanserin 1.0 mg/kg 10 minutes before DOI 0.1 mg/kg resulted in an increase in the open/total arm entry ratio that was lower than with either drug on its own and was not significantly greater than that of control animals (Table 4.11 and Fig 4.20). The total number of arm entries was increased.

4.9.6 ICI 169,369 and DOI.

ICI 169,369 at 1.0 mg/kg did not affect the open/total arm entry ratio but there was a significant reduction in the total number of arm entries (Table 4.11). DOI at 0.1 mg/kg significantly increased the open/total arm entry ratio without significantly changing the total number of arm entries (Table 4.11 and Fig. 4.21).

ICI 169,369 at 1.0 mg/kg administered 10 minutes before DOI at 0.1 mg/kg abolished the latter compounds increase in the open/total arm entry ratio (Table 4.11 and Fig 4.21). There was no change in the total number of arm entries.

4.9.7 Ritanserin and fenfluramine

Ritanserin at 1 mg/kg significantly increased the open/total arm entry ratio without affecting the total number of arm entries (Table 4.11 and Fig. 4.22). Fenfluramine at 1 mg/kg produced a significant reduction in the total number of arm entries but a non significant reduction in the open/total arm entry ratio (Table 4.11

and Fig. 4.22).

Ritanserin at 1 mg/kg administered 10 minutes before fenfluramine significantly increased the open/total arm entry ratio but did not affect the fenfluramine reduction of the total number of arm entries (Table 4.11 and Fig. 4.22).

4.10 Effect of chronic ritanserin.

Acute ritanserin at 1 mg/kg i.p. produced a significant increase in the open/total arm entry ratio without affecting the total number of arm entries. Neither the test nor control rats drunk much of the tartaric acid suspension for the first 3 days so ritanserin (or vehicle for control animals) was administered intraperitoneally for those three days. When they started drinking normally the rats drank less of the ritanserin/ tartaric acid suspension (20-25 ml/rat/day) than the control animals drank tartaric acid (25-30 ml/rat/day). The concentration of ritanserin in the suspension was adjusted to give them 10 mg/kg per rat per day. There was no weight difference between the ritanserin and control animals during the study.

Ritanserin administered chronically to rats at 10 mg/kg orally did not produce changes in the open/total arm entry ratio on testing on day 14, day 21, day 28 and 3 days after withdrawing ritanserin (day 31) (Table 4.12 and Fig 4.23). On day 14 there was a reduction in the total number of arm entries. This effect was not observed at any other time (Table 4.12).

When the drug was withdrawn after 28 days there were no perceivable changes in gross behaviour when rats were

tested in the X-maze 3 days after drug withdrawal.

4.11 Effects of 8-OH-DPAT and yohimbine on xylazine inhibition of pinna reflex.

8-OH-DPAT at 1 and 10 mg/kg subcutaneously (s.c) and yohimbine 1 mg/kg s.c. had no effects on pinna reflex in the mouse 20 and 40 minutes after injection (Table 4.13).

Xylazine 1 mg/kg s.c. abolished the pinna reflex 20 and 40 minutes after injection in half the animals tested. At 2.0 mg/kg s.c. the pinna reflex was abolished in all mice tested 20 and 40 minutes after injection (Table 4.13 and Fig. 4.24).

8-OH-DPAT 1 and 10 mg/kg s.c. had no effects on the inhibition of pinna reflex by xylazine 2 mg/kg (Table 4.13). Yohimbine 1 mg/kg abolished the inhibitory effects of xylazine 2 mg/kg on pinna reflex in mice (Table 4.11 and Fig. 4.24).

4.12 Behavioural profile of DOI in mice.

DOI at 0.2 - 10 mg/kg) intraperitoneally produced dose-dependent increases in: startle and touch responses, pinna reflex, fearfulness, scratching with self and mutual grooming, sniffing, restlessness, reactivity and head-twiches (see Table 4.14 and Fig 4.25). At 10 mg/kg there was appreciable cyanosis.

4.13 Behavioural profile of DOI in rats.

DOI at 0.5 - 5.0 mg/kg produced wet-dog shakes and intermittent forepaw tremor. At 5 mg/kg flat body posture occurred intermittently (score intensity 2 on a scale 0

-3), forepaw treading (score 1) and gnawing and chewing (score 1) and sniffing (see Table 4.15 and Fig 4.26).

DISCUSSION

8-OH-DPAT had an anxiogenic profile in the elevated X-maze but for reasons we have not been able to isolate there was a phase when the compound's anxiogenic effects were not always obtainable. Eventually the dose necessary to produce this anxiogenic response had to be raised from 0.05 to 0.2 mg/kg.

Gepirone did not have any effects on anxiety in the X-maze although it potentiated the anxiogenic-like effects of 8-OH-DPAT. The compound has been reported to be anxiolytic in several animal models of anxiety including punished drinking, punished responding and shock-induced aggression (Dourish 1987, see Chapter 1) and to have anxiolytic effects in humans (Cott et al 1985) and the hence the failure of the X-maze model to detect its anxiolytic effects shows a limitation of the model's predictive value.

Buspirone at 1 mg/kg did not have any anxiolytic effects both administered acutely or sub-chronically. The lack of acute effects in this test concurs with the findings of other investigators (Critchley and Handley 1987, Johnston and File 1988). However some other investigators have found the compound anxiogenic in this test (Dourish 1987, Moser 1987). The compound is also inactive in the four plate test and in the CER tests (Dourish 1987). The lack of anxiolytic effects on subchronic administration concurs with the findings of Wettstein (1988) who found the compound inactive in punished responding after 12 day treatment. Sub-chronic treatment with buspirone was used because it has been reported that the drug has to be administered over several days to reach full anxiolytic effectiveness in humans (Jacobson et al 1985).

BAY R 1531 was, like 8-OH-DPAT anxiogenic on the X-maze model of anxiety. This compound is a selective 5-HT1A agonist (Glaser et al 1987). In addition to the anxiogenic-like effects the compound produced a dramatic behaviour pattern where animals walked down and off the maze. This is a unique effect and is difficult to ascribe it to 5-HT receptors as it was not observed with other compounds. The 5-HT syndrome especially flat body posture was evident with the higher doses of the compound which is expected of a 5-HT1A agonist.

Ipsapirone on this model behaved like a 5-HT1A receptor antagonist being the only one of the 5-HT1 ligands to show anxiolytic effects while the others were either anxiogenic or inactive. However the anxiolytic effects of this compound were not always observable. Some batches of rats were not responsive to the compounds effects but the factors accounting for this variability could not be isolated. This variability with the compound probably explains why some observers report the compound inactive on this model of anxiety (Johnston and File 1988, Pellow et al 1987). The compound also reversed the anxiogenic-like effects of BAY R 1531 and RU 24969 even when its anxiolytic effects were not observable. These findings indicate that ipsapirone acts as a 5-HT1A antagonist and concur with those of Critchley and Handley (1987) who found that ipsapirone reversed the anxiogenic effect of 8-OH-DPAT. Ipsapirone has previously been reported to have 5-HT1A antagonist properties in both electrophysiological (Marsden and Martin 1986) and behavioural (Goodwin et al 1986) experiments.

That ipsapirone reversed the anxiogenic-like effects of RU 24969 suggests that the anxiogenic effects of RU 24969 are

mediated by 5-HT1A receptors rather than 5-HT1B since ipsapirone is selective for 5-HT1A receptors [Ki values, nanomolar for 5-HT1A = 2.9, 5-HT1B = 52000] (Peroutka 1987).

The selective 5-HT1A ligands, 8-OH-DPAT, ipsapirone and buspirone are all partial agonists at this receptor as measured by inhibition of adenyl cyclase in hippocampal preparations (De Vivo & Maayani 1986, Traber & Glaser 1987). There are no similar studies using BAY R 1531. Their effects in the X-maze form a continuum with 8-OH-DPAT and BAY R 1531 being anxiogenic and ipsapirone anxiolytic and the other two being inactive. In this model ipsapirone therefore acts as an antagonist and 8-OH-DPAT and BAY R 1531 as full agonists. That ipsapirone antagonises the anxiogenic effects of BAY R 1531 and RU 24969 while gepirone had a slight additive effect to the anxiogenic effect of 8-OH-DPAT fits this pattern. Gepirone had no effect alone, but Moser (1989) did find anxiogenic effects in this dose range.

The 5-HT1B/1C agonists mCPP and TFMPP (Hoyer and Schoeffter 1989, Kennett and Curzon 1988) were anxiogenic in the X-maze. However, there was a tendency for both compounds to reduce total entries. The anxiogenic effects are consistent with findings that both compounds are anxiogenic in the social interaction test in rats (Kennett and Curzon 1989) and that mCPP is anxiogenic in humans (Murphy et al 1989).

The 5-HT2/1C agonist DOI was anxiolytic in the X-maze model. The anxiolytic property is difficult to explain in the light of the anxiolytic effects of the 5-HT2 antagonist ritanserin. The 5-HT1C agonist activity would be expected in parallel with mCPP and TFMPP, to give

anxiogenic effects to the compound. It is conceivable that the hallucinogenic effects (Glennon 1987, Titeler et al 1988) a property of its 5-HT2 agonist activity render the rats oblivious to the anxiety-provoking characteristics other rats associate with the open arms of the X-maze.

Of the 5-HT2/1C antagonists ritanserin was anxiolytic while ICI 169,369 was inactive. The contribution of inhibition of 5-HT1C receptors to the anxiolytic activity of ritanserin is difficult to determine. Ketanserin which is a 5-HT2 antagonist with little 5-HT1C binding affinity (Hoyer 1988) is anxiolytic on this model (Critchley 1988) though to a smaller extent. Ketanserin binds to alpha-1-adrenoceptors (Colpaert et al 1985) and since alpha-1 antagonists are anxiolytic in the X-maze (Handley and Mithani 1984a) this could account for its anxiolytic effects. There are no selective 5-HT1C antagonists available to work with.

The combination of anxiolytic doses of DOI and ritanserin was inactive which is puzzling. ICI 169,369 also abolished the anxiolytic effects of DOI. It would appear that partial occupancy of the receptors of both agents negates the anxiolytic effects of both.

The histamine antagonist mepyramine was without effect on the X-maze. This would suggest that ritanserin's anxiolytic effects are unlikely to be mediated by histamine receptors for which the compound has high affinity for H1 receptors [Ki =11 nM] (Leysen, unpublished data).

Chronic ritanserin was not anxiolytic. Chronic ritanserin has been found to produce down-regulation of 5-HT2 receptors in frontal cortex (Leysen et al 1987a, b, Twist

et al 1989). This might have been expected to have effects on anxiety as measured on this model. Withdrawal from chronic ritanserin did not produce changes in rat behaviour. This suggests that chronic use of ritanserin might be free of the withdrawal symptoms that accompany long term benzodiazepines usage (Griffiths and Sannerud 1987, Snyder 1986).

All the 5-HT3 antagonists were inactive. The lack of effects of 5-HT3 antagonists is puzzling. These compounds have been reported to be anxiolytic in the mouse light/dark discrimination test (Costall et al 1987, Tyers et al 1987) the social interaction test in rats (Tyers et al 1987, Piper et al 1988) and in the marmoset provocation test (Tyers et al 1987).

This lack of effects of 5-HT3 antagonist in X-maze and social interaction have also been reported by other investigators (Johnston et al 1988).

Both zimeldine and fenfluramine were anxiogenic but the higher doses reduced the number of total entries. It appears that enhancement of 5-HT transmission reduces locomotor activity.

Electrophysiological studies have suggested that 8-OH-DPAT has alpha-2-adrenoceptor antagonist effects (Crist & Suprenant 1987). Xylazine, a selective alpha-adrenoceptor agonist (Colpaert and Raeymaekers 1986) abolished the pinna reflex in mice. This is a property of alpha-2-adrenoceptor agonists (Brown & Handley 1980). The alpha-2-adrenoceptor antagonist yohimbine reversed this effect of xylazine. 8-OH-DPAT, even at the relatively high dose of 10 mg/kg neither reduced the incidence of the pinna reflex nor affected the inhibition of the pinna reflex by xylazine.

Thus in this behavioural model 8-OH-DPAT did not exhibit alpha-2- adrenoceptor antagonist effects. It is therefore unlikely that the 8-OH-DPAT anxiogenic effect was due to antagonist effects at alpha-2 adrenoceptors.

However, 8-OH-DPAT produces alpha-2-adrenoceptor agonist effects such as mydriasis by facilitating noradrenaline release (Heal et al 1989). Alpha-2-adrenoceptor agonist effects were not seen in the present study. Unlike xylazine, 8-OH-DPAT did not abolish the pinna reflex indicating lack of alpha-2-adrenoceptor agonist effects.

If the compound's anxiogenic effect had been mediated by agonism at alpha-2-adrenoceptor receptors then it would have been blocked by idazoxan, an alpha-2-adrenoceptor antagonist. On the contrary idazoxan had additive effects to 8-OH-DPAT anxiogenesis.

The behavioural profile of DOI is consistent with selective 5-HT2 receptor stimulation at doses below 5 mg/kg in both rats and mice. Signs of probable 5-HT1 receptor stimulation occurred at higher doses and the chewing behaviour may be dopaminergic. The intermittent forepaw treading and flat body posture seen at 5 mg/kg are signs of 5-HT1 receptor stimulation. This might possibly be due to the compound's affinity for 5-HT1C receptors (Hoyer et al 1989).

Head twitches and wet-dog shakes are rapid involuntary flicks of the head or head and upper body that seem to be directed at shaking off irritant material (Boulton and Handley 1973). They are considered to be 5-HT2 receptor mediated (Green et al 1983, Middlemiss 1986b) and that DOI had these effects at doses which did not produce 5-HT1 mediated behaviour shows the compound's potency at 5-HT2

receptors. 5-HT2 agonists, including DOI, are probably hallucinogenic (Glennon 1987, Titeler et al 1988) and the contribution of such an effect to the observed actions in the X-maze cannot be determined.

That all wet-dog shakes observed in the X-maze occurred in the enclosed arms and the central square rather than in the open arms, suggests that novelty/anxiety suppress wet dog shakes until rats return to the more secure enclosed arms.

Table 4.1: Effect of 5-HT1A agonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arm	Total entries	Open/total
8-OH-DPAT			
0.05	4.2(1.5) [3.7(0.6)]	14.3 (2.1) [10.3 (1.6)]	0.28 (.08) [0.35 (.01)]
0.1	2.0(1.0)* [5.0(1.0)]	16.0 (4.2) [16.8 (2.2)]	0.09(.04)* [0.30(.04)]
0.2	1.3(1.0)** [3.7(0.6)]	16.3 (2.9) [10.3 (1.6)]	0.10 (.06)** [0.35 (.01)]
Gepirone			
0.1	7.5(1.0) [8.0(0.8)]	19.2 (2.1) [22.3 (1.6)]	0.39 (.02) [0.36 (.02)]
1.0	7.0(0.6) [8.0(0.8)]	19.0 (1.2) [22.3 (1.6)]	0.37 (.02) [0.36 (.02)]
2.5	7.3(1.5) [6.5(0.8)]	16.3 (2.0) [15.5 (1.4)]	0.40 (.03) [0.42 (.02)]
5.0	4.6(1.0) [6.5(0.8)]	10.8 (2.6)* 15.5 (1.4)]	0.40 (.03) [0.42 (.02)]
BAY R 1531			
0.1	2.2(1.2)* [5.8(1.2)]	10.0(1.3) [13.8(2.2)]	0.19(.09)* [0.40(.03)]
0.3	1.2(0.7)* [5.8(1.2)]	10.0(1.8) [13.8(2.2)]	0.10(.05** [0.40(.03)]
0.6	0.8(0.8)** [6.4(1.2)]	16.2(4.2) [15.2(1.6)]	0.07(.07)** [0.41(.05)]
1.2	0.6(0.6)** [6.4(1.2)]	16.2(4.2) [15.2(1.6)]	0.02(.02)** [0.41(.05)]
Ipsapirone 1	8.3(0.7) [6.8(1.4)]	20.5(2.1) [19.7(2.6)]	0.41(.01)* [0.33(.05)
Buspirone 1			
Acute	3.8(0.6)* [5.8(0.7)]	14.7(1.6)* [20.0(2.0)]	0.26(.02) [0.29(.03)]
Sub-chronic (5 day)	4.8(1.1) [5.8(0.7)]	16.3(1.9) [20.0(2.0)]	0.29(.04) [0.29(.03)]

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$, **=$p<0.01$ relative to vehicle controls.

Table 4.2: Effect of 5-HT1B and 5-HT1C agonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arms	Total entries	Open/total
TFMPP			
0.25	6.8(1.0) [5.0(1.0)]	20.0(1.5) [18.4(2.6)]	0.33(.03) [0.27(.03)]
1.0	3.0(1.1) [5.0(1.0)]	18.4(1.8) [18.4(2.6)]	0.15(.05)* [0.27(.03)]
5.0	0.4(0.2)** [5.0(1.0)]	4.6(1.3)* [18.4(2.6)]	0.05(.03)** [0.27(.03)]
mCPP			
0.1	2.8(0.7) [2.0(0.7)	9.8 (2.3) [8.2 (2.2)]	0.29 (.04) [0.22(.06)]
0.3	3.0(0.8) [2.0(0.7)	11.0(1.6) [8.2 (2.2)]	0.25(.05) [0.22(.06)]
1.0	0.6(0.4)* [2.0(0.7)	3.8 (1.3)* [8.2 (2.2)]	0.09(.06)* [0.22(.06)]
RU 24969			
0.5	7.4(1.9) [5.0(0.9)]	11.8(3.0) [13.0(2.0)]	0.28(.04)* [0.38(.05)]
1.0	0.4(0.2)* [3.6(0.5)]	5.0(1.6)* [13.2(1.4)]	0.05(.03)* [0.27(.02)]
2.0	2.2(0.6)* [5.0(0.9)]	27.8(5.6)** [13.0(2.0)]	0.10(.04)** [0.38(.05)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.3: Effect of DOI on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arms	Total entries	Open/total
DOI			
0.1	8.4(0.6) [6.0(0.7)]	20.1(1.2) [16.8(1.6)]	0.42(.01)** [0.35(.02)]
0.5	7.2(0.3) [6.0(0.7)]	18.1(1.1) [16.8(1.6)]	0.40(.01)* [0.35(.02)]
1.0	10.4(1.1) [7.0(0)]	23.8(2.4) [19.2(0.4)]	0.44(.03)** [0.37(.01)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.4: Effect of DOI on wet dog shakes during X-maze exploration.

Number of wet dog shakes in 10 min (s.e.m.)

DOI mg/kg	Wet dog shakes
0	0
0.1	0.8 (0,8)**
0.5	2.2 (0.9)**
1.0	5.2 (1.5)**

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.5: Effect of 5-HT2 antagonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arm	Total	Open/total
Ritanserin			
1.0(i.p.)	7.0(1.4) [3.3(1.8)]	15.5(2.1) [10.0(1.8)]	0.44(.04)* [0.31(.03)]
10 (oral)	4.4(0.6) [4.4(0.5)]	10.7(0.6) [11.4(0.6)]	0.40(.04) [0.38(.02)]
ICI 169,369			
0.3	5.2(1.1) [4.4(1.1)]	18.3(2.5) [15.4(1.5)]	0.28(.03) [0.25(.04)]
1.0	6.0(0.7) [4.4(1.1)]	21.4(2.8) [15.4(1.5)]	0.28(.01) [0.25(.04)]
3.0	5.0(1.1) [4.4(1.1)]	17.2(1.7) [15.4(1.5)]	0.27(.04) [0.25(.04)]

Statistical comparisons were made on raw data using a t-test. * = $p<0.05$ ** = $p<0.01$ relative to vehicle controls.

Table 4.6: Effect of 5-HT3 antagonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arm	Total entries	Open/total
BRL 24924			
0.1	7.8 (0.7) [8.0 (0.9)]	22.3 (1.6) [21.3 (2.2)]	0.35 (.02) [0.37 (.02)]
1.0	5.8 (1.0) [8.0 (0.9)]	17.2 (3.0) [21.3 (2.2)]	0.33 (.02) [0.37 (.02)]
ICS 205-930			
0.01	4.0 (0.6) [4.4 (0.4)]	12.3 (1.5) [13.6 (0.5)]	0.32(.02) [0.33(.03)]
0.1	5.0 (0.6) [4.4 (0.4)]	14.7 (1.5) [13.6 (0.5)]	0.33(.02) [0.33(.03)]
GR 38032F			
0.001	3.0 (0.3) [4.2 (0.2)]	10.0 (0.9)* [13.4 (0.5)]	0.30 (.01) [0.31 (.01)]
0.01	3.6 (0.5) [4.2 (0.2)]	11.2 (0.9) [13.4 (0.5)]	0.32 (.01) [0.31 (.01)]
0.1	3.2 (0.6) [3.7 (0.2)]	9.7 (1.3) [11.8 (1.0)]	0.31 (.04) [0.32 (.01)]
MDL 72222			
0.01	7.2 (1.0) [5.2 (0.4)]	18.6 (1.7) [15.5 (1.2)]	0.38(.02) [0.33(.01)]
0.1	3.3 (0.5) [3.2 (0.3)]	10.8 (2.1) [10.8 (0.9)]	0.32 (.02) [0.32 (.02)]
1.0	5.0 (0.8) [5.2 (0.4)]	14.8 (1.4) [15.5 (1.2)]	0.33 (.03) [0.33 (.01)]

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$, **= $p<0.01$ relative to vehicle controls.

Table 4.7: Effect of fenfluramine and zimeldine on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Fenfluramine			
1.0	1.8(0.8) [3.2(0.9)]	8.2(1.9) [11.7(2.5)]	0.16(.06) [0.26(.04)]
2.5	0.7(0.2)* [3.2(0.9)]	4.3(0.6)* [11.7 (2.5]	0.14(.05)* [0.26(.04)]
5.0	0* [4.8(0.7)]	3.2(1.0)** [12.8(1.0)]	0** [0.36(.04)]
Zimeldine			
0.3	6.5(1.2) [5.8(0.5)]	18.8(3.1) [15.3(1.0)]	0.35(.03) [0.41(.04)]
3.0	6.7(1.7) [5.8(0.5)]	20.8(3.1) [15.3(1.0)]	0.30(.03)* [0.41(.04)]
10	3.2(1.1) [5.7(1.1)]	12.8(2.6)* [17.8(2.4)]	0.19(.06)** [0.31(.02)]

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.8: Effect of mepyramine on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Mepyramine			
1	6.7(0.4) [5.2(0.4)]	19.3(1.4) [16.2(0.6)]	0.34(.02) [0.31(.03)]
5	7.5(0.8) [5.2(0.4)]	21.0(2.0)* [16.2(0.6)]	0.36(.01) [0.31(.03)]
10	4.8(0.6) [4.0(0.6)]	15.3(2.6) [14.0(1.8)]	0.31(.02) [0.28(.02)]

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.9: Effect of ipsapirone on the anxiogenic effects of RU 24969 and BAY R 1531.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Saline	3.6(0.5)	13.2(1.4)	0.27(.02)
RU 24969 1.0	0.4(0.2)*	5.0(1.6)*	0.05(.03)*
Ipsapirone 1.0	5.2(0.6)	16.4(0.9)	0.31(.02)
Ipsapirone 1.0/ RU 24969	4.4(0.9)	19.0(3.8)	0.25(.04)
F=(A X B)=6.3	DF=1/16	p<0.05	
Saline	4.4(1.5)	11.2(2.9)	0.32(.09)
BAY R 1531 .3	0.4(0.2)**	6.6(2.2)**	0.04(.02)**
Ipsapirone 1	4.6(1.1)	12.2(2.4)	0.36(.05)
Ipsapirone/ BAY R 1531	1.6(0.8)**	8.8(1.4)	0.14(.07)**
F=(A X B)=15.5	DF=1/16	p<0.01	

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= p<0.05 **= p<0.01 relative to vehicle controls.

Table 4.10: Effect of gepirone and idazoxan on 8-OH-DPAT anxiogenic effect.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Saline	2.3(0.5)	8.6(1.0)	0.29(.04)
Gepirone 2.5	3.0(0.5)	9.2(2.0)	0.33(.02)
8-OH-DPAT .2	3.2(1.6)	13.0(2.1)	0.22(.09)*
Gepirone/ 8-OH-DPAT	1.4(0.2)	8(1.3)	0.19(.03)*
F (B)= 4.4	DF= 1/16		
Saline	4.3(0.4)	11.4(1.0)	0.37(.03)
Gepirone 1	2.2(0.5)	7.0(0.3)	0.30(.05)
8-OH-DPAT .2	0.8(0.4)	9.0(1.5)	0.07(.05)*
Gepirone/ 8-OH-DPAT	0.2(0.2)	11.6(1.4)	0.01(.01)*
F(B)= 5.3	DF= 1/16	p<0.01	
Saline	6.2(1.3)	15.4(2.2)	0.39(.07)
8-OH-DPAT .2	3.2(1.3)	12.6(0.9)	0.23(.07)*
Idazoxan 1	2.0(0.3)*	7.0(1.6)*	0.30(.03)
Idazoxan/ 8-OH-DPAT	1.6(0.7)*	9.2(1.5)*	0.16(.07)*
F (A x B)= 0.025	Non-significant.		
F(A) = 5.2	DF= 1/16	p<0.05	

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= p<0.05 **= p<0.01 relative to vehicle controls.

Table 4.11: Effect of 5-HT2 antagonists on DOI and fenfluramine anxiogenic effects.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arms	Total entries	Open/total
Saline	3.3(0.8)	10.0(1.8)	0.31(.03)
Ritanserin 1.0	7.0(1.4)*	15.5(2.1)	0.44(.04)*
DOI 0.1	7.0(0.4)*	17.8(1.3)*	0.39(.01)*
Ritanserin 1.0/ DOI 0.1	6.2(1.0)	16.2(1.7)*	0.37(.03)
	F(A X B) = 5.07	DF = 1/16	p<0.05
Saline	7.0(0.9)	20.8(3.9)	0.33(.03)
ICI 169369 1	4.5(1.2)*	15.0(0.6)	0.29(.07)
DOI 0.1	6.9(0.5)	17.6(1.2)	0.39(.02)*
ICI 169369 1/ DOI 0.1	6.0(1.4)	18.4(2.2)	0.32(.03)
	F(A) = 4.51	DF = 1/16	p<0.05
Saline	4.8(1.3)	14.6(2.4)	0.33(.08)
Rit 1	5.7(0.7)	14.2(1.2)	0.40(.03)*
Fenfluramine 1	1.6(0.7)	6.0(1.5)	0.27(.08)
Rit/Fen	3.0(0.8)	6.8(1.1)	0.42(.07)*
	F(A X B) = 0.30	Non-significant	

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= p<0.05 **= p<0.01 relative to vehicle controls.

Table 4.12: Effect of chronic ritanserin on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open arm	Total entries	Open/total
Day 1 (i.p.)			
Ritanserin 1	5.7(0.8)	13.1(1.5)	0.45(.03)*
Vehicle	[4.8(0.8)]	[14.9(1.1)]	[0.37(.03)]
Day 14 (oral)			
Ritanserin	3.7(0.5)*	11.9(1.3)*	0.31(.04)
Vehicle	[5.7(0.8)]	[15.6(1.3)]	[0.36(.02)]
Day 21 (oral)			
Ritanserin	3.8(0.6)	11.0(1.8)	0.33(.03)
Vehicle	[4.6(0.8)]	[13.7(2.0)]	[0.33(.03)]
Day 28 (oral)			
Ritanserin	3.6(0.7)	10.9(1.7)	0.32(.03)
Vehicle	[4.3(0.9)]	[13.2(1.8)]	[0.32(.03)]
Day 31 (3 days after drug withdrawal)			
Ritanserin	3.0(0.8)	8.7(1.2)	0.31(.04)
Vehicle	[4.3(1.2)]	[11.9(1.9)]	[0.33(.04)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 4.13: Effect of xylazine, 8-OH-DPAT and yohimbine on pinna reflex.

Drug/dose (mg/kg)		Mice with pinna reflex intact (%) 20 min	40 min	60 min
Xylazine	1.0	10	20	50
	2.0	0	0	20
8-OH-DPAT	1.0	100	100	100
	10.0	100	100	100
Yohimbine	1.0	100	100	100
8-OH-DPAT 1.0/ xylazine	2.0	0	0	0
8-OH-DPAT 10/ xylazine	2.0	0	0	0
Yohimbine 1.0/ xylazine	2.0	100	100	100

0 = pinna reflex abolished.

100 = pinna reflex intact.

Table 4.14: Effect of DOI on mouse behaviour 15 Mins after i.p. injection.

	DOSE (MG/KG)		
	0.2	1	10
Fighting	0	0	0
Vocalisation	0	0	0
Pain (untestable)	0	0	0
Straub tail	0	0	0
Restlessness	0	0	2
Twitches	2	4	6
Convulsions	0	0	0
Tremor	0	0	2
Sniffing	0	4	4
Fearfulness	0	2	4
Respiration	0	0	0
Grooming & scratching	0	4	6
Ptosis	0	0	2
Alertness	0	0	0
Body sag	0	0	4
Spontaneous activity	0	-2	-2
Startle response	2	2	6
Touch response	0	2	6
Gait	0	0	0
Passivity	0	-2	0
Pinna reflex	0	2	4
Corneal reflex	0	0	4
Cyanosis	0	2	6

Intensity of behaviour scored on a scale of -6 to +6.

Table 4.15: Effect of DOI at 5 mg/kg on rats gross behaviour.

	TIME (minutes)		
	5	15	30
Wet dog shakes	3	3	3
Chewing	1	1	1
Gnawing	1	1	1
Flat body postures	1	2	2
Fore limb tremor	0	1	1
Fore paw treading	0	1	1

Intensity of 5-HT mediated behaviour was scored on a scale of 0 to 3 at 5, 15 and 30 min. after i.p. injection of DOI.

FIG 4.1 EFFECT OF 8-OHDPAT ON X-MAZE BEHAVIOUR.

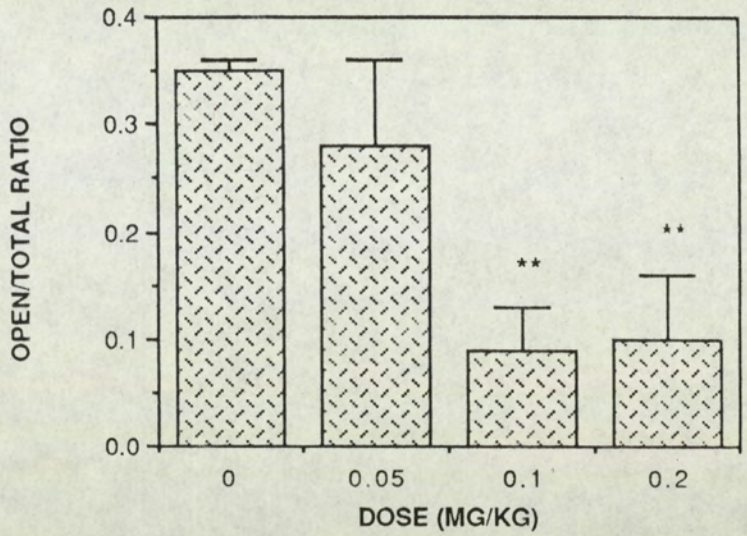
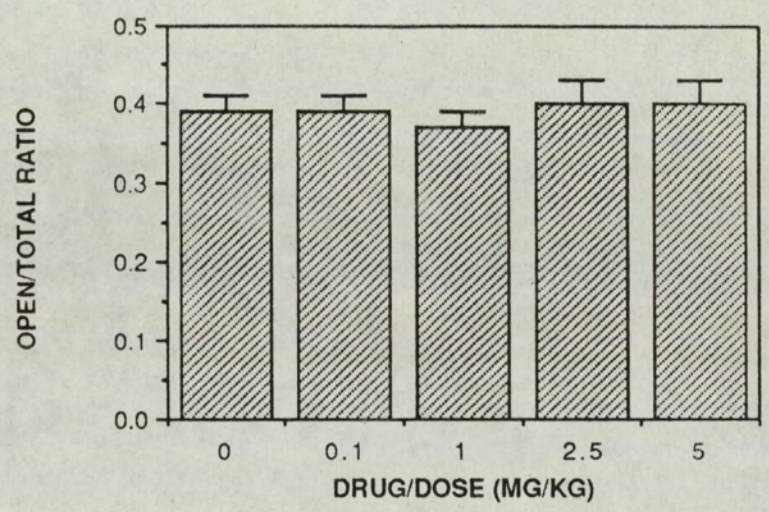


FIG. 4.2 EFFECT OF GEPIRONE ON X-MAZE BEHAVIOUR



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 4.3 EFFECT OF BAY R 1531 0N X-MAZE BEHAVIOR.

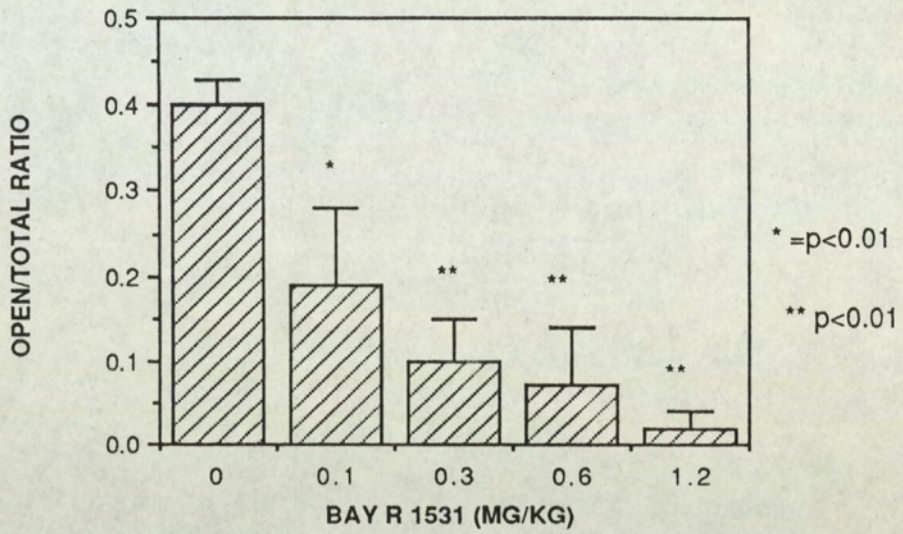
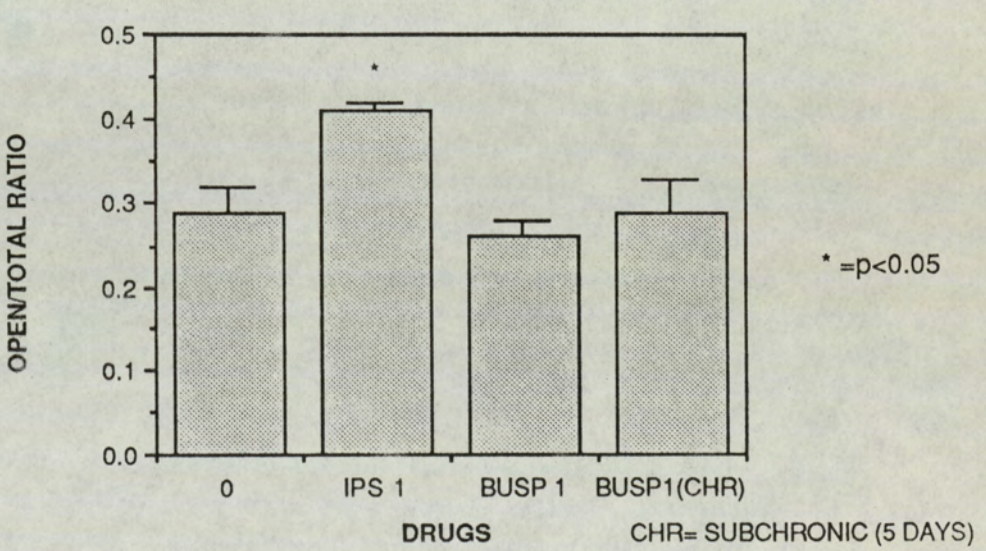


FIG 4.4 EFFECT OF IPSAPIRONE AND BUSPIRONE IN X-MAZE.



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 4.5 EFFECT OF TFMPP ON X-MAZE BEHAVIOUR

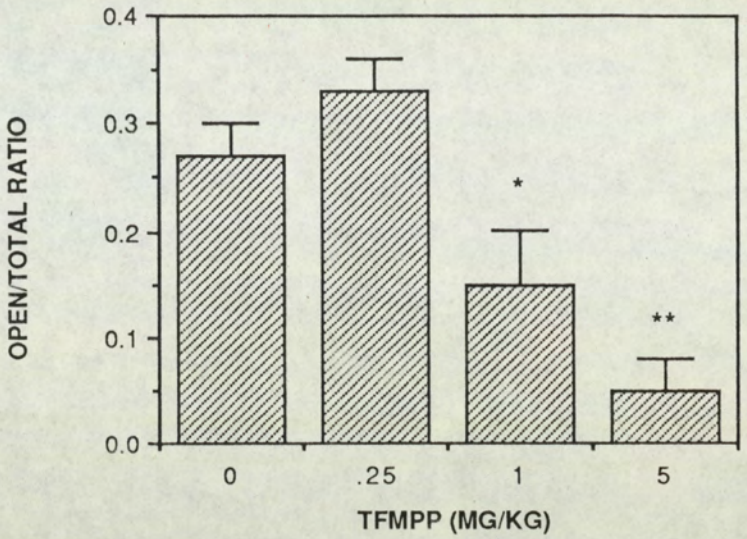
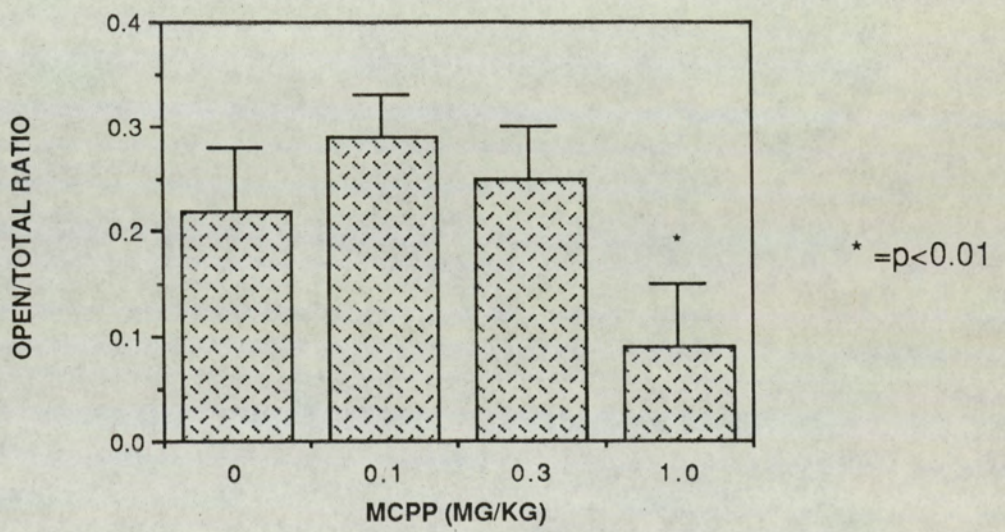
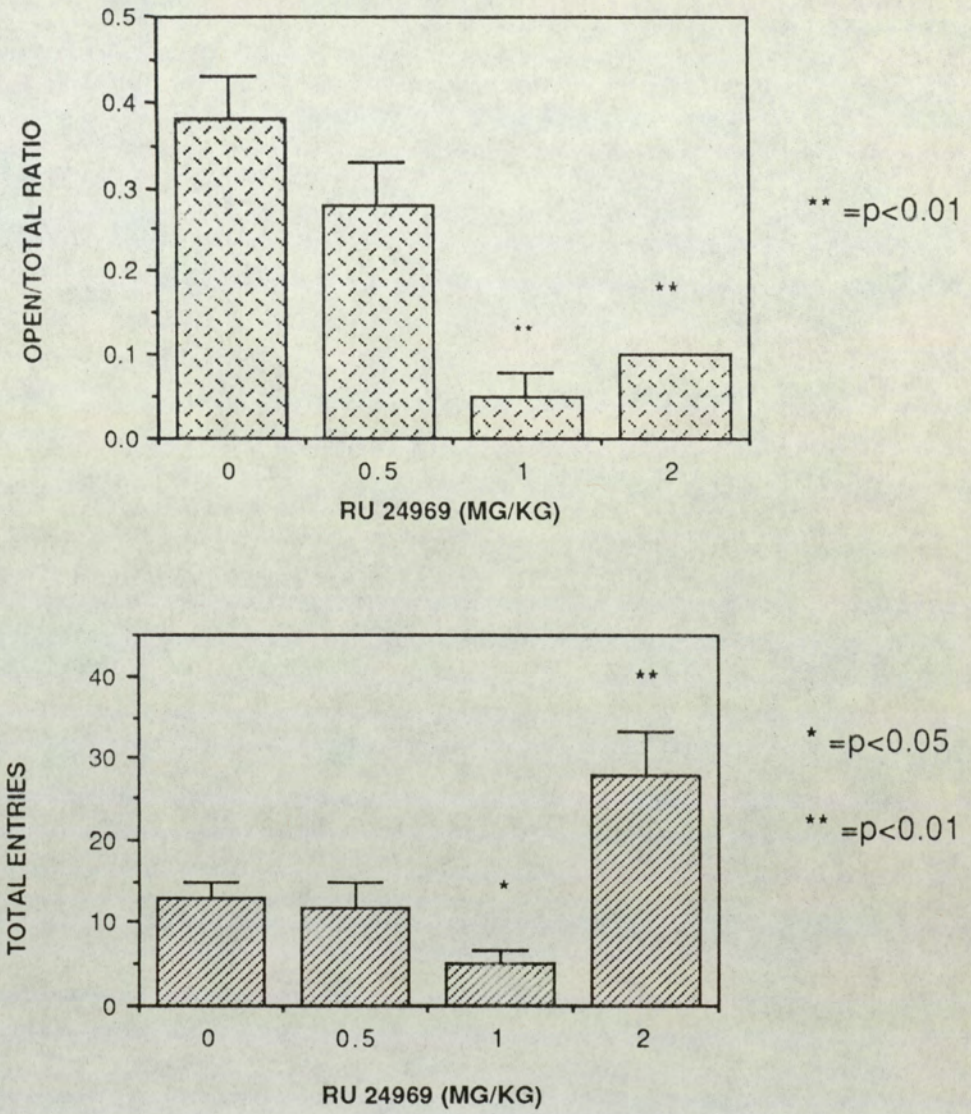


FIG. 4.6 EFFECT OF MCPP ON X-MAZE BEHAVIOUR



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 4.7 EFFECT OF RU 24969 ON X-MAZE BEHAVIOUR.



Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 4.8 EFFECT OF DOI ON X-MAZE BEHAVIOUR.

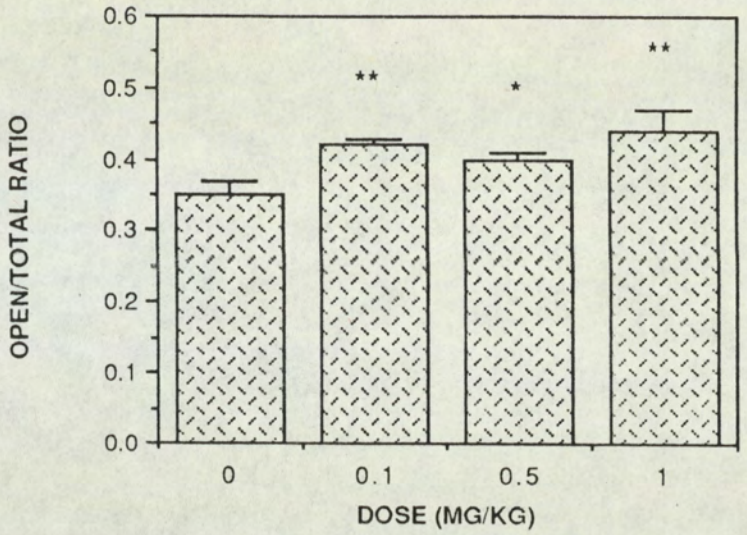
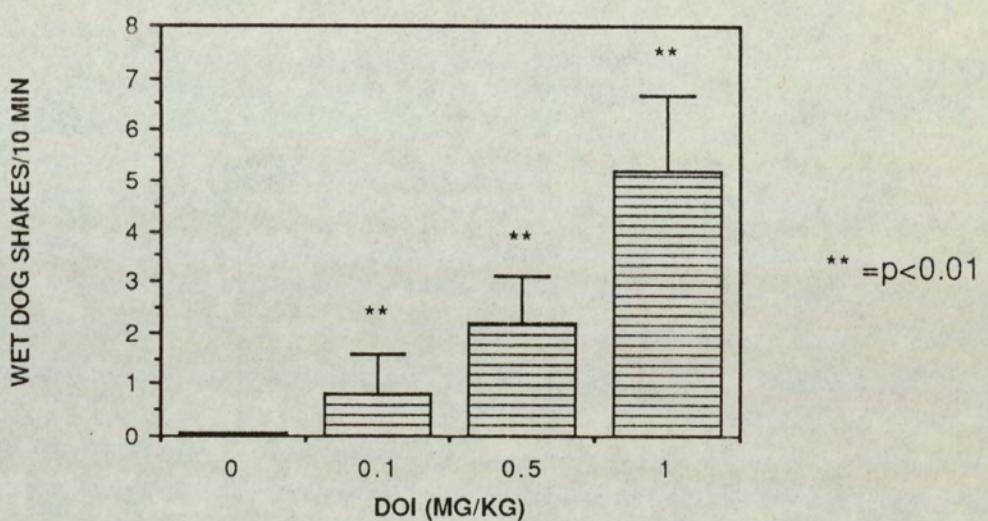


FIG 4.9 DOI INDUCTION OF WET DOG SHAKES IN X-MAZE.



Effects of DOI on the open/total entry ratio (Fig 4.8) and in inducing wet dog shakes (Fig 4.9) during X-maze exploration . Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 4.10 EFFECT OF ICI 169,369 ON X-MAZE BEHAVIOUR.

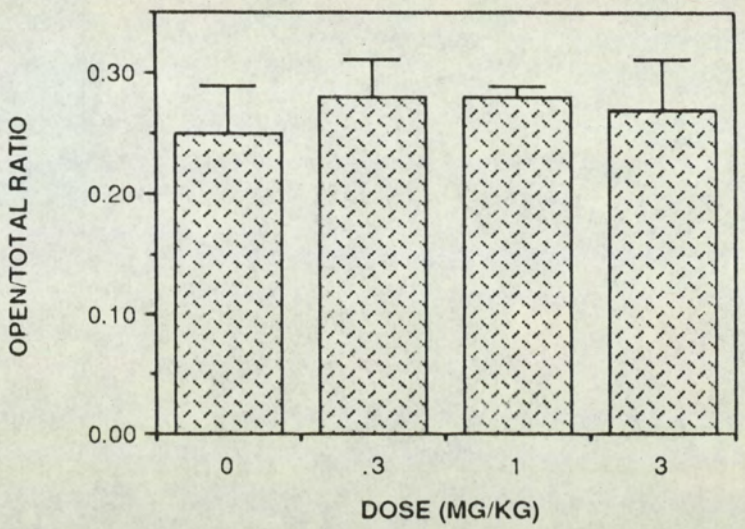
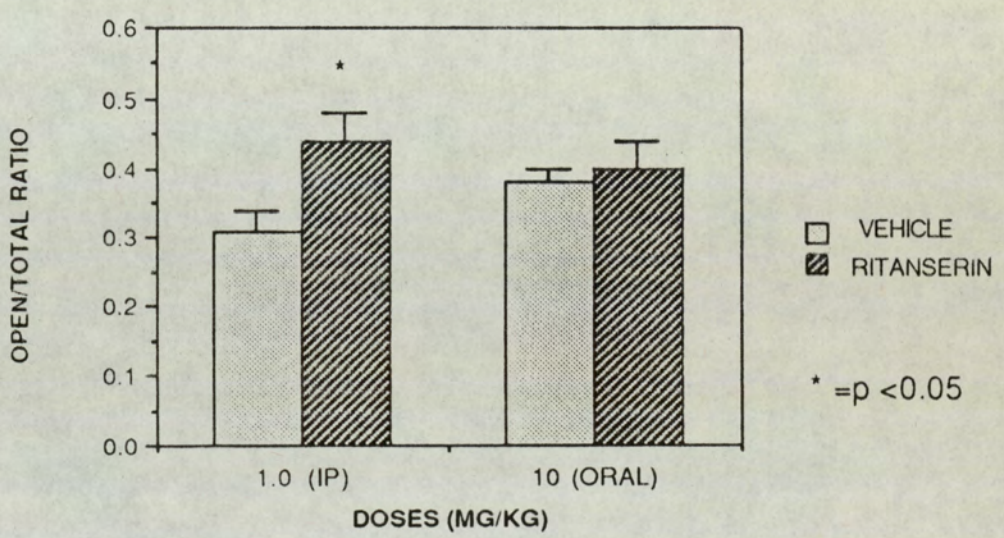


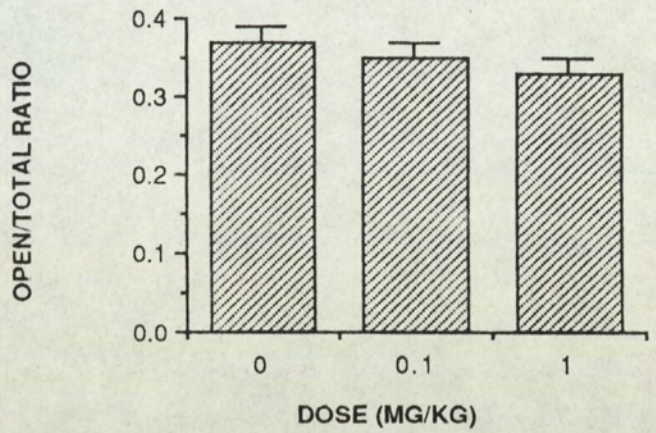
FIG 4.11 EFFECT OF RITANSERIN ON X-MAZE BEHAVIOUR.



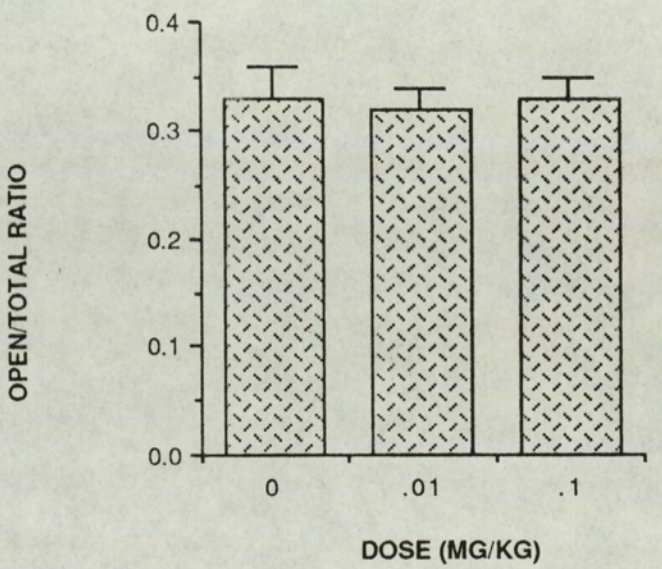
Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG.4.12.1 EFFECT OF BRL 24924 AND ICS 205-930

ON X-MAZE BEHAVIOUR



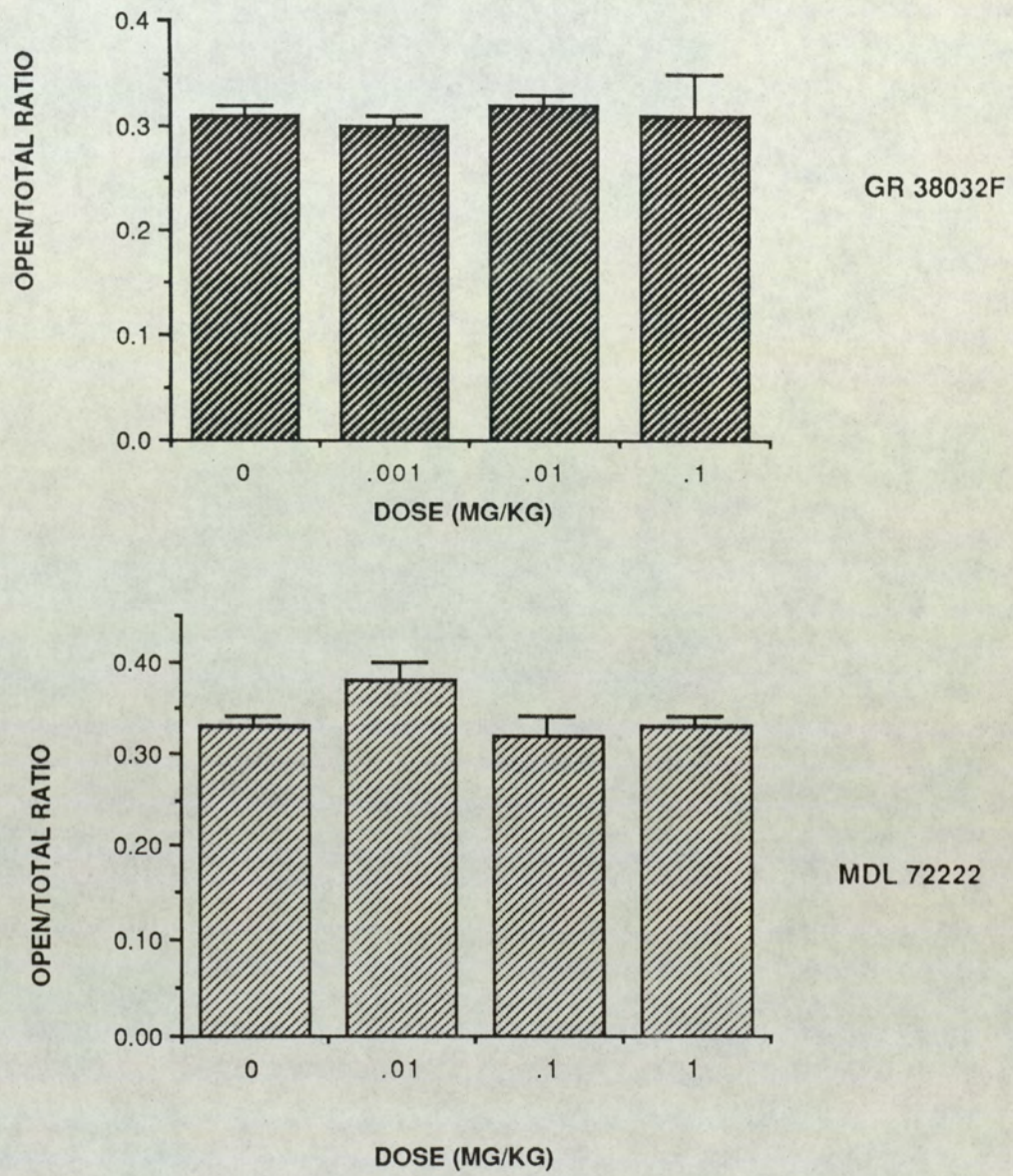
BRL 24924



ICS 205-930

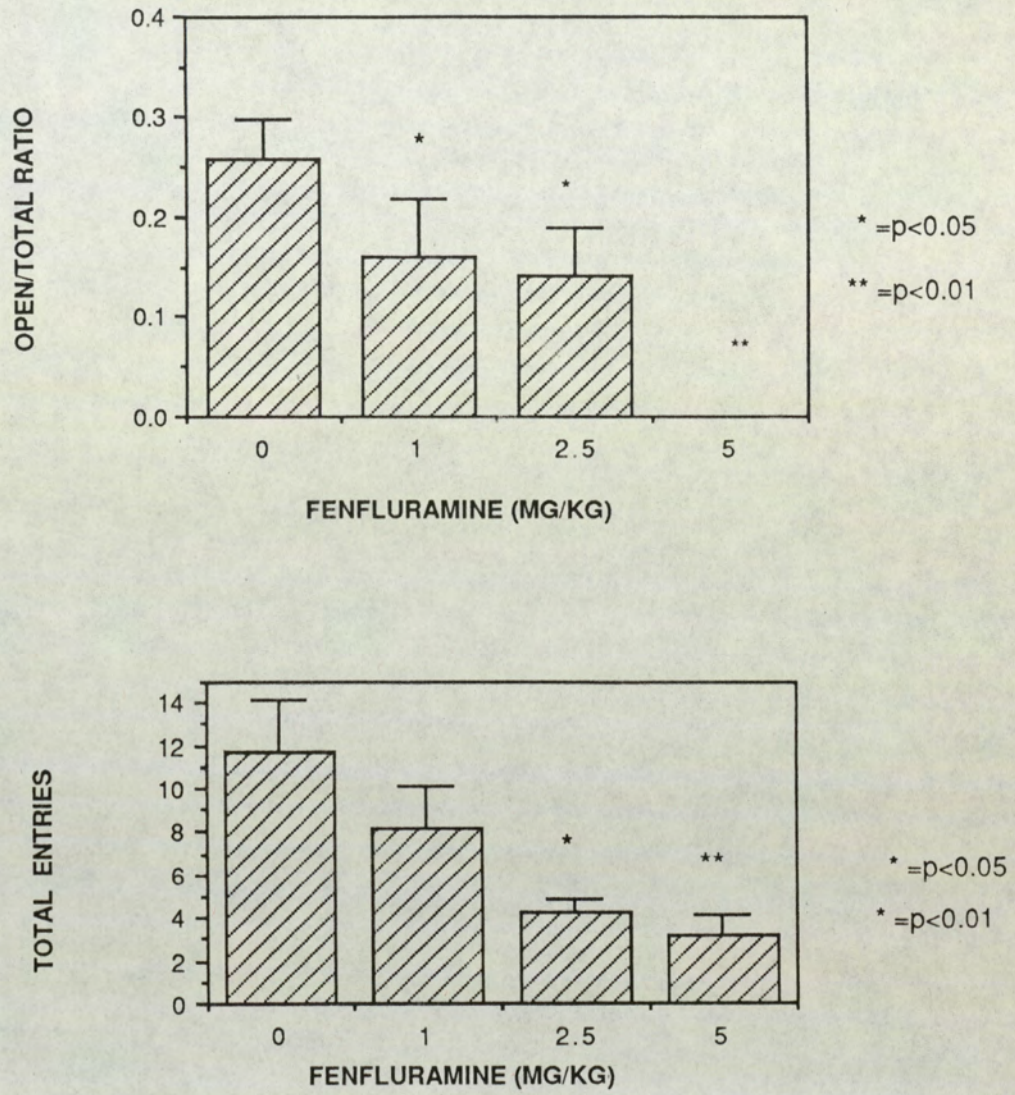
Effects on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 4.12.2 EFFECT OF GR 38032F AND MDL 72222 ON X-MAZE BEHAVIOUR



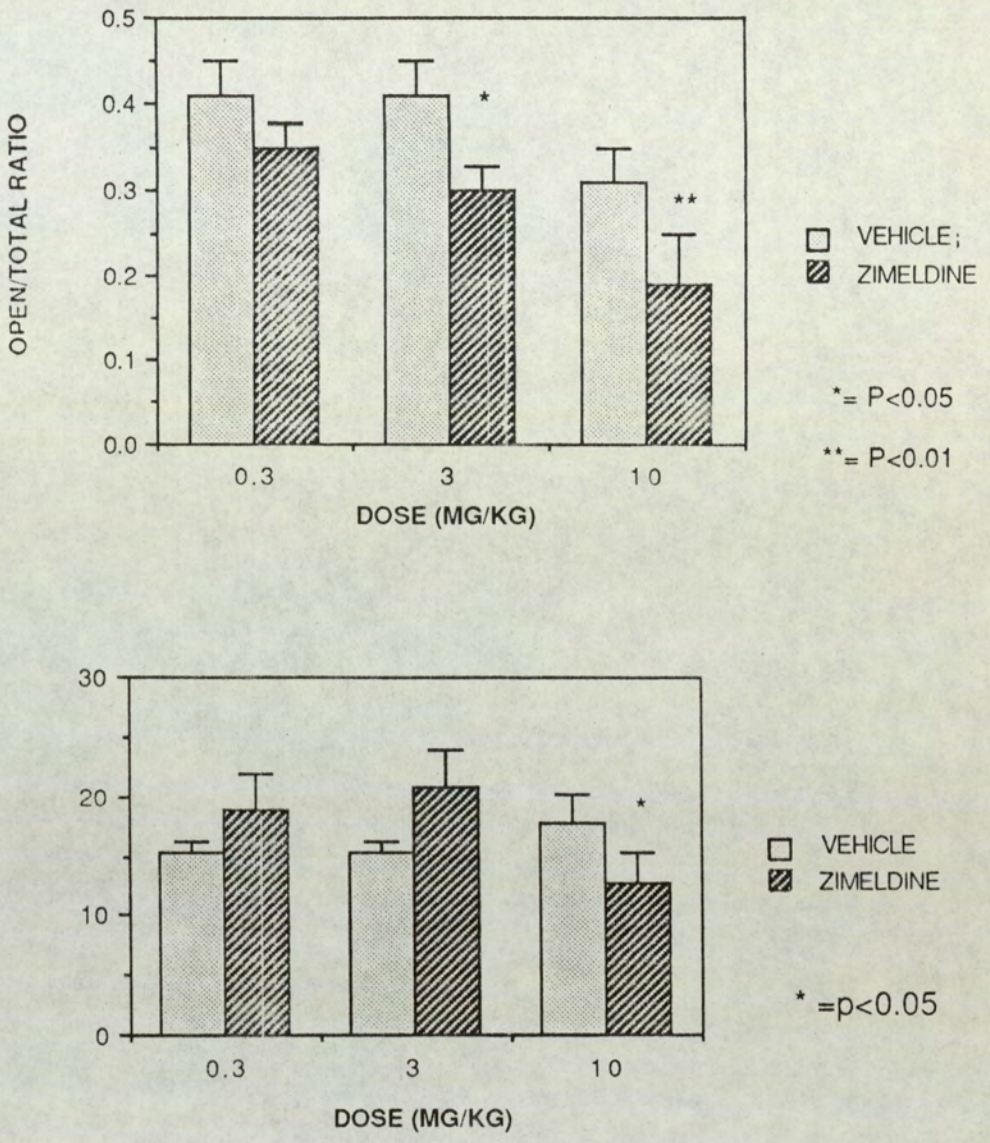
Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 4.13 EFFECT OF FENFLURAMINE ON X-MAZE BEHAVIOUR



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 4.14 EFFECT OF ZIMELDINE IN X-MAZE.



Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 4.15 EFFECT OF MEPYRAMINE ON X-MAZE BEHAVIOUR

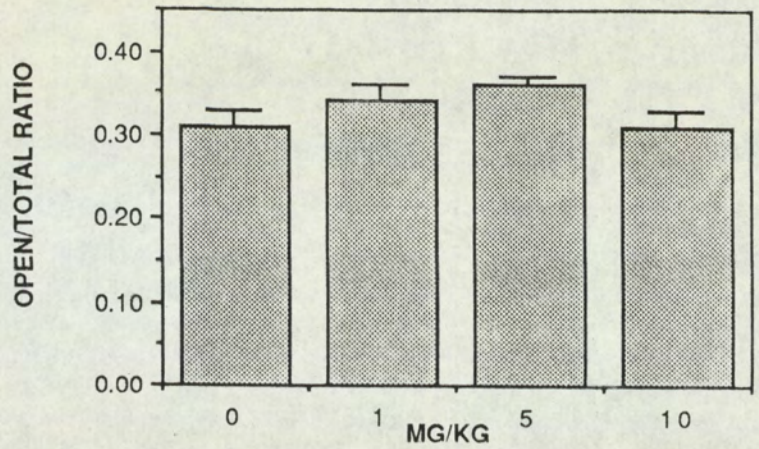
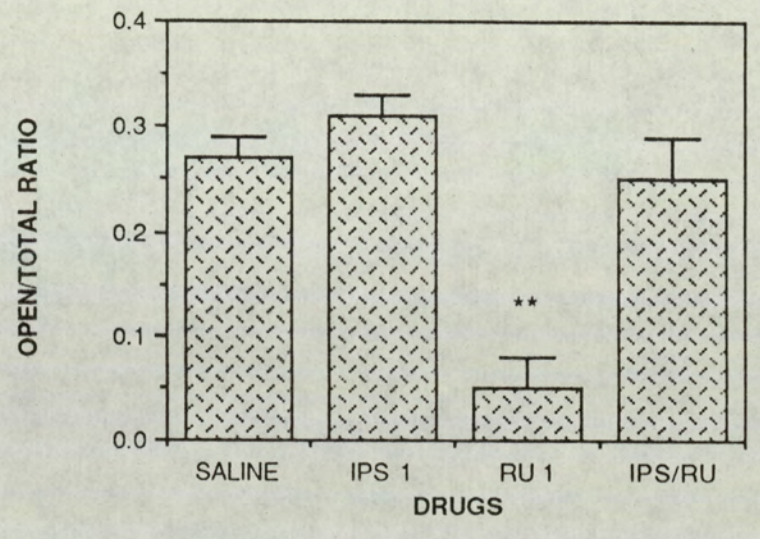


FIG 4.16 EFFECT OF IPSAPIRONE ON RU 24969 ANXIOGENIC EFFECT



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test (Fig 4.15) or a 2-way ANOVA (Fig 4.16).

FIG 4.17 EFFECT OF IPSAPIRONE ON ANXIOGENIC EFFECTS OF BAYR 1531.

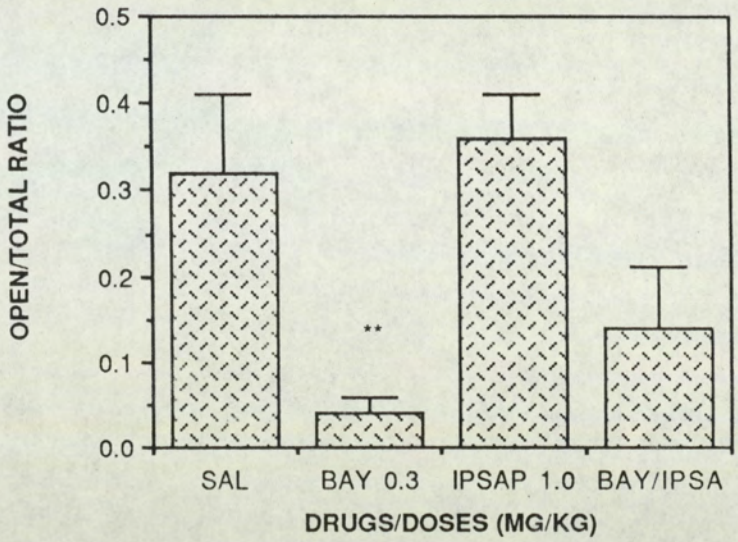
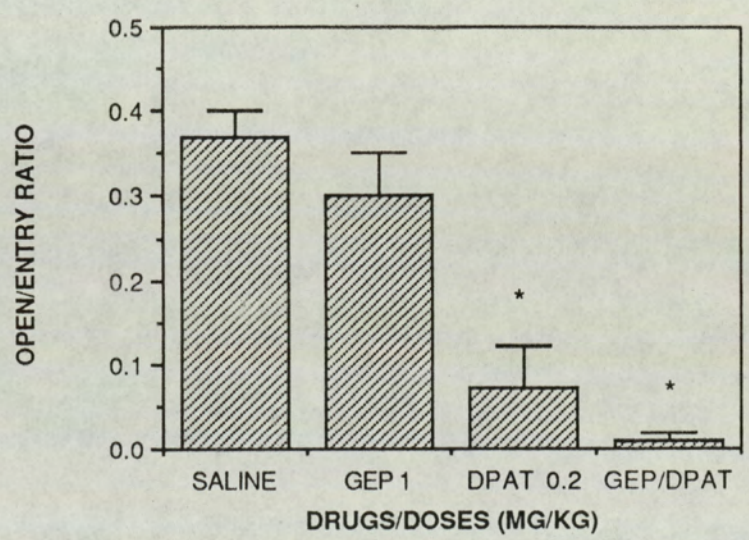
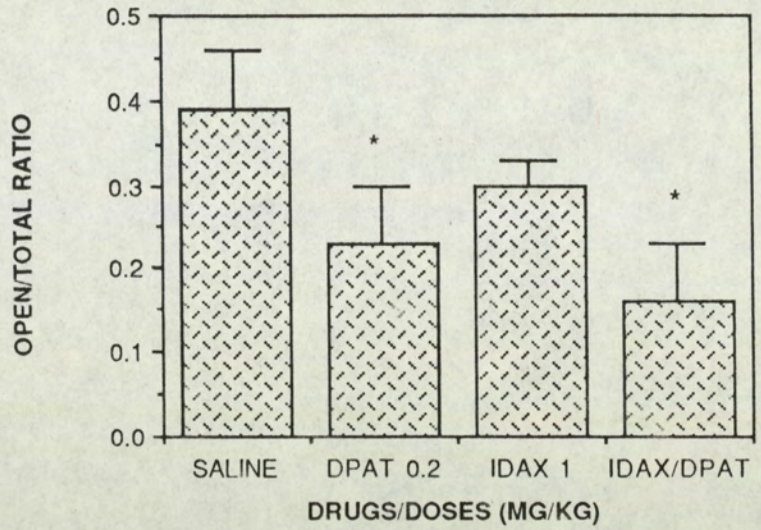


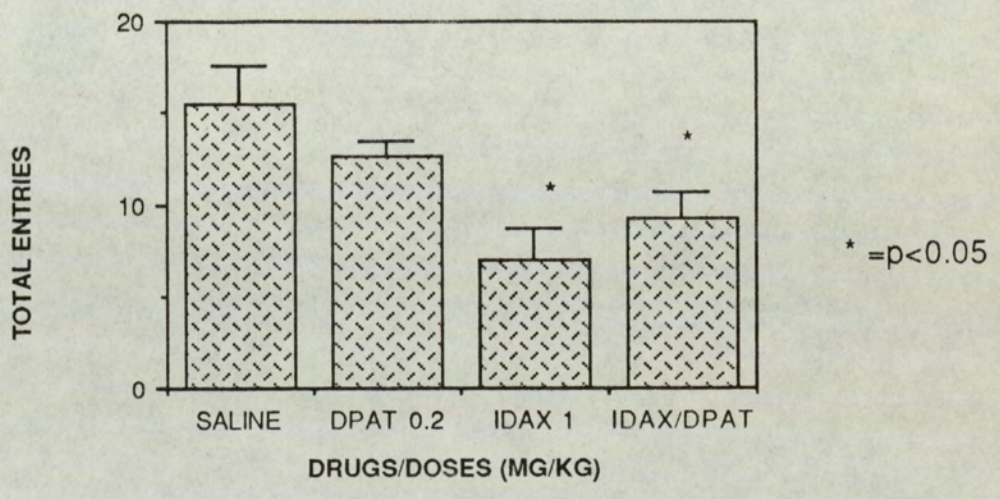
FIG 4.18 EFFECT OF GEPIRONE ON 8-OHDPAT ANXIOGENIC EFFECT.



Effect on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 4.19 IDAXOZAN/8-OHDPAT INTERACTION IN X-MAZE





Effect on the total/open entry ratio and on the number of total entries. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 4.20 INTERACTION OF RITANSERIN AND DOI IN X-MAZE.

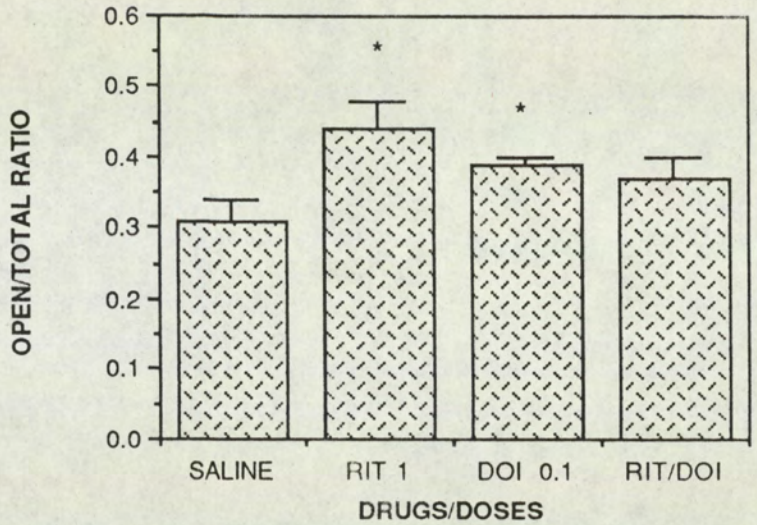
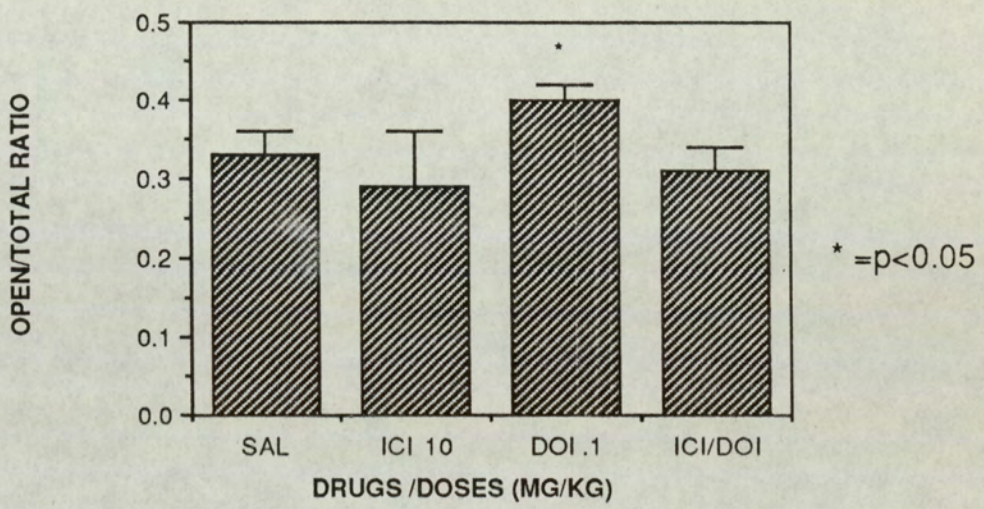
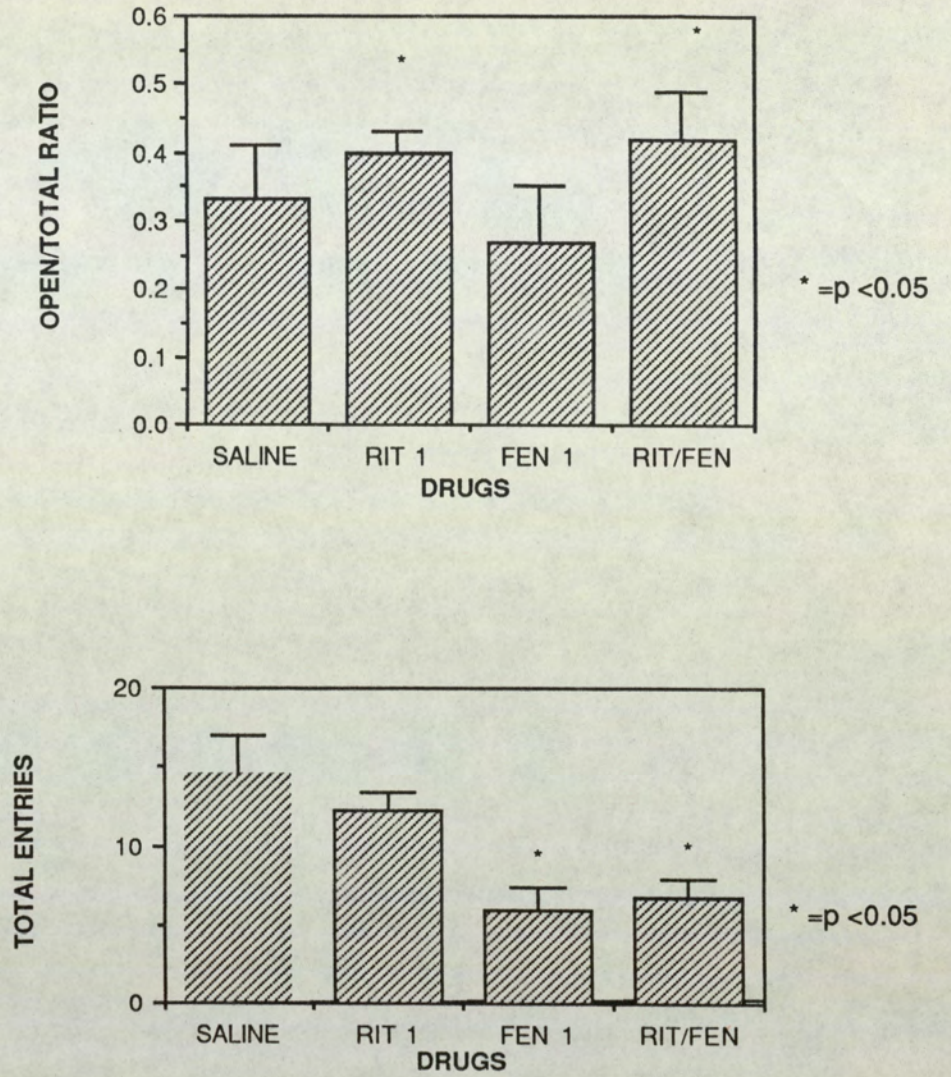


FIG. 4.21 EFFECT OF ICI 169,369 ON DOI EFFECTS IN X-MAZE.



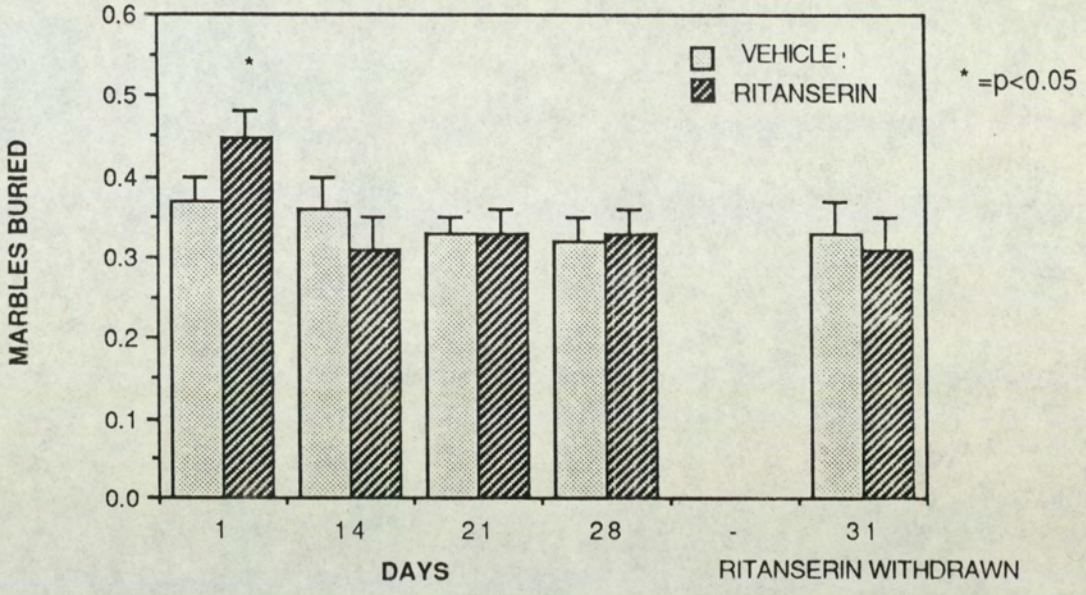
Effect on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 4.22 INTERACTION OF RITANSERIN AND FENFLURAMINE IN X-MAZE



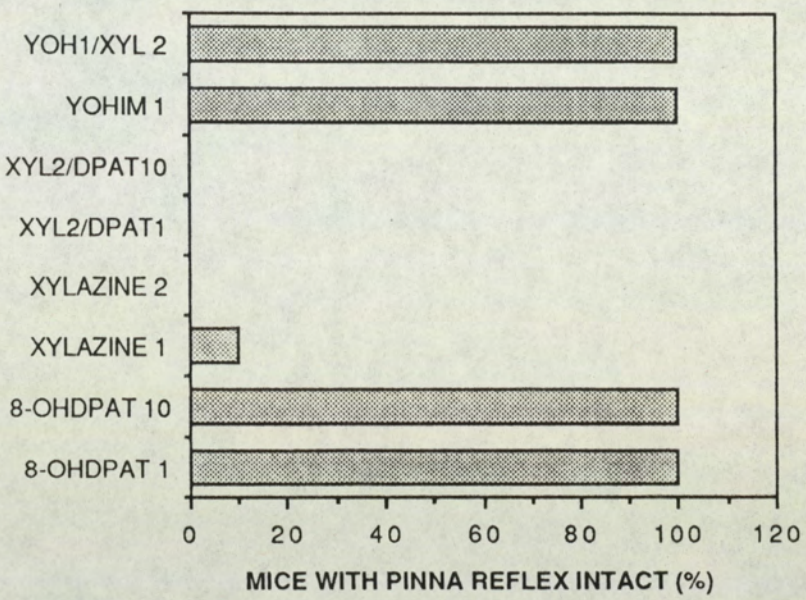
Effect on the total/open entry ratio and on the number of total entries. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 4.23 EFFECT OF CHRONIC RITANSERIN IN X-MAZE.



Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 4.24 EFFECT OF XYLAZINE,YOHIMBINE AND 8-OHDPAT ON PINNA REFLEX IN MICE.

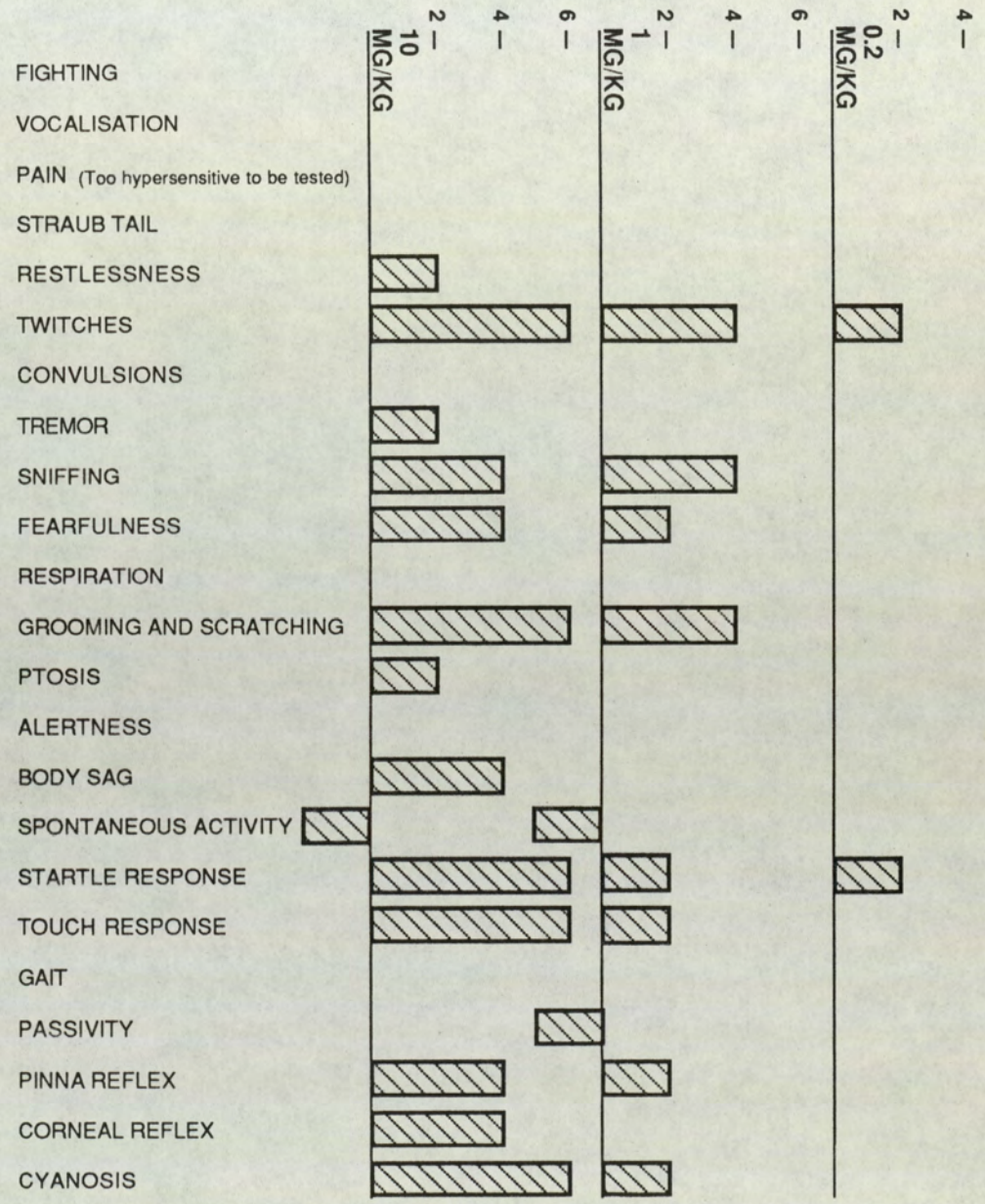


The percentage of mice with the pinna reflex intact on testing 40 min. after drug injection. Yoh = yohimbine, DPAT = 8-OH-DPAT, Xyl = xylazine. Doses are in mg/kg.

100 % - pinna reflex present in all mice in group.

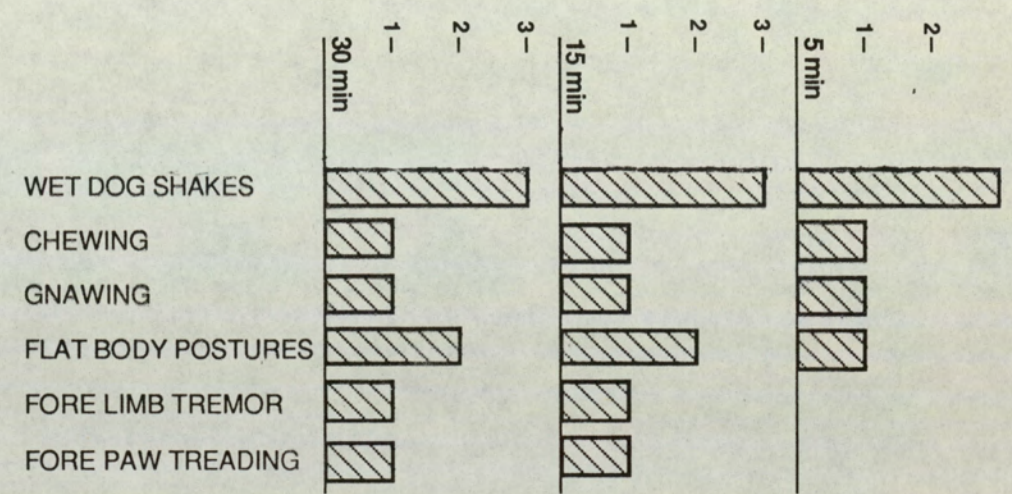
0 % - pinna reflex abolished in all mice.

FIG. 4.25 Effect of (±) DOI on mouse behaviour intensity 15 min after IP injection



Behaviour intensity was scored on the scale +6 to -6 using the criteria of Irwin (1968) 15 minutes after the injection of 0.2, 1.0 and 10 mg/kg of DOI i.p. in mice.

Fig. 4.26 Effect of (±) DOI 5 mg/kg on rat gross behaviour



Intensity of 5-HT mediated behaviour was scored on a scale of 0 to 3 at 5, 15 and 30 minutes after i.p. injection of DOI at 5 mg/kg.

CHAPTER 5.

EFFECTS OF AGENTS ACTING ON BETA-ADRENOCEPTORS IN THE ELEVATED X-MAZE MODEL OF ANXIETY.

INTRODUCTION.

Beta-adrenoceptors antagonists have been known to be effective in alleviating symptoms of anxiety in humans for a long time (Harris et al 1964). These workers showed that blockade of beta-adrenoceptors with pronethalol reduced the circulatory and respiratory changes associated with induced fear and anger. Subsequently, Harris (1965) reported that propranolol reduces the haemodynamic symptoms in chronic anxiety states. The first comprehensive study (Granville-Grossman and Turner 1966) showed that propranolol was preferred to placebo on psychiatrists' rating of anxious patients although the improvement in anxiety and tension was not significant. Since then other beta- adrenoceptor antagonists including alprenolol (Nordenfelt et al 1968), oxprenolol (Krishnan 1976), practolol (Bonn et al 1972), metoprolol (Chaturvedi 1985) have been shown to be superior to placebo in clinical trials.

Controlled studies with beta-adrenoceptor antagonists in patients with anxiety disorders indicate that these compounds produce improvements in somatic symptoms like palpitations, tremor and sweating but had little effect on psychological symptoms (Granville-Grossman and Turner 1966, Tyrer and Lader 1974, Kelly 1980, Noyes 1985). Unlike diazepam, propranolol had no effect on self-reported anxiety in patients with psychic anxiety (Tyrer and Lader 1974). That this anxiolytic activity is

primarily mediated peripherally is supported by the finding that practolol, which enters the CNS only very poorly, produced improvement in anxious patients (Bonn et al 1972).

Most controlled trials have found beta-adrenoceptor antagonists less effective than benzodiazepines in the treatment of anxiety (Tyrer and Lader 1974, Noyes 1985). Consequently these agents have not found much clinical application in the treatment of anxiety. They have however proved to be effective in stress-related anxiety states such as stage fright (Noyes 1985, Kelly 1980) and examination nerves (Krishnan 1976).

In animal models of anxiety these compounds have produced conflicting results. Propranolol was reported to have no activity in the punished responding (Sepinwall et al 1983, Sepinwall and Cook 1978) and social interaction models of anxiety (File 1980). Oxprenolol was inactive in conditioned emotional response in the pigeon (Meissner and Zeier 1976). On the other hand, Durel et al (1986) reported that both propranolol and atenolol increased punished responding in pigeons. Pindolol was anxiolytic in the elevated X-maze at low doses and anxiogenic at high doses while alprenolol was without effect (Critchley and Handley 1987, Critchley 1988).

Beta-adrenoceptor antagonists that are not selective for either beta-1 and beta-2 adrenoceptors bind to 5-HT1

receptors at nanomolar concentrations (Middlemiss et al 1977, Green et al 1983, Nahorski and Willcocks 1983). Those that block either beta- 1 or beta-2 receptors have very low affinity for 5-HT receptors ((Middlemiss et al 1977, Green et al 1983, Nahorski and Willcocks 1983). The non-selective beta-1 and beta-2 adrenoceptor antagonists block 5-HT mediated behavioural effects such as hyperactivity, hypothermia and the behavioural syndrome (Costain and Green 1985, Goodwin and Green 1985, see Chapter 1).

The anxiolytic activity in man of selective beta-1-adrenoceptor antagonists like metoprolol (Chaturvedi 1985) and practolol (Bonn et al 1972) which lack effects at 5-HT receptors suggests that the clinical anxiolytic effects of these compounds are mediated by beta-adrenoceptors rather than 5-HT receptors. There are no reports of the effects of these compounds in animal models of anxiety.

In contrast to the anxiolytic effects of the beta-adrenoceptor antagonists, some beta-adrenoceptor agonists have been reported to induce anxiety in humans (Gorman et al 1987, Noyes 1985, Pitts and Allen 1982). Intravenous adrenaline provoked panic anxiety in patients who are prone to panic attacks but not in normal subjects (Pitts and Allen 1982, Suzman 1981). Isoprenaline, a beta-adrenoceptor agonist which does not readily penetrate

the blood brain barrier induced panic anxiety in patients with a history of panic attacks (Gorman et al 1987, Pitts and Allen 1982). Isoprenaline-induced panic attacks were blocked by intravenous propranolol (Gorman et al 1987). Isoprenaline induced panic attacks occur more commonly in panic prone patients than in normal subjects (Gorman et al 1987, Pitts and Allen 1982). However, even in anxiety-prone patients isoprenaline did not always provoke panic attacks (Gorman et al 1987, Pitts and Allen 1982). There are no reports of selective beta-1 or beta-2 adrenoceptor agonists such as clenbuterol and salbutamol having anxiogenic effects in humans.

The beta-adrenoceptor agonists dobutamine and clenbuterol stimulate central 5-HT turnover (Waldmeier 1981) and facilitate 5-HT mediated hyperactivity (Cowen et al 1982, Ortmann et al 1981), tremor (Hallberg 1986), hypothermia and motor responses (Green et al 1986, Goodwin et al 1987) in animals. Both the beta- 1 adrenoceptor agonists dobutamine and procaterol and the beta- 2 adrenoceptor agonists clenbuterol and salbutamol potentiated 5-HT mediated head twitch (Handley and Singh 1984, 1986). As discussed in Chapter 1 these effects are mediated by beta-adrenoceptors rather than 5-HT receptors since they are blocked by selective beta-1 and beta-2 antagonists which have very low affinity for 5-HT receptors.

This work sought to establish whether beta-adrenoceptors

agonists and antagonists have any effects on the elevated X-maze model of anxiety and whether such effects are mediated by beta-adrenoceptors or by 5-HT receptors. With the antagonists the choice of doses used in the study was governed by the experience with propranolol where only the highest doses had effects on the open/total arm entry ratio. Drug doses were increased until some behavioural effect was obtained or we ran into solubility problems. With the beta-2 adrenoceptor agonists the doses were limited by reduction in the number of total arm entries which occured at relatively low doses.

RESULTS.

5.1 Effects of beta-adrenoceptor antagonists.

5.1.1 Effects of propranolol.

Propranolol at 0.1-0.5 mg/kg produced a slight increase in the open/total arm entry ratio without changing the total number of arm entries. At 1 and 2.5 mg/kg there was no change in either the open/total arm entry ratio or the total number of arm entries. Propranolol at 5 and 10 mg/kg produced a significant decrease in the open/total arm entry ratio without changing the total number of arm entries (Table 5.1 and Fig. 5.1).

5.1.2 Effects of timolol and sotalol.

Timolol at 3 to 40 mg/kg and sotalol at 1 to 10 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 5.2 and Fig. 5.2).

5.1.3 Effects of ICI 118,551.

ICI 118,551 at 0.5 to 2 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 5.3 and Fig. 5.3).

5.1.4 Effects of metoprolol and practolol.

Metoprolol at 1 to 10 mg/kg and practolol at 1 to 10 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 5.3 and Fig 5.3).

5.2 Effects of beta-adrenoceptors agonists.

5.2.1 Effects of clenbuterol.

Clenbuterol at 0.01 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries. At 0.03 to 2.5 mg/kg, clenbuterol produced a significant reduction in both the open/total arm entry

ratio and total number of arm entries (Table 5.4 and Fig. 5.4).

5.2.2 Effects of terbutaline.

Terbutaline at 0.1 and 0.5 mg/kg decreased the total number of arm entries but had no effects on the open/total arm entry ratio (Table 5.4 and Fig. 5.5).

5.2.3 Effects of dobutamine.

Dobutamine at 0.3 to 3.0 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 5.5 and Fig. 5.6).

5.2.4 Effects of adrenaline.

Adrenaline at 0.03 to 0.15 mg/kg produced gross effects on animals. These included writhing movements, exophthalmia, motor incoordination including dragging of hindlimbs and obvious discomfort. At 0.03 and 0.06 mg/kg adrenaline did not change either the open/total arm entry ratio or the total number of arm entries but at 0.15 mg/kg both the measures were sharply decreased (Table 5.5 and 5.7).

5.3 Drug interactions.

5.3.1 Timolol and 8-OH-DPAT.

Timolol at 3 and 40 mg/kg had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 5.6 and Fig. 5.8).

8-OH-DPAT at 0.2 mg/kg reduced open total arm entry ratio without changing total arm entries.

Timolol at 3 and 40 mg/kg administered 20 minutes before 8-OH-DPAT reduced the 8-OH-DPAT reduction of the open/total arm entry ratio without affecting total arm entries (Table 5.6 and Fig. 5.8).

5.3.2 Propranolol and 8-OH-DPAT.

Propranolol at 3 mg/kg had no effects on either the

open/total arm entry ratio or the total number of arm entries (Table 5.6 and Fig 5.9).

8-OH-DPAT at 0.1 mg/kg produced a significant reduction in the open/total arm entry ratio but had no effect on the total number of arm entries (Table 5.6 and Fig 5.9).

Propranolol at 3 mg/kg administered 20 minutes before 8-OH-DPAT blocked the latter compounds effect on the open/ total arm entry ratio. The drug combination had no effects on the total number of arm entries (Table 5.6 and Fig 5.9).

5.3.3 Metoprolol and 8-OH-DPAT.

Metoprolol at 10 mg/kg had no effects on either open/total arm entry ratio or total arm entries (Table 5.7 and Fig. 5.10).

8-OH-DPAT at 0.2 mg/kg reduced open total arm entry ratio without affecting total arm entries.

Metoprolol at 10 mg/kg administered 20 minutes before 8-OH-DPAT did not significantly affect the 8-OH-DPAT reduction of the open/total arm entry ratio. The combination did not affect the total number of arm entries (Table 5.7 and Fig. 5.10).

5.3.4 ICI 118,551 and 8-OH-DPAT.

ICI 118,551 at 10 mg/kg had no effects on either open total arm entry ratio or total arm entries (Table 5.7 and Fig. 5.11).

8-OH-DPAT at 0.2 mg/kg reduced open total arm entry ratio without affecting total arm entries.

ICI 118,551 at 10 mg/kg administered 20 minutes before 8-OH-DPAT did not significantly affect the 8-OH-DPAT reduction of the open/total arm entry ratio. The combination did not affect the total number of arm entries (Table 5.7 and Fig. 5.11).

5.3.5 Clenbuterol and 8-OH-DPAT.

Clenbuterol at 0.05 mg/kg produced a large increase in the open total arm entry ratio and a sharp decrease in the total number of arm entries. This increase in the ratio was due to 2 rats that made only one arm entry, into an open arm.

8-OH-DPAT at 0.2 mg/kg reduced open total arm entry ratio without affecting total arm entries.

The beta-2-adrenoceptor agonist clenbuterol 10 minutes before did not affect 8-OH-DPAT anxiogenic like effects but it reduced the total number of arm entries (Table 5.8 and Fig. 5.12).

5.3.6 Ipsapirone and Timolol.

Neither timolol at 10 mg/kg nor ipsapirone at 1 mg/kg had any effects on either open total arm entry ratio or total arm entries (Table 5.9 and Fig. 5.13).

Timolol at 10 mg/kg administered 10 minutes before ipsapirone produced a slight but insignificant increase in the open/total arm entry ratio. There was no change in the total number of arm entries (Table 5.9 and Fig. 5.13).

DISCUSSION

Beta-adrenoceptors antagonists other than propranolol did not produce any effects on their own in the X-maze model of anxiety. High doses of propranolol (5 and 10 mg/kg) had anxiogenic effects while at lower doses there was a tendency to anxiolytic effects. A similar biphasic effect has been reported with pindolol which was anxiolytic at low doses and anxiogenic at higher doses (Critchley and Handley 1987). This effect is most probably due to the fact that both compounds have mixed 5-HT agonist/antagonist properties (Maura et al 1987, Hjorth and Carlsson 1986).

That both compounds have antagonist properties is well documented (Costain and Green 1978, Goodwin and Green 1985, Middlemiss 1986a, see Chapter 1). Hjorth and Carlsson (1986) found that pindolol had 5-HT agonist effects and in a dose dependent manner reduced brain 5-HT synthesis and release/turnover. They proposed that pindolol acts as an agonist at the 5-HT autoreceptor and as antagonist at the postsynaptic 5-HT receptor. However, Maura et al (1987) found that propranolol and cyanopindolol had agonist and antagonist properties at the 5-HT autoreceptor depending on experimental conditions. Whether these compounds act as agonists or antagonists at 5-HT receptors in vivo depends on several factors including the concentration of the transmitter at the receptor biophase (Maura et al 1987).

The combined beta-1/beta-2 adrenoceptor antagonists, timolol and propranolol, while having no effects alone in the X-maze reversed the anxiogenic-like effects of 8-OH-DPAT. The selective beta-1-adrenoceptor antagonist metoprolol (Ablad et al 1975) and the selective beta-2-adrenoceptor antagonist ICI 118,551 (Bilski et al 1983) had no effects in X-maze and did not reverse the

anxiogenic-like effects of 8-OH-DPAT. This tallies with the finding that combined beta-1/beta-2 antagonists but not the selective beta-1 and beta-2-adrenoceptor antagonists bind to 5-HT1 receptors (Middlemiss et al 1977, Nahorski and Willcocks 1983) and antagonise 5-HT mediated behaviour (Costain and Green 1978). This would indicate that their effects on the anxiogenic-like effects of 8-OH-DPAT were due to their 5-HT1A effects rather than their effects on beta-adrenoceptors.

Timolol had additive effects to the effects of ipsapirone in the X-maze. This is in contrast to the findings of Boaventura et al (1987) who reported that the beta-adrenoceptor antagonists pindolol and penbutolol reversed the anxiolytic effect of ipsapirone on 3 animal models of anxiety :the staircase, 4-plate and Vogel conflict tests. This suggests that on this model both agents are acting in the same way, most probably as antagonists at 5-HT1 receptors. There is evidence that mixed beta-1/ beta-2 adrenoceptor antagonists exert their 5-HT antagonist effects at [5-HT1A] cell-body autoreceptors (Middlemiss 1986a) and at [5-HT1B] presynaptic terminal autoreceptors (Hamon et al 1986). Antagonist effects at either receptor would be analogous as agonists at terminal 5-HT1B receptors reduce 5-HT release and agonists at 5-HT1A autoreceptors reduce firing of 5-HT neurones.

Of the beta-2-adrenoceptor agonists, terbutaline reduced only locomotor activity without altering the open/total ratio. The effects of clenbuterol are difficult to interpret. The compound reduced the open/enclosed arm ratio but this was accompanied by a reduction in the number of total arm entries, suggesting the effects may not be specific to anxiety. Since terbutaline hardly enters the CNS (Bodin et al 1972) it appears that the reduction in the number of arm entries by beta-2-adrenoceptor agonists could be peripherally

mediated. There is evidence that the motor suppressant effect of beta-adrenoceptor agonists has a peripheral component: practolol, a beta-adrenoceptor antagonist which penetrates the brain very poorly attenuates the inhibition of locomotor activity by isoprenaline (Frances et al 1979, 1987). This would suggest that the anxiogenic element seen with clenbuterol but not with terbutaline is centrally mediated; unlike terbutaline clenbuterol enters the CNS (Frances et al 1983).

The beta-1-adrenoceptor agonist dobutamine did not affect X-maze behaviour while the non-selective agonist adrenaline produced gross behavioural effects and only reduced the open total arm entry ratio and total number of arm entries at the highest dose used.

The anxiolytic effects of beta-adrenoceptor antagonists in humans (Bonn et al 1972, Noyes 1985, Kelly 1980) and the anxiogenic effects of adrenaline (Pitts and Allen 1982) are both considered to be peripherally-mediated. The inactivity of both beta-adrenoceptor agonists and antagonists on the X-maze suggest that the test is not sensitive to peripheral effects on anxiety. It also suggests that the anxiogenic effects of high doses of propranolol reported here are most likely to be mediated by the compounds central effects on 5-HT1A receptors. This concurs with the finding that pindolol, which has greater binding affinity at 5-HT1 receptors than propranolol (Middlemiss et al 1977) had pronounced effects on the X-maze test (Critchley and Handley 1987).

Table 5.1: Effect of propranolol on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Propranolol			
0.1	3.6(1.3) [4.2(0.7)]	9.0(2.9) [11.3(1.2)]	0.40(.03) [0.36(.03)]
0.25	3.2(0.7) [2.0(0.5)]	10.6(1.7) [6.8(1.2)]	0.29(.13) [0.29(.13)]
0.5	4.7(1.0) [4.2(0.7)]	10.3(0.9) [11.3(1.2)]	0.44(.07) [0.36(.03)]
1.0	3.5(1.0) [5.0(0.7)]	12.2(3.1) [15.7(1.4)]	0.28(.02) [0.31(.02)]
2.5	3.0(0.6) [3.6(0.8)]	10.8(1.6) [10.8(1.8)]	0.27(.03) [0.33(.01)]
5.0	3.2(0.5) [3.6(0.8)]	13.5(1.4) [10.8(1.8)]	0.22(.02)** [0.33(.01)]
10	2.2(0.5)** [5.0(0.7)]	11.7(1.7) [15.7(1.4)]	0.18(.03)** [0.31(.02)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.2: Effect of timolol and sotalol on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values]

Drug/dose	Open	Total	Open/total
Timolol			
3	5.0(0.3) [5.0(1.0)]	13.0(1.3) [16.8(2.2)]	0.39(.02) [0.30(.04)]
10	5.5(0.8) [5.7(0.7)]	14.3(1.1) [16.8(1.6)]	0.38(.04) [0.33(.02)]
20	5.2(0.8) [6.0(0.9)]	16.2(2.3) [19.3(1.9)]	0.32(.03) [0.31(.03)]
40	7.4(0.9) [6.6(0.9)]	21.4(2.0) [17.6(2.4)]	0.35(.03) [0.37(.01)]
Sotalol			
1	6.5(1.1) [6.5(0.6)]	13.8(1.2) [16.0(1.2)]	0.42(.04) [0.40(.02)]
10	8.2(0.9) [6.5(0.6)]	19.0(0.8) [16.0(1.2)]	0.43(.04) [0.40(.02)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.3: Effects of selective beta-1 and beta-2 adrenoceptor antagonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m.)

[vehicle control values]

Drug/dose	Open	Total	Open/total
ICI 118,551			
0.5	5.6(1.0) [4.7(0.9)]	15.2(1.3) [15.4(2.1)]	0.39(.03) [0.35(.01)]
1.0	5.8(0.9) [6.0(0.6)]	15.5(1.4) [16.5(1.4)]	0.37(.04) [0.37(.03)]
2.0	5.7(0.8) [6.0(0.6)]	16.2(1.8) [16.5(1.4)]	0.34(.02) [0.37(.03)]
Metoprolol			
1	4.3(0.3) [4.3(1.0)]	13.3(0.8) [12.2(2.0)]	0.33(.01) [0.31(.05)]
3	4.7(0.6) [6.8(1.2)]	13.4(1.2) [16.8(2.9)]	0.35(.03) [0.38(.02)]
10	4.0(0.9) [6.8(1.2)]	11.8(1.6) [16.8(2.9)]	0.32(.03) [0.38(.02)]
Practolol			
1	2.5(0.5) [3.4(0.8)]	7.2(0.9) [10.8(2.6)]	0.33(.04) [0.32(.02)
2.5	6.8(1.5) [6.0(1.5)]	15.2(2.6) [15.3(3.3)]	0.42(.02) [0.39(.03)]
5	4.4(1.5) [3.4(0.8)]	11.4(2.4) [10.8(2.6)]	0.32(.04) [0.32(.02)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.4: Effect of selective beta-2-adrenoceptor agonists on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values] N = 6

Drug/dose	Open entries	Total entries	Open/total
Clenbuterol			
0.01	4.5(0.6) [5.8(0.3)]	13.0(1.0) [16.0(0.5)]	0.34(.03) 0.36(.03)
0.03	3.5(0.4)* [8.7(1.0)]	9.3(1.5)* 18.0(2.1)	0.39(.03)* 0.48(.02)
0.1	2.5(0.6)* [8.7(1.0)]	5.5(1.5)** [18.0(2.1)]	0.23(.05)* 0.33(.02)
1.0	0.2(0.2)** [4.5(0.9)]	1.7(0.3)* [13.0(1.8)]	0.06(.06)** [0.33(.03)]
2.5	0.7(0.3)** [4.5(0.9)]	4.0(0.7)* [13.0(1.8)]	0.13(.06)* [0.33(.03)]
Terbutaline			
0.1	4.7(0.8)* [7.5(0.4)]	12.7(1.6)* [19.0(1.3)]	0.36(.03) [0.40(.02)]
0.5	4.8(1.1)* [7.5(0.4)]	10.4(2.2)** [19.0(1.3)]	0.38(.04) [0.40(.02)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.5: Effect of dobutamine and adrenaline on X-maze behaviour.

Mean number of arm entries in 10 minutes (s.e.m)

[vehicle control values] N = 6

Drug/dose	Open entries	Total entries	Open/total
Dobutamine			
0.3	3.5(0.8) [3.2(0.8)]	10.8(1.3) [9.2(2.2)]	0.31(.04) [0.35(.04)]
1.0	6.8(1.1) [6.0(1.3)]	16.2(2.4) [13.8(2.1)]	0.42(.03) [0.41(.05)]
3.0	6.5(0.8) [6.0(1.3)]	14.5(2.0) [13.8(2.1)]	0.45(.02) [0.41(.05)]
Adrenaline			
0.03	2.8(0.8) [2.5(0.6)]	8.2(1.6) [8.2(1.0)]	0.32(.08) [0.30(.04)]
0.06	4.5(0.8) [5.2(0.8)]	11.2(1.7) [13.7(1.5)]	0.40).04) [0.38(.04)]
0.15	0.6(0.2) [2.5(0.6)]	4.6(0.9)* [8.2(1.0)]	0.16(.09)* [0.30(.04)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.6: Effects of mixed beta-1/beta-2 adrenoceptor antagonists on anxiogenic-like effects of 8-OH-DPAT.

Mean number of arm entries in 10 minutes (s.e.m)

Drug/dose	Open	Total	Open/total
Saline	5.0(1.0)	16.8(2.2)	0.30(.04)
Timolol 3	5.0(0.3)	13.0(1.3)	0.39(.02)
8-OH-DPAT 0.1	2.0(1.0)*	16.0 (4.2)	0.09(.04)**
Timolol 3/ 8-OH-DPAT 0.1	7.2(1.0)	21.6(1.7)	0.33(.04)
F (A x B)= 4.6	DF=1/16	p<0.01	
Saline	6.6(0.9)	17.6(2.4)	0.37(.01)
Timolol 40	7.4(0.9)	21.4(2.0)	0.35(.03)
8-OH-DPAT 0.1	4.2(0.8)*	18.2(1.1)	0.23(.04)*
Timolol 40/ 8-OH-DPAT 0.1	7.2(0.6)	22.4(2.9)	0.34(.02)
F (A x B)= 6.9	DF=1/16	p<0.05	
Saline	6.0(0.9)	18.0(2.1)	0.33(.01)
Propranolol 3	5.2(0.7)	16.2(1.1)	0.32(.03)
8-OH-DPAT 0.1	4.3(0.8)	16.4(2.9)	0.24(.03)*
Propranolol 3/ 8-OH-DPAT 0.1	5.6(0.7)	20.0(1.9)	0.28(.03)
F (A x B)= 4.5	DF=1/16	P<0.05	

Statistical comparisons were made on raw data using a two-way ANOVA followed by a Tukeys test for unconfounded means. *= p<0.05 **= p<0.01 relative to vehicle controls.

Table 5.7: Effects of selective beta1- and beta-2 adrenoceptor antagonists on the anxiogenic-like effects of 8-OH-DPAT.

Mean number of arm entries in 10 minutes (s.e.m)

Drug/dose	Open arm	Total entries	Open/total
Saline	4.6(.36)	15.2(1.2)	0.30(.01)
Metoprolol 3	4.8(.33)	15.4(.8)	0.31(.02)
8-OH-DPAT 0.1	2.0(.61)*	18.3(1.1)	0.11(.03)**
Metoprolol/ 8-OH-DPAT 0.1	3.4(1.1)	18.6(2.2)	0.17(.05)*

F (A x B)= 0.65 Non-significant

Saline	5.8(.72)	18.4(1.6)	0.31(.01)
ICI 118,551 1	5.6(.73)	17.8(1.8)	0.31(.01)
8-OH-DPAT 0.1	6.6(.67)	26.8(3.2)	0.25(.01)*
ICI 118,551/ 8-OH-DPAT 0.1	6.8(.52)	27.6(1.0)	0.25(.01)*

F (A x B)= 0.06 Non-significant

Statistical comparisons were made on raw data using a two-way ANOVA followed by a Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.8: Effects of clenbuterol on the anxiogenic-like effects of 8-OH-DPAT.

Mean number of arm entries in 10 minutes (s.e.m)

Drug/dose	Open arm	Total entries	Open/total
Saline	3.8(0.4)	10.6(1.8)	0.34(.05)
8-OH-DPAT 0.2	0.6(0.6)**	9.8(2.3)	0.04(.04)**
Clen 0.05	2.0(0.5)	4.4(1.1)*	0.55(.02)
Clen/8-OH-DPAT	0.4(0.4)	4.0(0.8)*	0.10(.10)

F(A X B) = 0.35 Non-significant

F(B) = 9.6 DF = 1/16 $p<0.01$

F(A) = 1.2

Statistical comparisons were made on raw data using a two-way ANOVA followed by a Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 5.9: Effects of interaction of timolol on ipsapirone on X-maze behaviour.

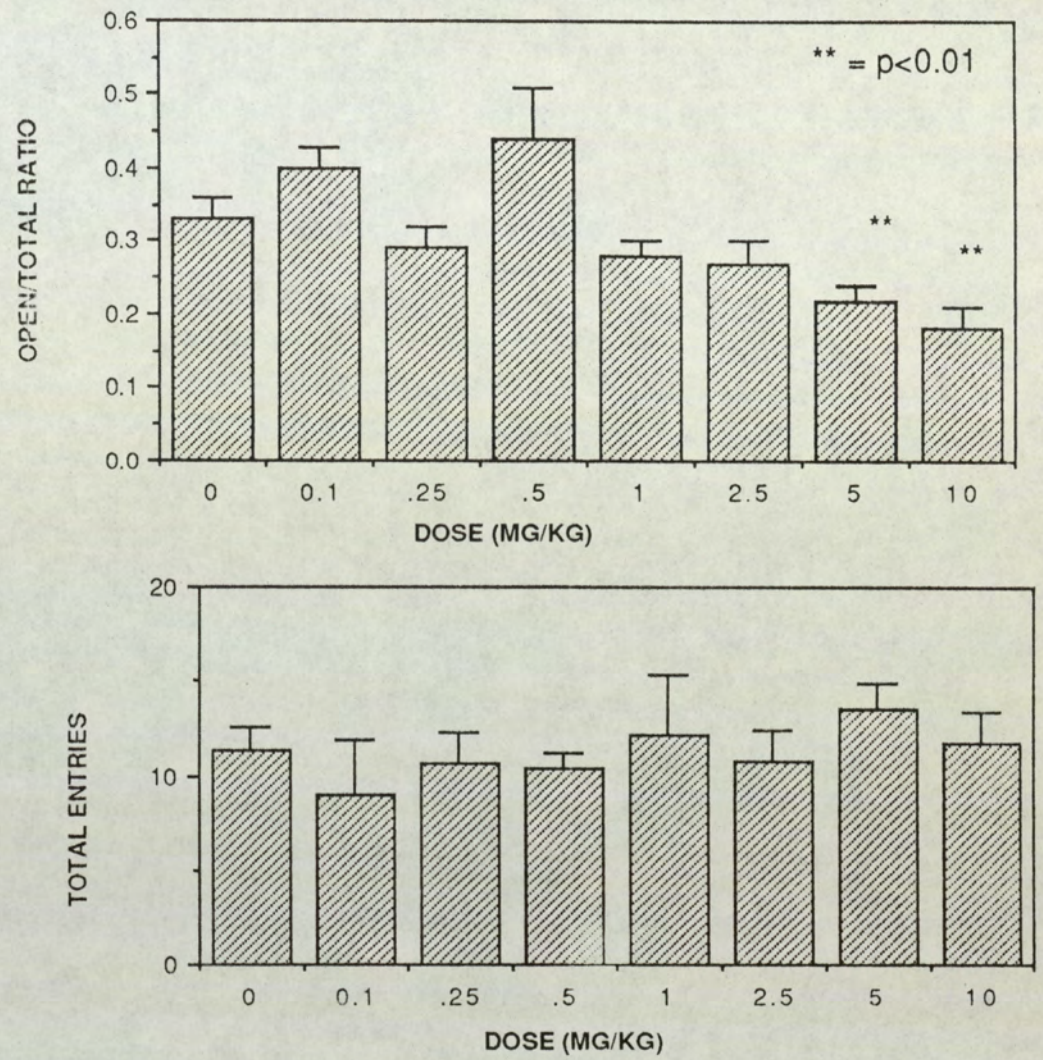
Mean number of arm entries in 10 minutes (s.e.m)

Drug/dose	Open	Total	Open/total
Saline	5.2(0.7)	15.6 (1.8)	0.33(.01)
Ipsapirone 1	7.0(1.0)	18.8 (2.4)	0.37(.01)
Timolol 3	5.0(0.7)	14.4 (1.2)	0.35(.04)
Timolol 3/ Ipsapirone 1	7.0(0.8)	16.8 (2.0)	0.42(.04)

F (A x B)= 4.51 DF=1/16 $p<0.05$

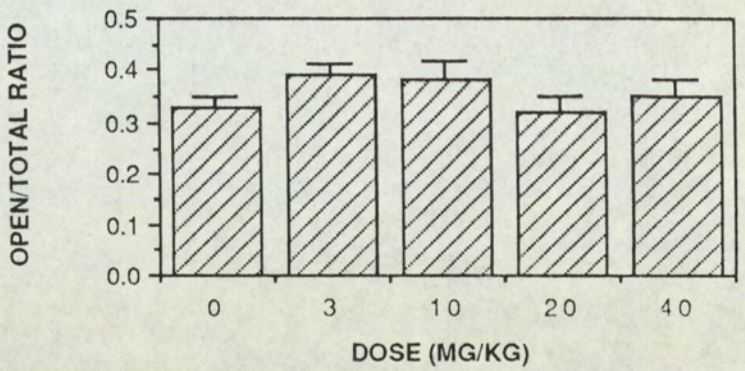
Statistical comparisons were made on raw data using an ANOVA. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

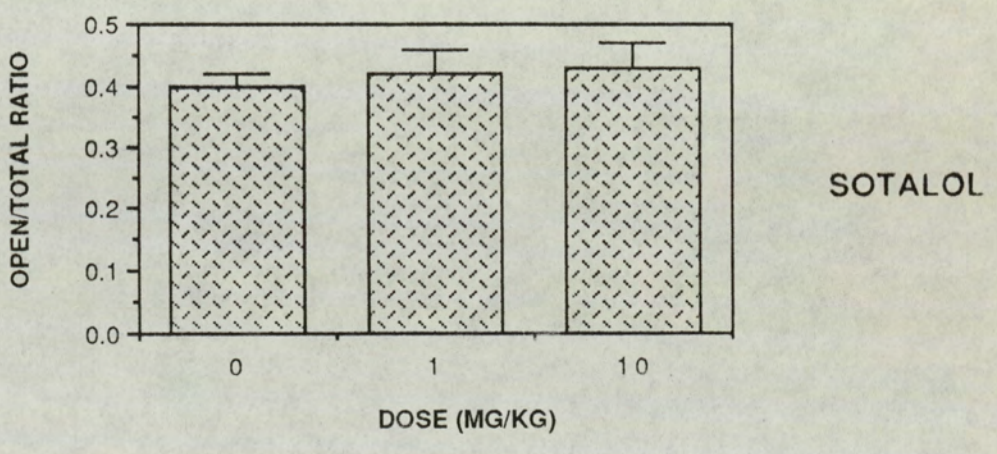
FIG. 5.1 EFFECT OF PROPRANOLOL ON X-MAZE BEHAVIOUR



Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

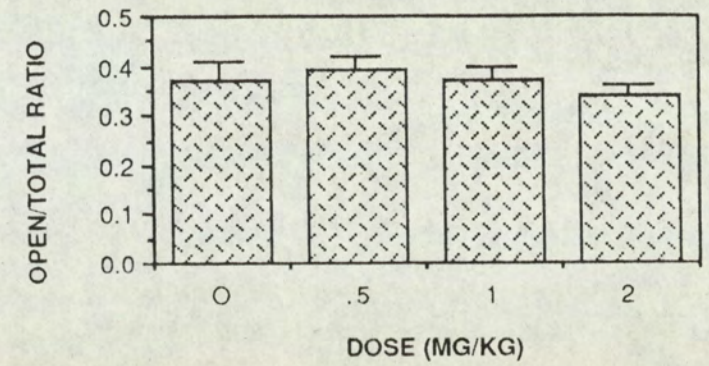
FIG. 5.2 EFFECT OF TIMOLOL AND SOTALOL ON X-MAZE BEHAVIOUR



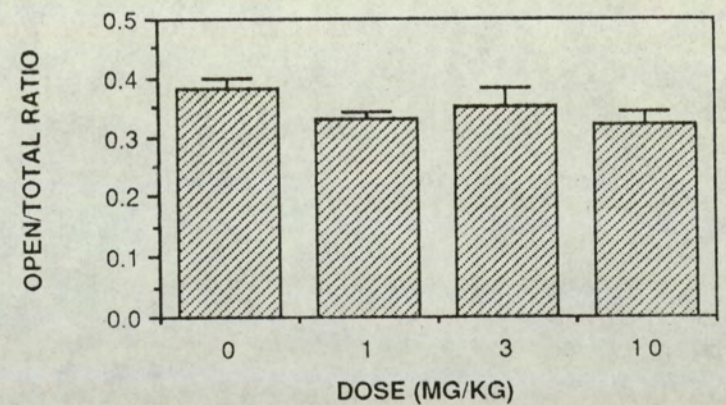


Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

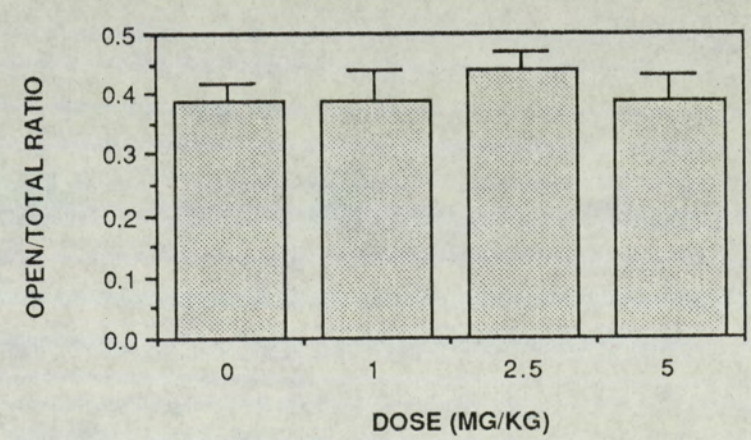
FIG 5.3 SELECTIVE BETA-ANTAGONISTS AND X-MAZE BEHAVIOUR



ICI 118,551



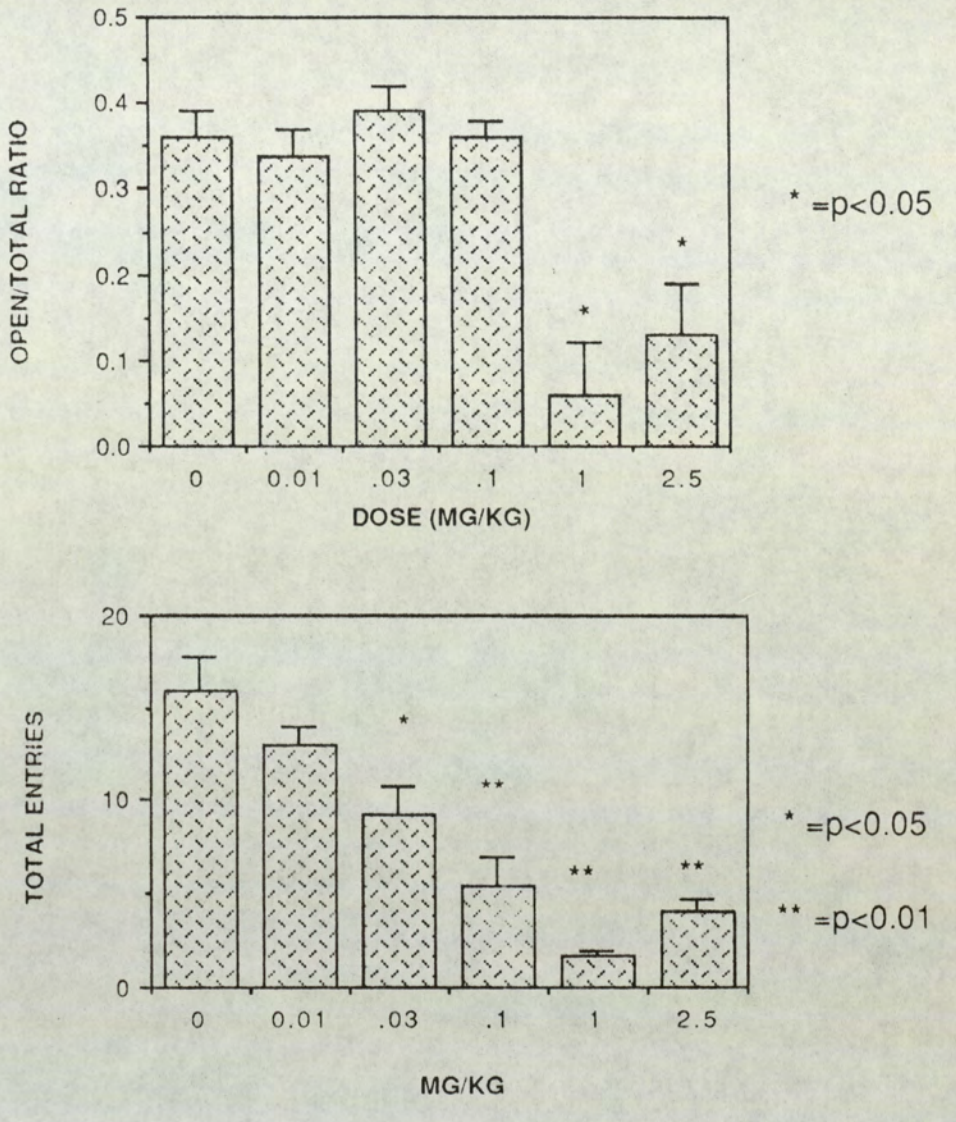
METOPROLOL



PRACTOLOL

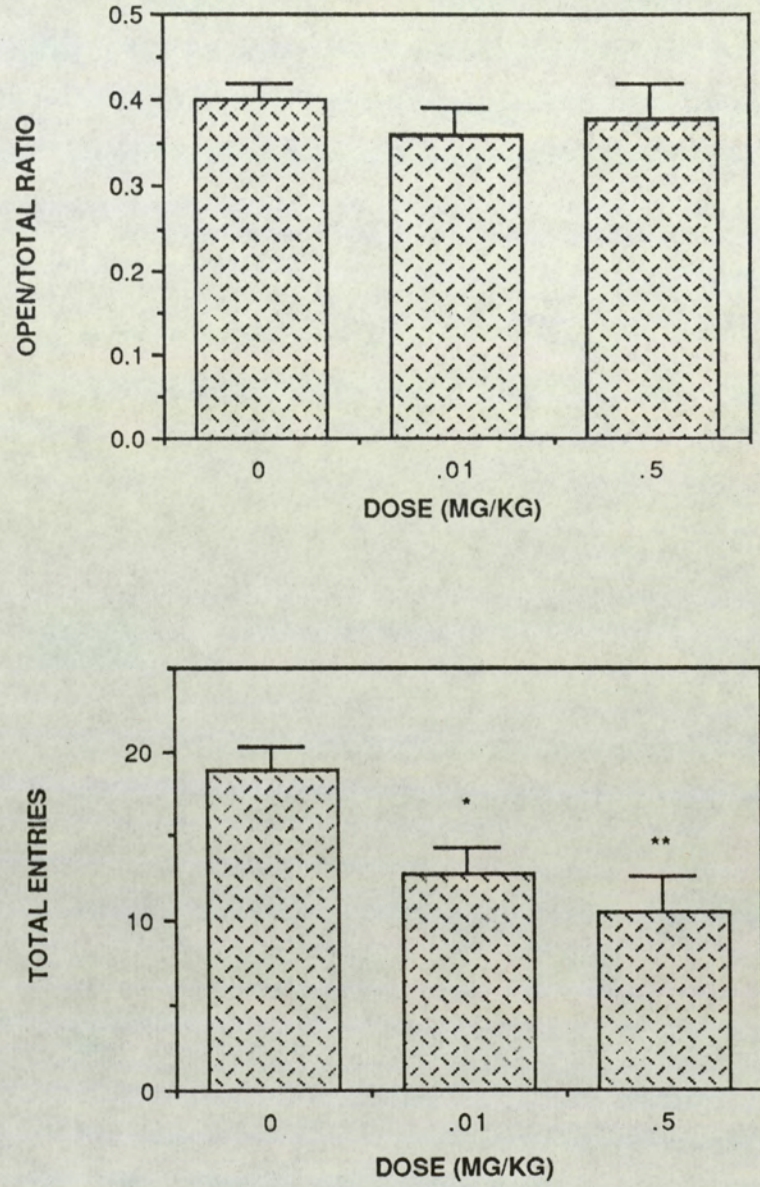
Effects on the open/total entry ratio during X-maze exploration. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 5.4 EFFECT OF CLENBUTEROL ON X-MAZE BEHAVIOUR



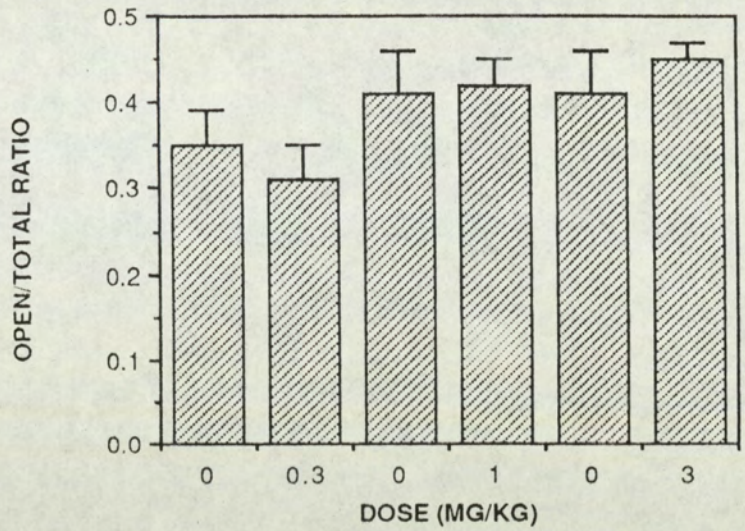
Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

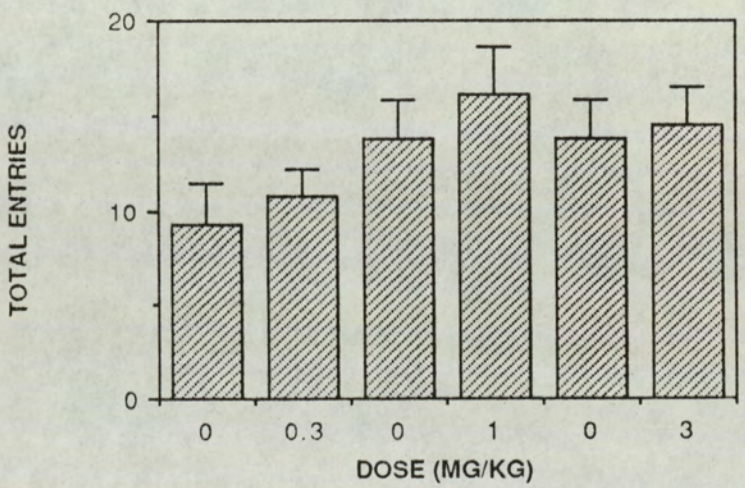
FIG 5.5 EFFECT OF TERBUTALINE ON X-MAZE BEHAVIOUR.



Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

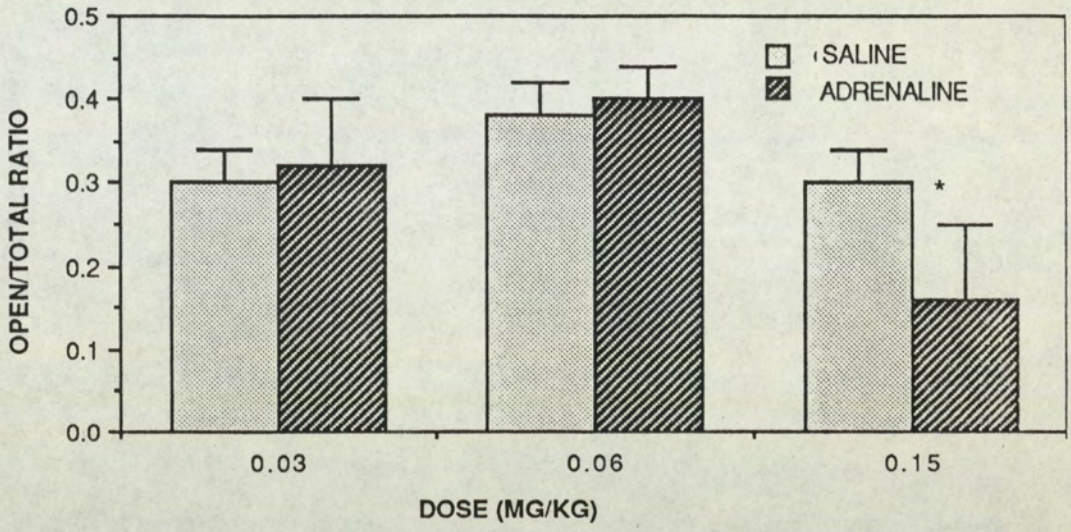
FIG. 5.6 EFFECT OF DOBUTAMINE ON X-MAZE BEHAVIOUR.

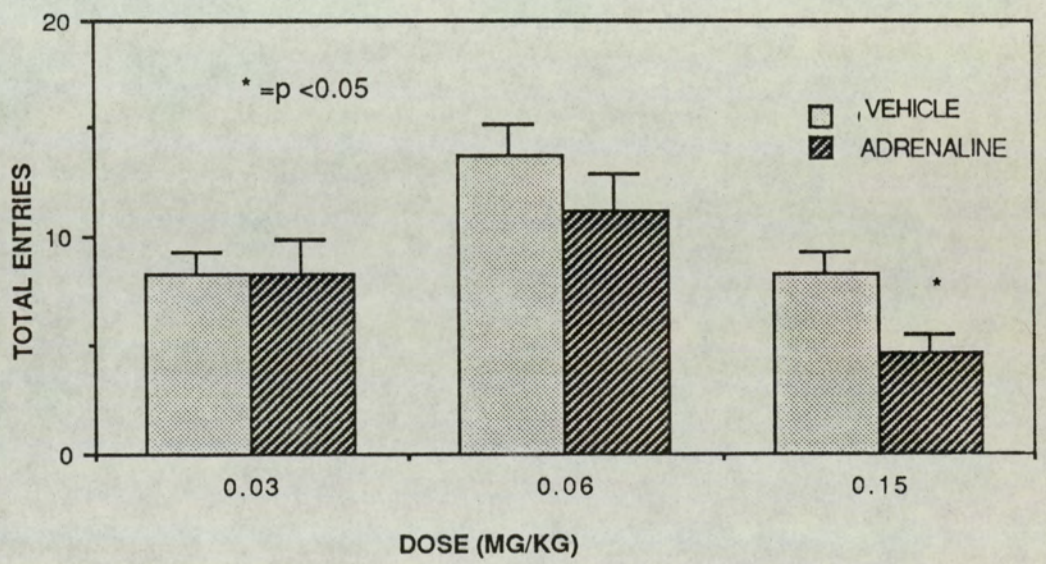




Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

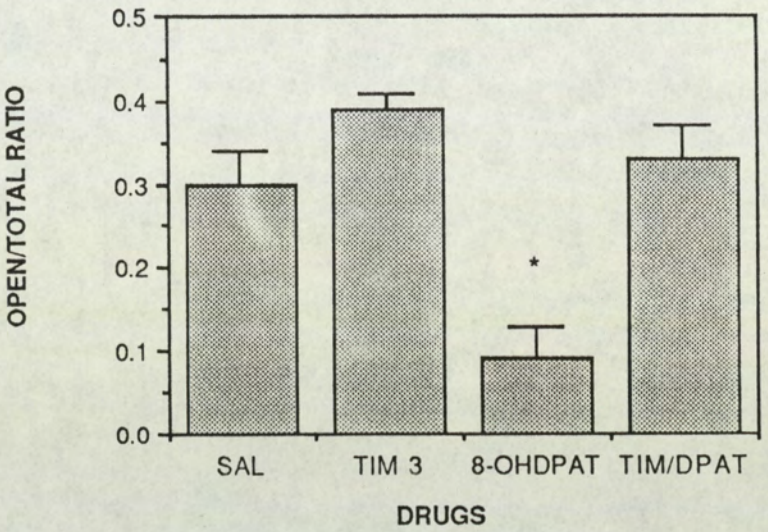
FIG. 5.7 EFFECT OF ADRENALINE ON X-MAZE BEHAVIOUR.





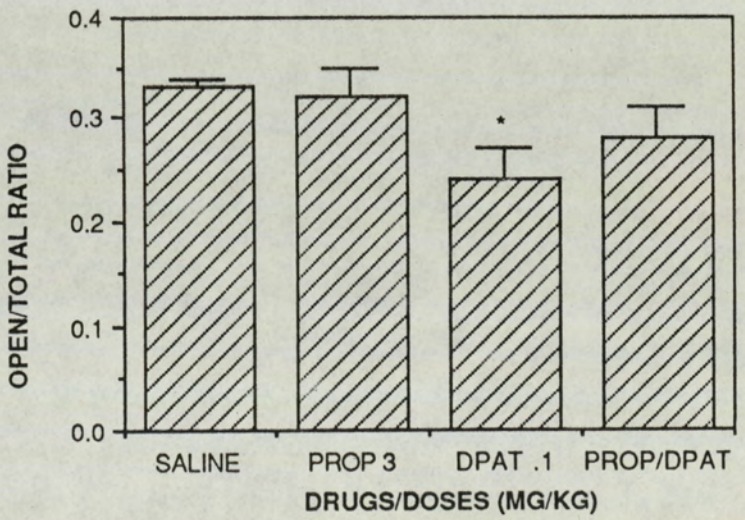
Effects on open/total entry ratio and total entries. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 5.8 INTERACTION OF TIMOLOL AND 8-OHDPAT IN X-MAZE.



* p<0.05

FIG 5.9 INTERACTION OF PROPRANOLOL AND 8-OHDPAT IN X-MAZE.



* =p <0.05

Effect on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 5.10 INTERACTION OF METOPROLOL AND 8-OHDPAT IN X-MAZE.

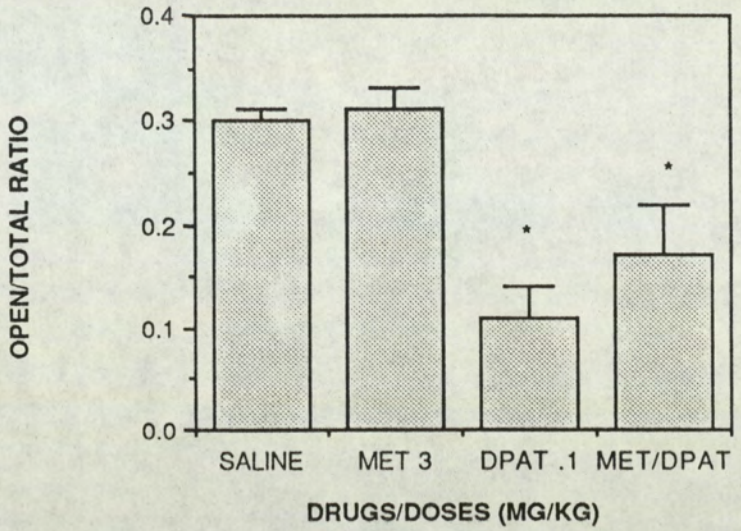
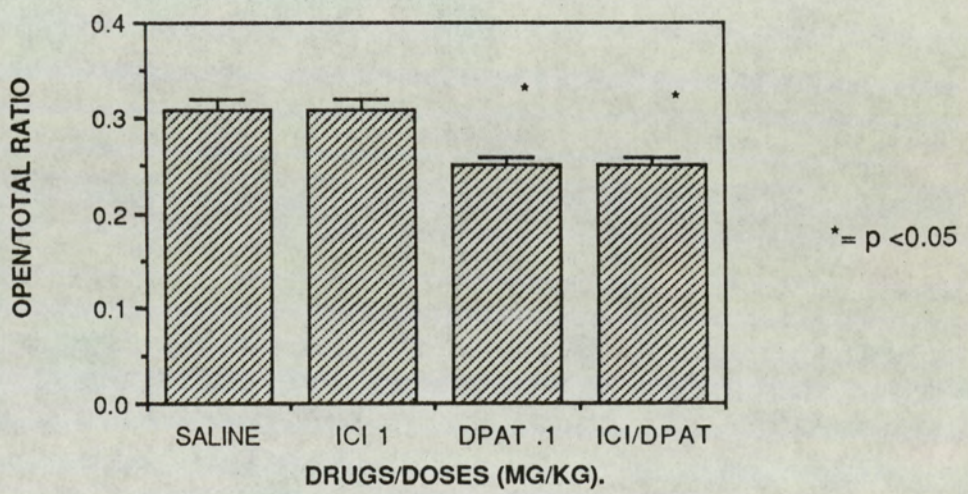
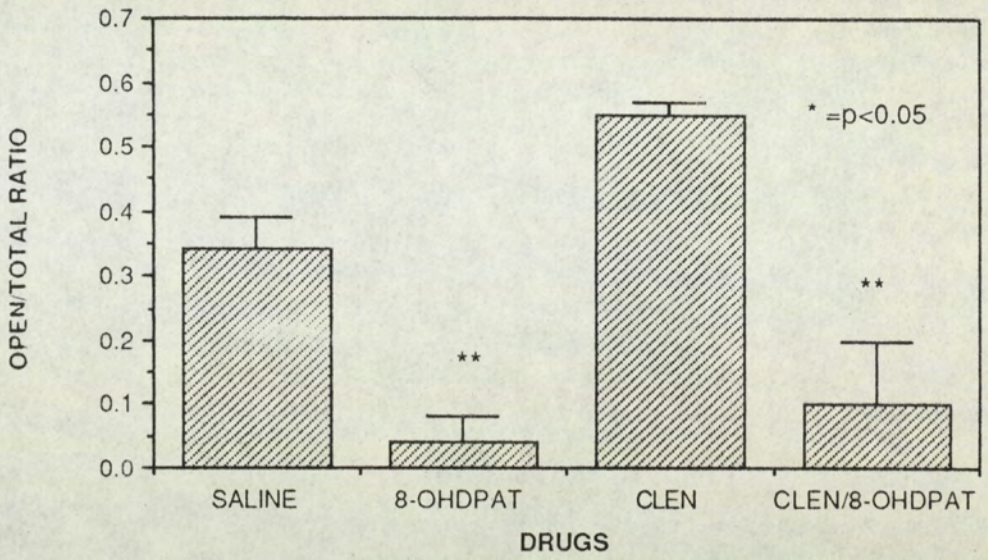


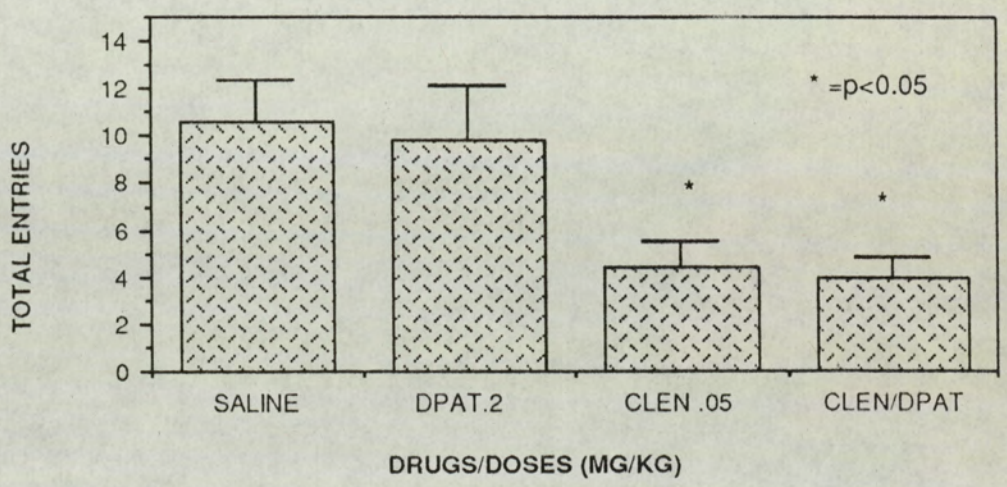
FIG 5.11 INTERACTION OF ICI 118,551 AND 8-OHDPAT IN X-MAZE.



Effect on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

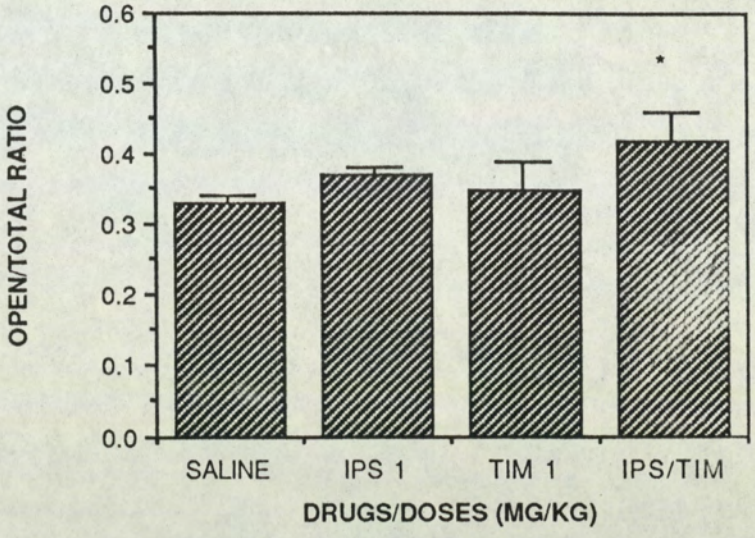
FIG. 5.12 INTERACTION OF CLENBUTEROL AND 8-OHDPAT IN X-MAZE





Effect on the total/open entry ratio and on the number of total entries. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 5.13 INTERACTION OF IPSAPIRONE AND TIMOLOL IN X-MAZE.



* =p <0.05

Effect on the open/total entry ratio. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

CHAPTER 6

EFFECTS OF 5-HT LIGANDS ON THE SOCIAL INTERACTION MODEL OF ANXIETY.

INTRODUCTION

The social interaction test was used to ascertain whether the results obtained in the X-maze anxiety test could be replicated in another animal model. This was important as some other investigators have obtained results different from those obtained here. For instance 8-OH-DPAT has been reported to be anxiolytic in Vogel conflict (Engel 1984) and in the elevated X-maze (Soderpalm et al 1988) and inactive in the X-maze (File et al 1987) and in the Geller-Seifter test (Critchley 1988). Further, this work was the first to report the effects of the 5-HT2 agonists DOI on an animal model of anxiety. Hence the need to widen the range of models used.

Social interaction was proposed as a model of anxiety by File and Hyde (1978) and has been widely used to evaluate many putative anxiolytics. As described by File and Hyde (1978) however, the test - measuring the duration of social interaction in pairs of rats (previously housed singly) tested in a novel arena under both high and low lighting conditions - did not detect the anxiolytic effects of acute benzodiazepine treatment. A version of the above method (Gardner & Guy 1984), which involves pair-housing; familiarising the test animals to the test arena and testing them with weight-matched partners in moderate lighting conditions, does detect the acute anxiolytic effects of benzodiazepines and was therefore used here.

As a routine screening test for anxiolytics the method is hampered by the large number of animals needed to test in different lighting and familiarity conditions and the need to use a pair of animals for every data point. As this method was used in this work mainly to confirm results obtained from the elevated X-maze, only those doses that

had proved most effective in the X-maze were used. The exception to this was GR 38032F which had no effects in the X-maze and the doses used here were those reported to be anxiolytic by other researchers (Costall et al 1987). The compound was also tested in different experimental designs, in one case using rats from Glaxo, Ware from a colony that in their the testing conditions was sensitive to the compound's anxiolytic effects.

RESULTS

6.1 Effects of diazepam.

Diazepam at 1.0 mg/kg significantly increased the duration of social interaction (Table 6.1 and Fig 6.1) without affecting either the number of walks or rears (Table 6.1 and Fig 6.1). At a lower dose, 0.5 mg/kg, there was a slight but non-significant increase in social interaction but no changes in number of walks or rears. At the higher dose, 2.0 mg/kg, the animals appeared sedated but the duration of social interaction and the number of walks were unaffected. The number of rears however, was reduced.

6.2 Effects of beta-CCE.

Beta-CCE at doses of 0.5 and 1.0 mg/kg produced a significant decrease in the duration of social interaction (Table 6.1 and Fig 6.2) but had no effects on either number of rears or walks (Table 6.1 and Fig 6.2).

6.3 Effects of 8-OH-DPAT

8-OH-DPAT at 0.05 and 0.1 mg/kg produced a significant decrease in duration of social interaction, to almost half of the vehicle control (Table 6.2 and Fig 6.3) and significantly reduced the number of rears. On the other hand, the number of walks was significantly increased (Table 6.2 and Fig 6.3).

6.4 Effects of RU 24969 and 5MeODMT on social interaction.

RU 24969 at a dose of 0.5 mg/kg produced a sharp fall in the duration of social interaction whilst producing a significant increase in the number of walks (Table 6.2 and Fig. 6.4). At the higher dose of 1.0 mg/kg, the fall in social interaction was still present and the number of walks showed an even greater increase. Neither dose affected the number of rears (Table 6.2 and Fig. 6.4).

5MeODMT at 0.25 mg/kg produced a significant fall in duration of social interaction whilst producing a non-significant fall in the number of rears (Table 6.2 and Fig. 6.5). At the higher dose of 1.0 mg/kg, time spent in social interaction fell drastically to only a tenth of control values as did the number of rears (Table 6.4 and Fig. 6.5). No changes were seen with either dose in the number of walks (Table 6.2 and Fig. 6.5).

6.5 Effect of ipsapirone on social interaction.

Ipsapirone at a dose of 1.0 mg/kg produced a significant increase in time spent in social interaction to virtually double the control values (Table 6.2 and Fig 6.6) whilst producing no changes in the number of walks or rears (Table 6.2 and Fig. 6.6).

6.6 Effects of pindolol on social interaction.

Pindolol at 0.1 mg/kg produced a significant increase in the duration of social interaction but had no effects on either the number of walks or rears (Table 6.2 and Fig. 6.6).

At 0.25 mg/kg pindolol produced a non-significant increase in social interaction but no change in either the number of walks or rears (Table 6.2 and Fig. 6.6).

6.7 Effects of DOI on social interaction.

DOI at 0.1 and 1.0 mg/kg produced a significant increase in the duration of social interaction , but did not affect the number of rears or walks (Table 6.3 and Fig. 6.7).

There was an appreciable number of wet-dog shakes during the course of the test which appeared to be dose-dependent (Table 6.5 and Fig. 6.10). The bulk of these wet-dog shakes ocurred near the walls of the social interaction

arena rather than in the middle. There was no association between occurence of the wet-dog shakes and the proximity of the other rat. Wet dog shakes occurred both when the rats were walking and when they were standing still.

6.8 Effects of ritanserin and ketanserin on social interaction.

Ritanserin at a dose of 1.0 mg/kg produced a slight but non-significant increase in duration of social interaction (Table 6.3 and Fig. 6.8). There was no change in the number of rears or walks (Table 6.3 and Fig. 6.8).

Ketanserin 0.5 mg/kg had no effect on either the duration of social interaction or the number of walks or rears (Table 6.3 and Fig. 6.8).

6.9 Effects of GR 38032F on social interaction.

Under the same conditions of testing as the other drugs (lighting 125 lux), GR 38032F at 10 and 100 micrograms/kg produced no changes in the duration of social interaction or in the number of rears and walks (Table 6.4 & Fig. 6.9).

In addition, GR 38032F was tested in a different experimental design under high light intensity (250 lux) and using two different measures of social interaction, one where sniffing was considered as part of social interaction (as in all the other experiments) and the other where social interaction did not include sniffing each other. In neither case was the duration of social interaction or the number of rears or walks affected (Table 6.4 and Fig. 6.9).

DISCUSSION

The test was validated by its ability to detect the anxiolytic effects of diazepam and the anxiogenic effects of beta-CCE. This is important as obtaining results with standard anxiolytic and anxiogenic agents under each laboratories operating conditions is necessary before a test can serve as an anxiety model.

The anxiogenic effects obtained with the 5-HT1 agonists 8-OH-DPAT, RU 24969 were in agreement with those obtained in the X-maze test. The 5-MeODMT anxiogenic effect concurs with the results in X-maze reported by Critchley and Handley 1986.

The anxiolytic effects of ipsapirone agree with the results obtained in the X-maze model, while the pindolol anxiolytic effects agree with X-maze results reported by Critchley and Handley (1986).

The lack of activity of the 5-HT2 antagonists ritanserin and ketanserin is surprising in view of the potent anxiolytic effects obtained with ritanserin in the X-maze model. Ritanserin only produced a slight but non-significant increase in social interaction. This lack of activity however concurs with Gardner (1986) who was unable to detect anxiolytic effects of the 5-HT2 antagonists in the social interaction test. The vehicle social interaction values appear high but are comparable with the mean for all the social interaction studies (41.6 [3.4]) with a range 27.3 - 62.4 seconds. The study has since been replicated and neither compound was anxiolytic though the vehicle controls were lower then.

The anxiolytic effects of DOI concur with those obtained

in the X-maze. That DOI was anxiolytic on two animal models brings a perplexing situation where both 5-HT2 antagonists and an agonists have the same effect.

The 5-HT3 antagonist GR 38032F was without effect on any of the components of social interaction behaviour. This was despite testing for the compound's effects under three different experimental set-ups: low lighting and and a familiar arena as used for other the drugs; high lighting conditions and an unfamiliar arena both with sniffing one anothers hindquarters included (as in the other experiments), and excluded, from social interaction. The rats used in the test under high lighting conditions were specially obtained from Glaxo, Ware from a colony workers there had found sensitive to the anxiolytic effects of the compound. Our testing conditions were also adapted - use of high lighting conditions and an unfamiliar arena, and exclusion of sniffing from social interaction - to match those used at Glaxo.

These results tend to suggest that the compounds effects are very sensitive to minor changes in the testing conditions. The one obvious difference between the testing conditions used here and at Glaxo is the route of administration: intraperitoneal as opposed to oral administration at Glaxo. These results continue a trend where the compound is found anxiolytic in some laboratories but not in others. For example, Costall et al (1987) and Piper et al (1988) found the compound anxiolytic while Johnston and File (1988) and Molewijk et al (1987) found it inactive in the social interaction test.

TABLE 6.1: Effects of diazepam and beta-CCE on social interaction.

Mean (s.e.m.) N=6 [vehicle control values]

DRUG/DOSE (mg/kg)	S.I.(sec)	REARS	WALKS
Diazepam			
0.5	47.8(6.4) [36.9(3.2)]	31.3(3.9) [36.0(2.3)]	11.8(2.1) [9.3(1.5)]
1.0	61.5(4.8)* [42.6(5.2)]	19.0(2.3) [25.5(2.3)]	11.2(0.8) [8.5(1.0)]
2.0	53.7(10.1) [48.8(4.9)]	10.7(3.1)** [31.2(4.1)]	13.4(3.7) [10.0(2.0)]
Beta-CCE			
0.5	25.6(6.1)** [46.5(4.8)]	33.0(2.1) [37.0(2.8)]	15.3(2.1) [10.7(1.6)]
1.0	18.4(4.1)* [34.4(4.3)]	32.0(3.0) [33.6(3.1)]	11.1(2.1) [10.8(1.2)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 6.2: Effect of 5-HT1 ligands on social interaction.

Mean (s.e.m.) N = 6 [vehicle control values]

DRUG/DOSE (mg/kg)	S.I.(sec)	REARS	WALKS
8-OHDPAT			
0.05	24.8(3.5)* [42.6(5.2)]	7.6(1.5)** [25.5(2.3)]	26.4(3.4)** [8.5(1.0)]
0.1	28.2(2.4)** [48.8(4.9)]	11.2(2.3)** [31.2(4.1)]	19.2(3.9)** [10.0(2.0)]
RU 24969			
0.5	14.6(3.9)* [34.4(4.3)]	25.6(1.5) [33.6(3.1)]	21.4(2.9)* [10.8(1.2)]
1.0	16.8(3.7)** [62.4(6.4)]	24.1(3.5) [27.8(1.9)]	32.7(3.5)** [9.4(1.4)]
5-MeODMT			
0.25	13.6(1.7)** [46.5(4.8)]	20.8(3.3)** [37.0(2.8)]	11.3(0.5) [13.7(1.6)]
1.0	3.8(1.8)** [62.4(6.4]	2.25(0.9)** [27.8(1.9)]	5.3(1.6) [9.4(1.4)]
Ipsapirone			
1.0	72.3(6.2)** [36.9(3.2)]	24.6(3.4) [36.0(2.3)]	6.3(0.3) [9.3(1.5)]
Pindolol			
0.25	50.6(5.5) [33.8(3.7)]	28.0(2.3) [31.5(1.5)]	10.8(1.8) [8.9(0.5)]
0.1	63.6(6.9)* [33.8(3.7)]	29.8(1.6) [31.5(1.5)]	10.7(1.6) [8.9(0.5)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **=$p<0.01$ relative to vehicle controls.

Table 6.3: Effect of 5-HT2 ligands on social interaction

Mean (s.e.m.) N=6 [vehicle control values]

DRUG/DOSE (mg/kg)	S.I.(sec)	REARS	WALKS
DOI			
0.1	43.0(3.7)* [27.3(2.5)]	42.3(4.7) [30.2(5.2)]	14.5(1.4) [11.5(1.2)]
1.0	58.5(7.0)** [27.3(2.5)]	26.2(2.9) [30.2(5.2)]	17.0(4.0) [11.5(1.2)]
Ritanserin			
1.0	53.0(8.3) [41.8(0.5)]	37.4(3.0) [33.9(1.3)]	8.8(2.5) [10.3(2.1)]
Ketanserin			
0.5	44.6(9.2) [41.8(0.5)]	37.0(1.2) [33.9(1.3)]	8.0(2.0) [10.3(2.1)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 6.4: Effect of GR 38032F on social interaction.

Mean (s.e.m.) N=6 [vehicle control values]

DRUG/DOSE (mg/kg)	S.I.(sec)	REARS	WALKS
Low light, familiar arena			
10mcg/kg	20.3(3.3) [18.8(4.5)]	22.4(3.0) [21.0(2.1)]	14.4(1.8) [13.8(2.2)]
100	17.8(3.0) [18.8(4.5)	19.8(2.0) [21.0(2.1)]	14.7(1.1) [13.8(2.2)]
High light, unfamiliar arena			
10mcg/kg	39.2(8.8) [44.3(5.7)]	27.2(2.2) [24.8(3.0)]	15.8(2.4) [22.5(2.9)]
100	28.0(4.4) [44.3(5.7)]	27.8(5.4) [24.8(3.0)]	23.8(4.2) [22.5(2.9)]
High light, unfamiliar (sniffing excluded from S.I.)			
10mcg/kg	42.2(8.9) [49.3(4.4)]	20.7(6.4) [22.7(2.3)]	22.7(4.3) [14.7(2.6)]
100	39.2(5.1) [49.3(4.4)]	17.8(2.1) [22.7(2.3)]	27.5(6.1) [14.7(2.6)]

High light = 250 lux, Low light = 125 lux.

Statistical comparisons were made on raw data relative to saline controls using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

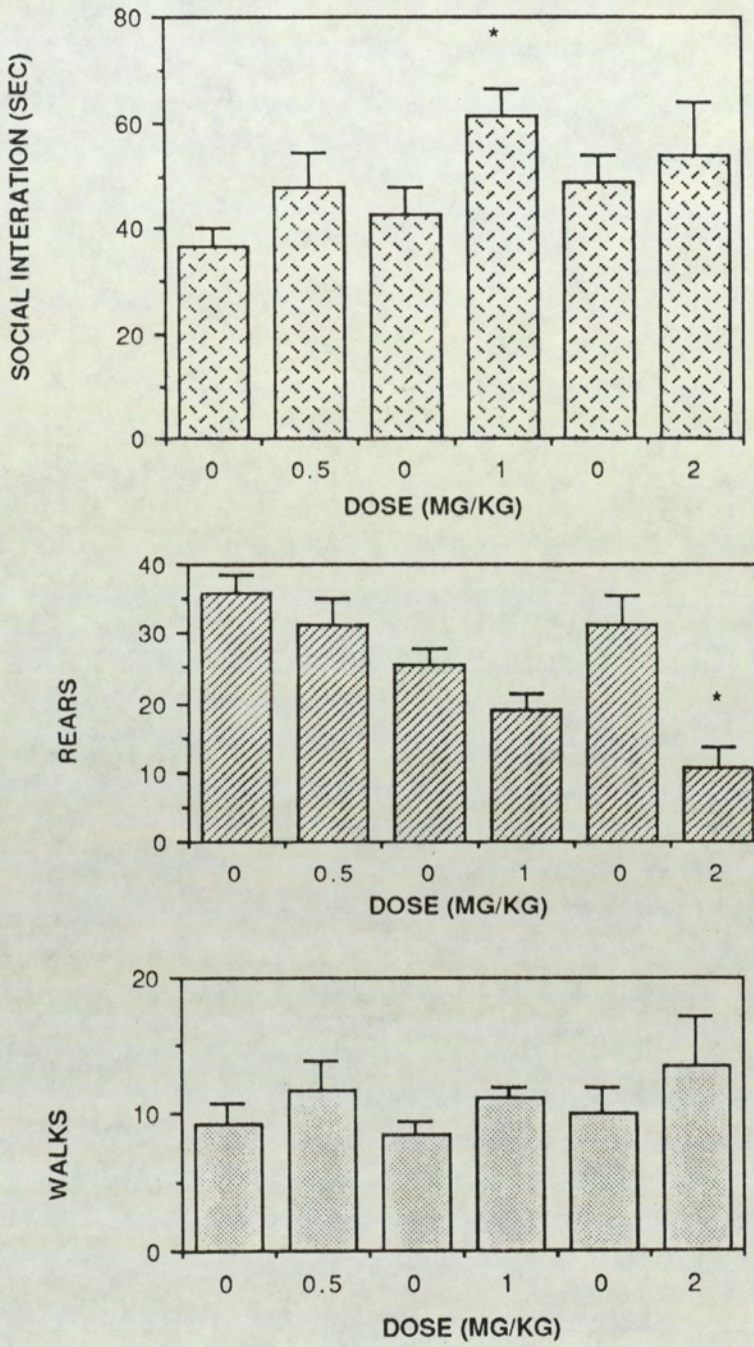
Table 6.5: DOI-induced wet dog shakes during social interaction.

Mean (s.e.m.) N = 12

DOI mg/kg	Wet-dog shakes/ 10 min
0	0
0.1	0.9 (0.5)**
1.0	6.0 (2.6)**

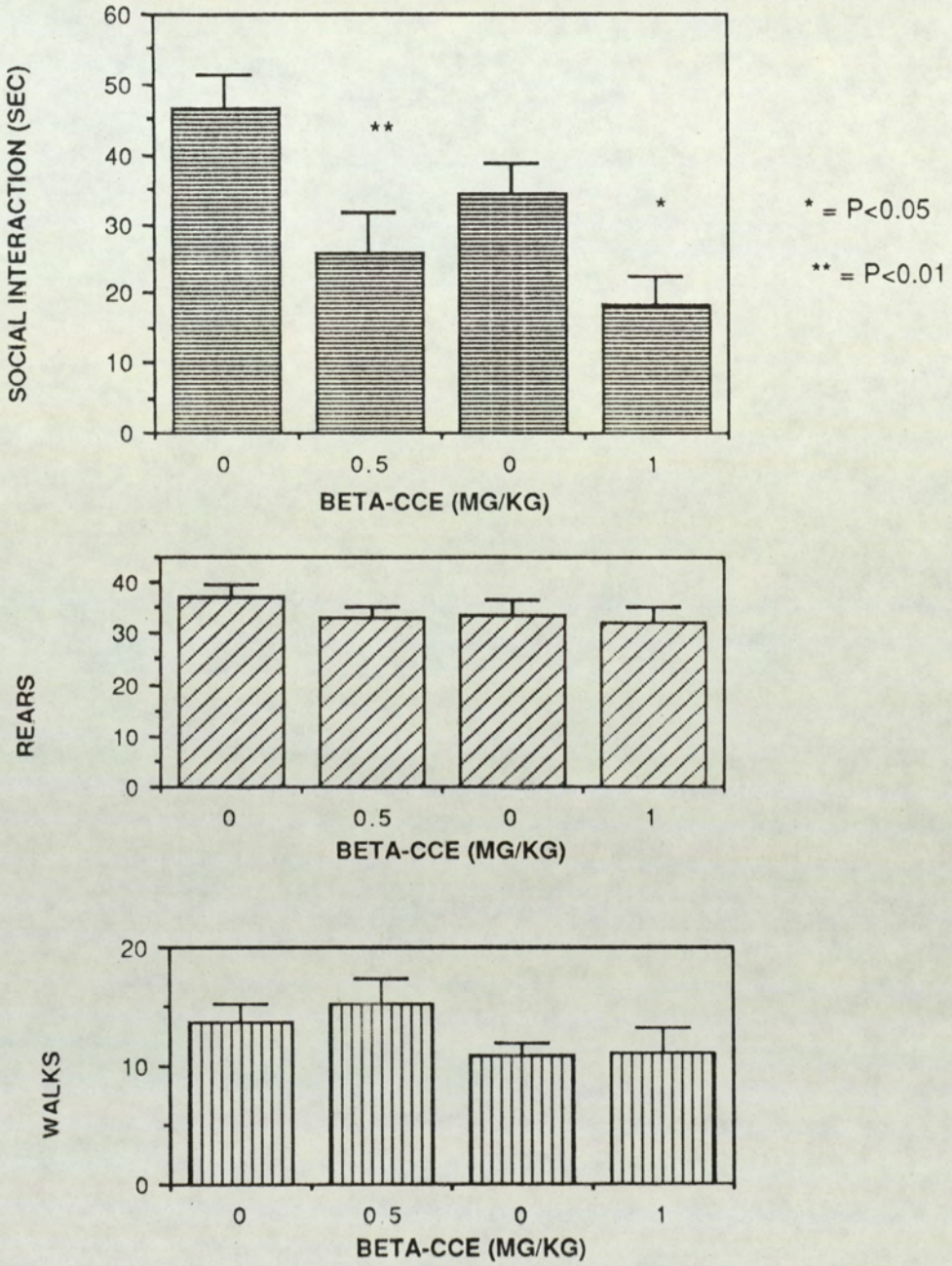
Wet dog shakes were counted in both rats during social interaction. Statistical comparisons were made on raw data relative to vehicle controls using a t-test.
*= $p<0.05$, **= $p<0.01$ relative to vehicle controls.

FIG. 6.1 EFFECT OF DIAZEPAM ON SOCIAL INTERACTION



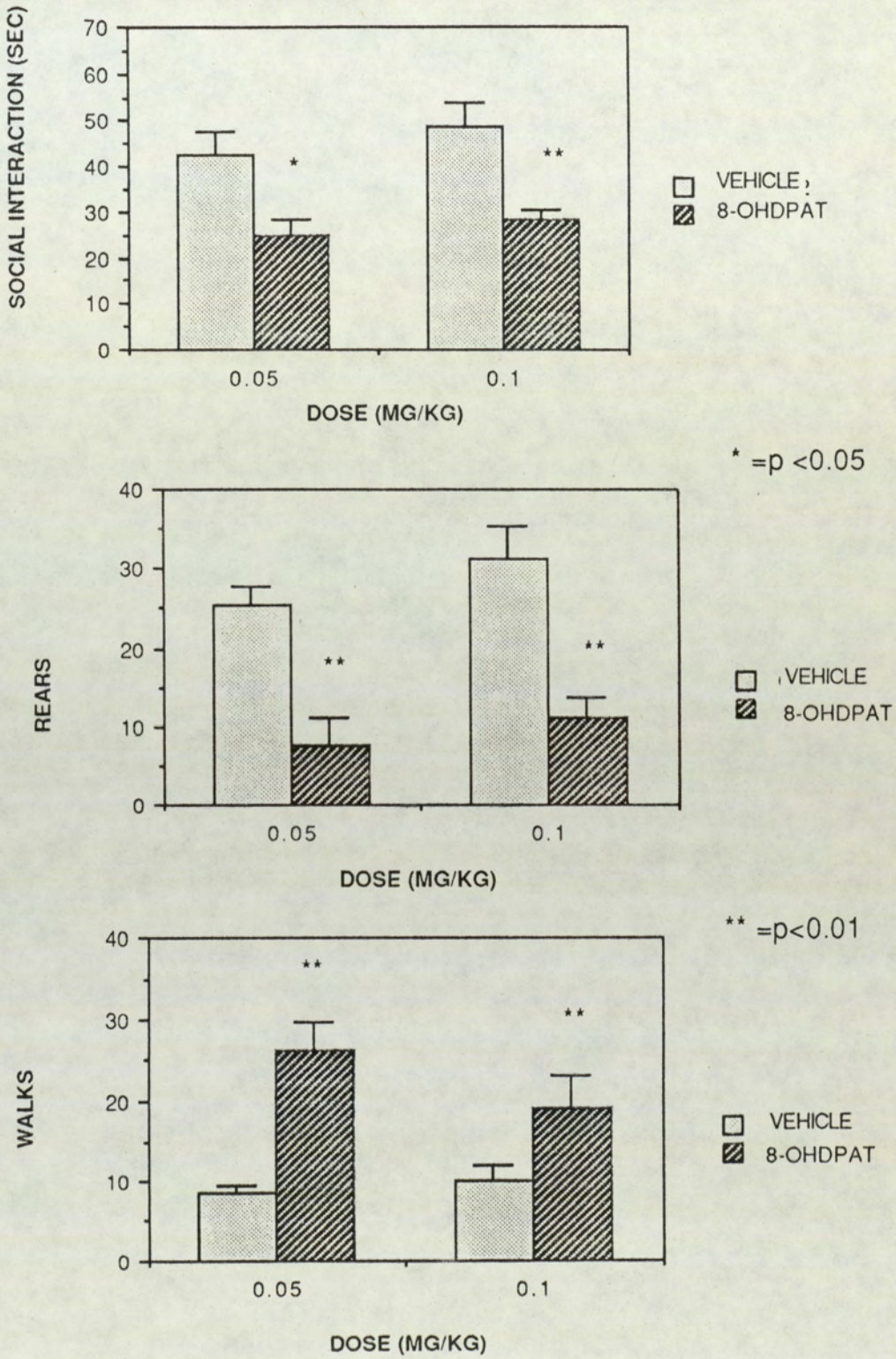
Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG.6.2 EFFECT OF BETA-CCE ON SOCIAL INTERACTION



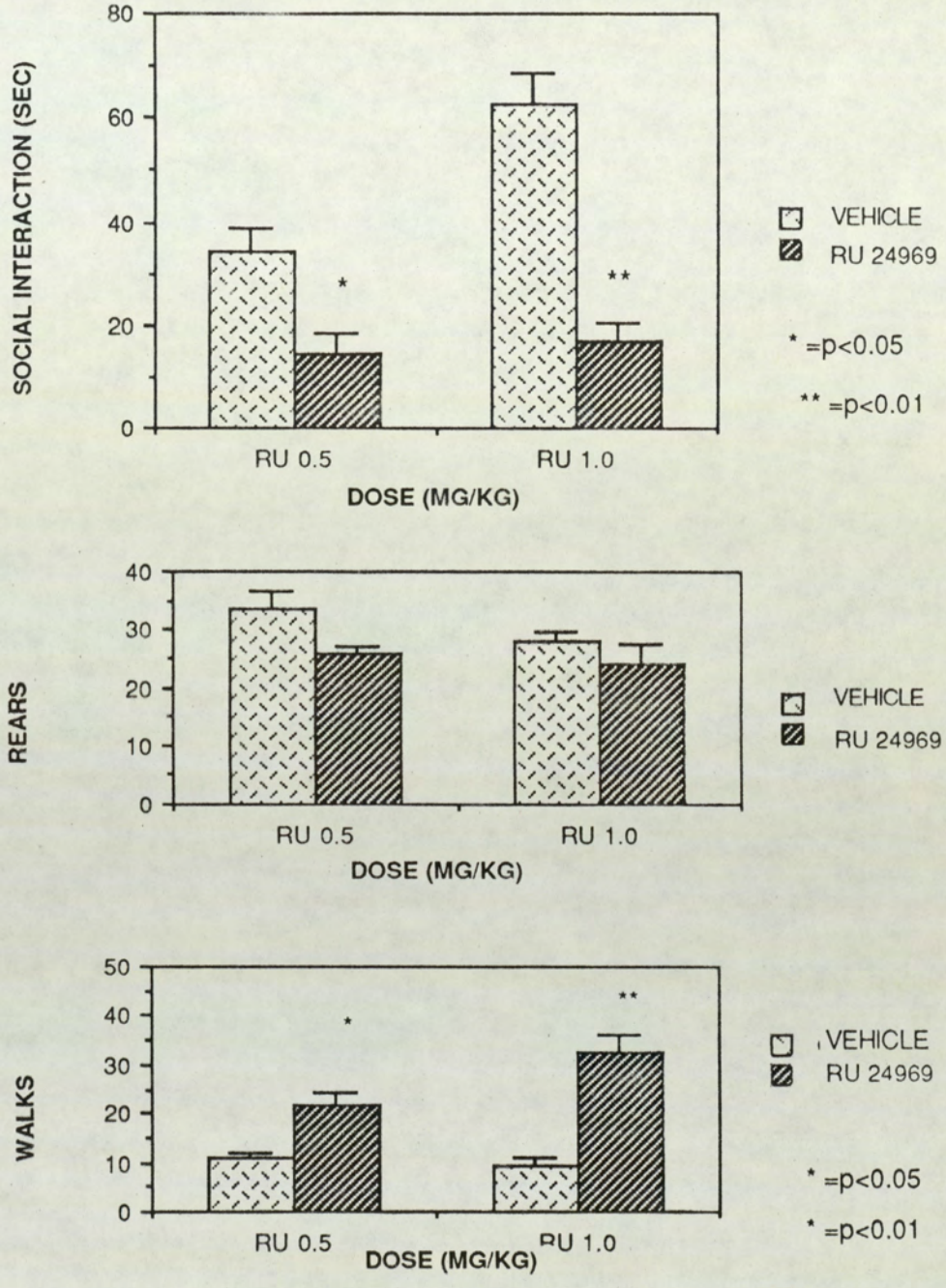
Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.3 EFFECT OF 8-OHDPAT ON SOCIAL INTERACTION



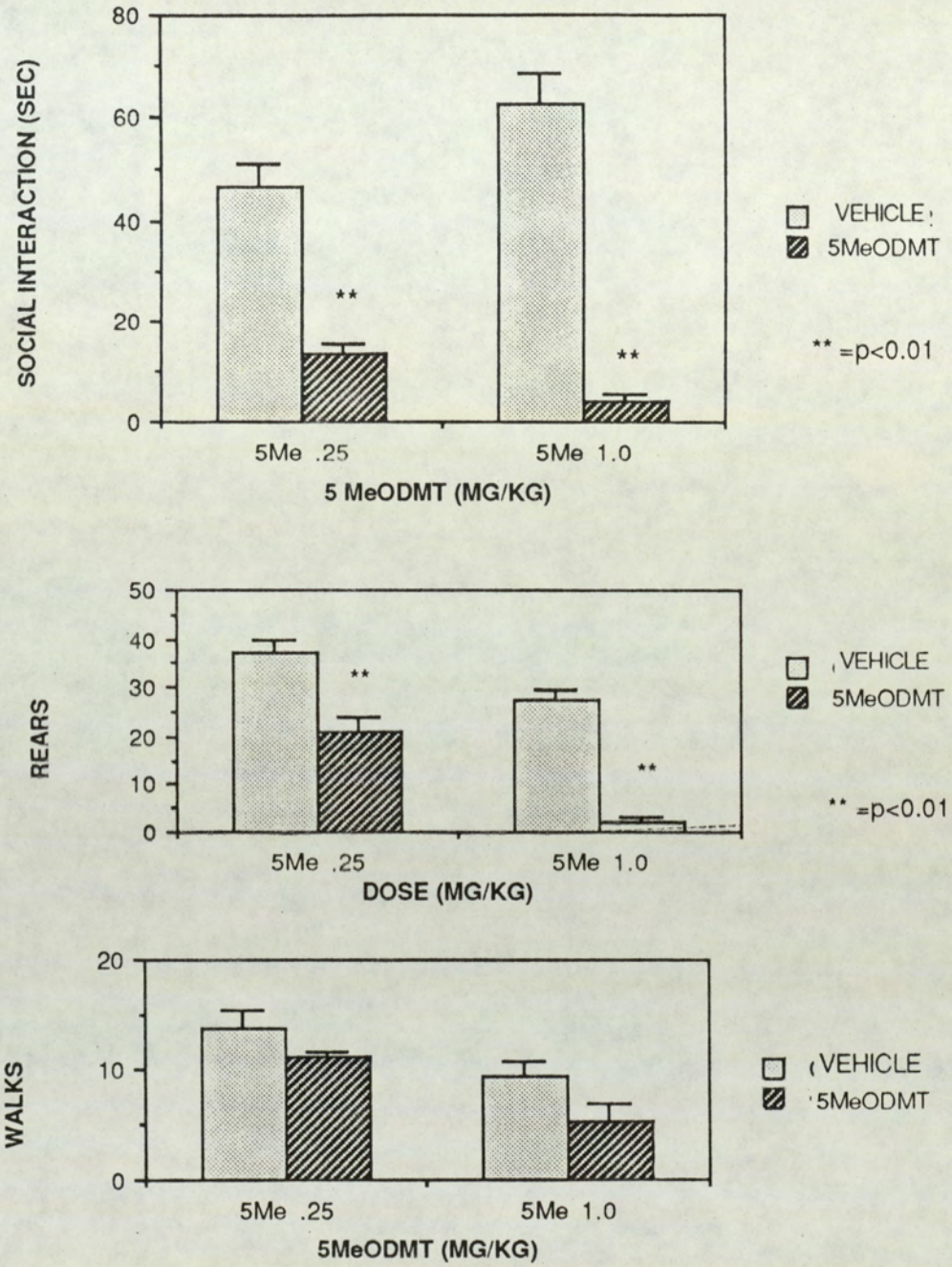
Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.4 EFFECT OF RU 24969 ON SOCIAL INTERACTION



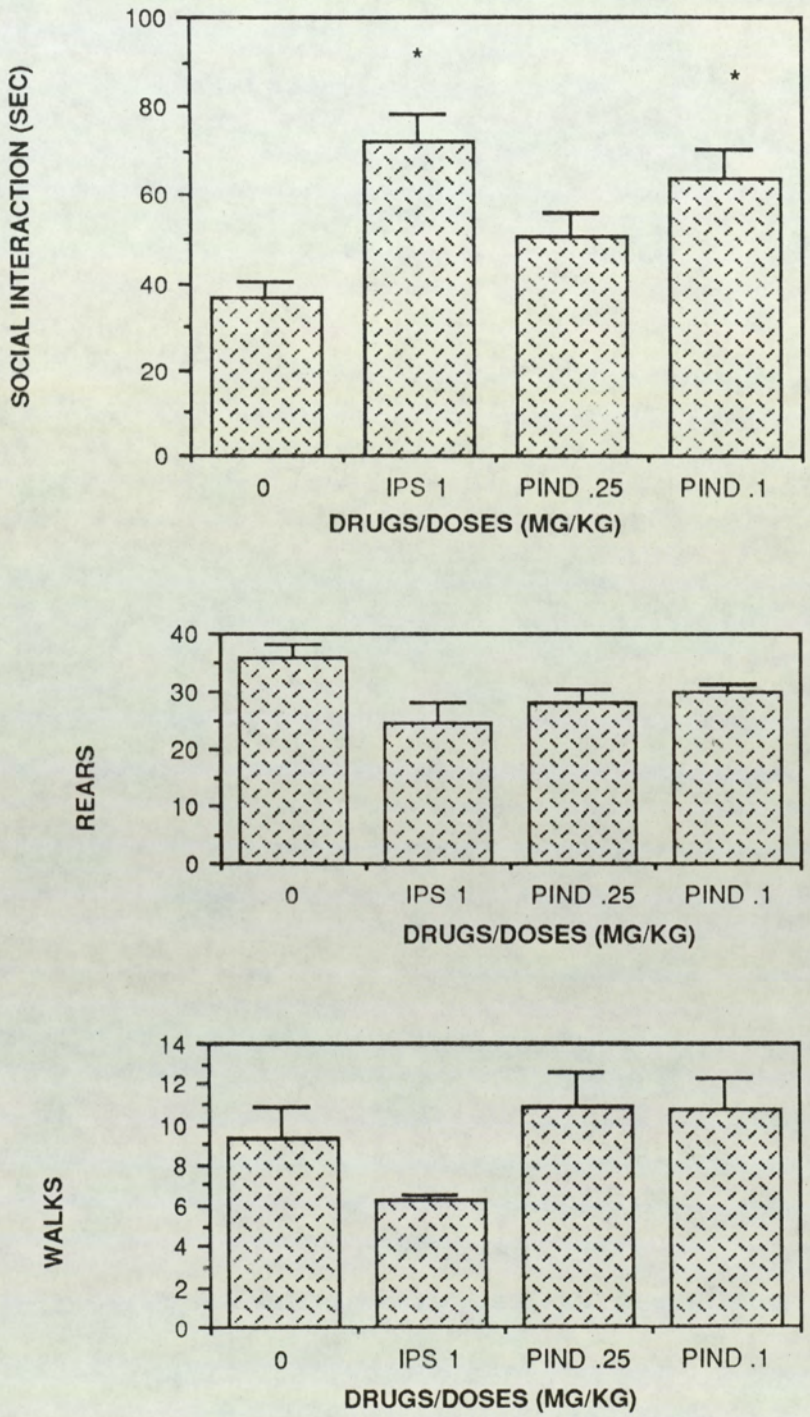
Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.5 EFFECT OF 5MeODMT ON SOCIAL INTERACTION.



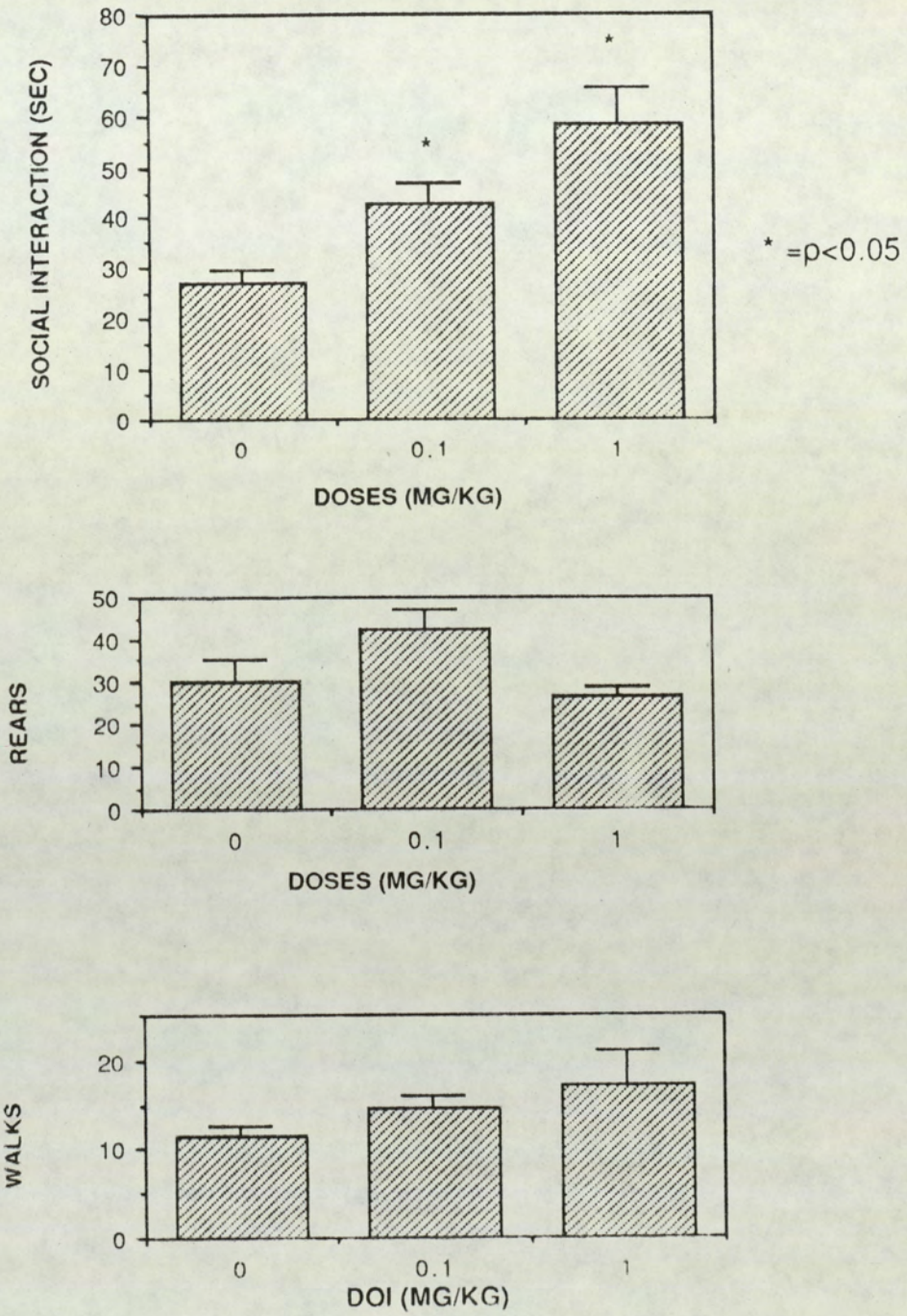
Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.6 EFFECT OF PINDOLOL AND IPSAPIRONE ON SOCIAL INTERACTION



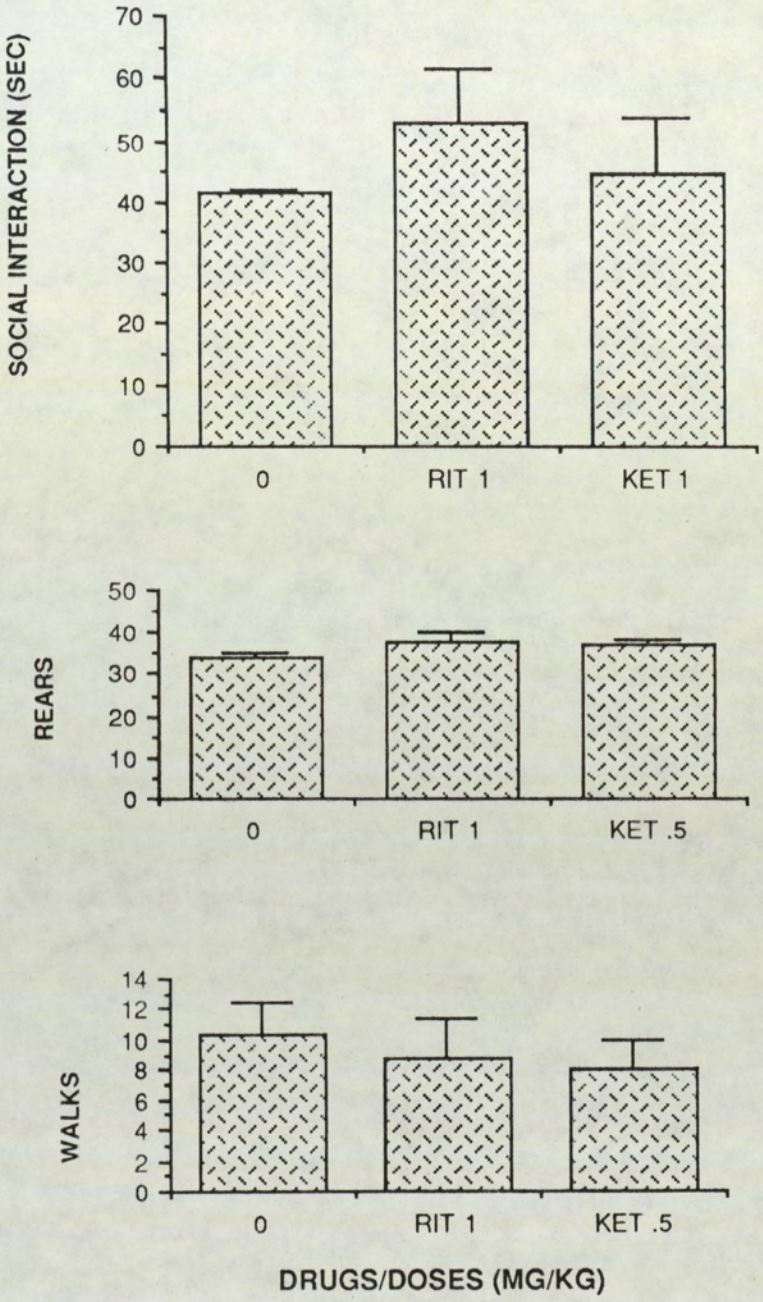
Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 6.7 EFFECT OF DOI ON SOCIAL INTERACTION



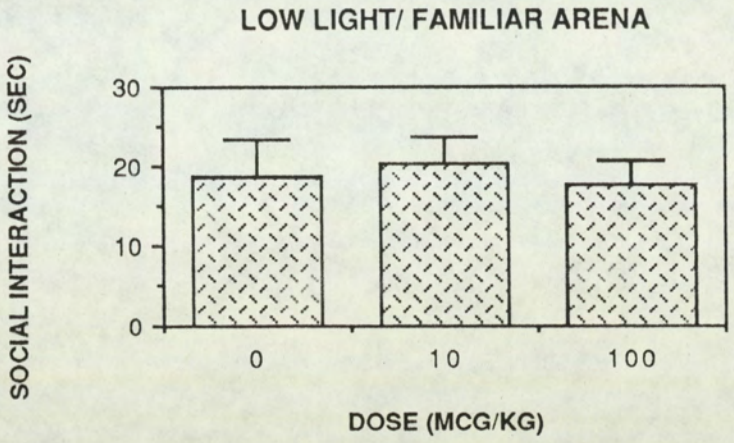
Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

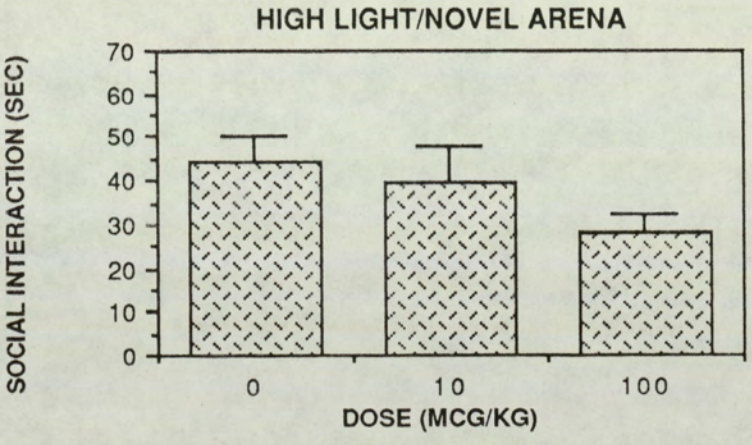
FIG 6.8 EFFECT OF RITANSERIN AND KETANSERIN ON SOCIAL INTERACTION

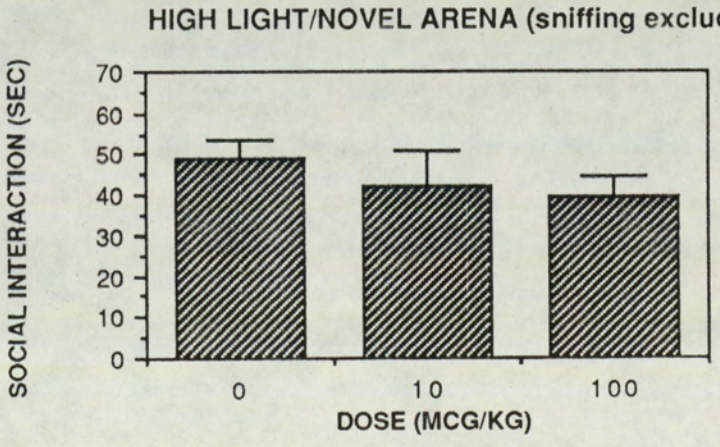


Effect on duration of social interaction and number of rears and walks. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.9 EFFECT OF GR 38032F ON SOCIAL INTERACTION

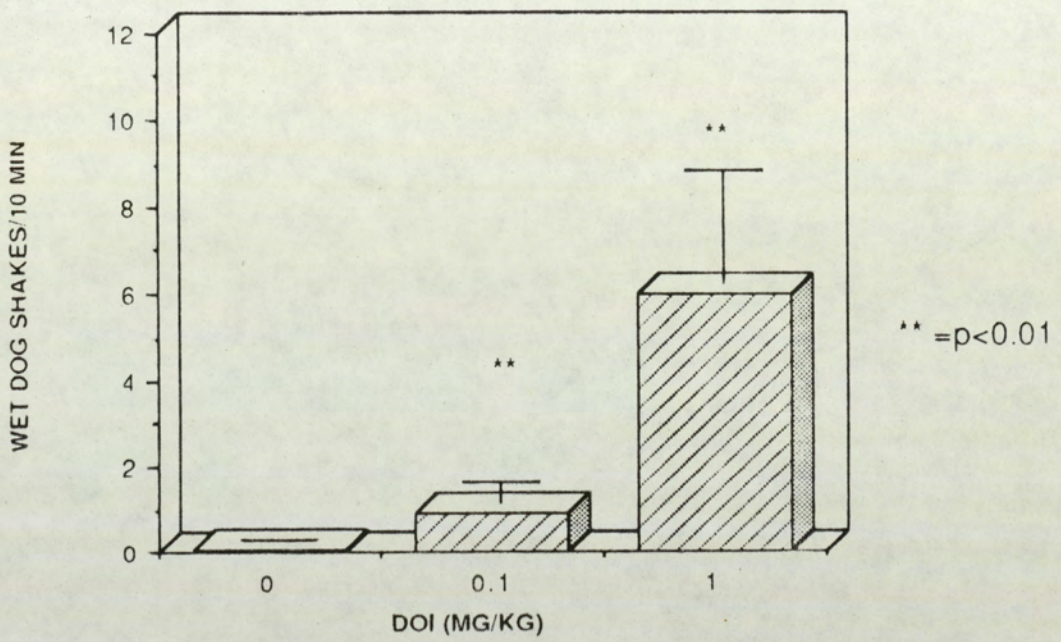






Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 6.10 DOI-INDUCED WET DOG SHAKES DURING SOCIAL INTERACTION



Number of wet dog shakes induced by DOI in rats during the social interaction test. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

CHAPTER 7.

ANALYSIS OF MARBLE BURYING BEHAVIOUR.

Introduction

Results.

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7.2 Effect of location of marbles in cages.

7.3 Effects of repeated testing.

7.3.1 Testing on consecutive days.

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7.8 Effect of anxiogenic compounds.

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7.11 Two compartment model.

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INTRODUCTION

Rodents generally use bedding material to bury noxious materials that are housed with them. Objects so buried include prods that give them electric shocks (Pinel & Treit 1978, Treit et al 1981, Harder & Maggio 1983, Terlecki et al 1979); rat chow pellets coated with quinine (Poling et al 1981); spouts of bottles containing bad tasting liquids e.g. pepper sauce (Wilkie et al 1979, Poling et al 1981); liquids to which they have developed taste aversion after concomitant injection with lithium chloride (Wilkie et al 1979) or amphetamine (Poling et al 1981); mouse traps that strike, flashcubes that discharge near them and hoses which direct airbursts into their faces (Terlecki et al 1979).

In addition rodents also bury harmless objects housed with them. Such objects include rat chow pellets - the rat's normal food (Poling et al 1981), flashcubes that do not flash (Terlecki et al 1979), and glass marbles (Poling et al 1981, Broekkamp et al 1986).

Both the duration and extent of burying of electrified prods by rats was reduced by the anxiolytic agents diazepam, chlordiazepoxide and pentobarbitone (Treit et al 1981).

Burying of glass marbles was reduced by anxiolytic agents including the benzodiazepines, ethanol and meprobamate at doses that did not reduce water-immersion induced grooming which was used to assess sedation. Non-anxiolytic centrally active compounds such as neuroleptics had non-selective effects reducing marble burying only at doses that reduced grooming (Broekkamp et al 1986).

Object burying behaviour in rodents has been proposed as an animal model of anxiety (Treit et al 1981, Broekkamp et al 1986). To establish if this is indeed a valid model of anxiety this project sought to investigate the factors that influence burying of harmless objects, in particular glass marbles, by mice. It was intended to establish how and why mice bury marbles. Is it neophobia or do mice find marbles aversive ? Alternatively does the presence of marbles stimulate digging behaviour generally ? It also sought to investigate the effect of anxiolytic and anxiogenic drugs on marble burying.

Sedative effects of drugs were assessed by measuring locomotor activity with an Animex counter. This was preferred to immersion-induced grooming as used by Broekkamp et al (1986) since 5-HT ligands such as DOI modulated grooming behaviour in rodents (Chapter 4, Heaton et al 1988).

Two different Animex counters were used in this work. Although both of them were tuned to the same sensitivity. One of them gave counts (in arbitrary units) of around 200 and the other around 500 when saline treated mice were placed on them for 5 minutes. Each drug test and its concurrently run control were performed on the same machine.

RESULTS.

7.1 How mice bury marbles

When mice were placed in a cage containing 20 glass marbles they generally approached the marbles, sniffed and grasped them with their forepaws, pushed them about with the snout, forepaws or hindlimbs and then moved away to other parts of the cage. When they returned to the marbles they would repeat the process or include variations such as pushing sawdust towards marbles with the snout or forelimbs or dig holes in the sawdust and push marbles in. Some mice in addition walked to a corner of the cage and pushed sawdust all over the cage with their hind limbs. This last activity did not appear to be specifically directed at the marbles though quite a number of marbles were buried in this way. The mean number of marbles buried overall by control mice after 30 minutes was 7.8 ± 0.2.

Some marbles were found buried several centimetres below the surface and it was difficult to visualise how the mouse got them there. Sometimes a number of the marbles were found gathered together in the centre or to one side of cage. Since mice also dug sawdust all over cage, some of the burying was most probably incidental to the digging.

7.2 Effect of location of marbles in cages.

Mice were found to bury most marbles when the marbles were spread evenly in the cage. The number of marbles buried fell almost by half when they were all placed in the centre or to one side of the cage (Table 7.1).

7.3 Effects of repeated testing.

7.3.1 Testing on consecutive days.

There was no significant change in the number of marbles buried when mice were tested daily for 5 consecutive days (Table 7.2 and Fig 7.1). The tests were performed at the same time daily (1000 hours). Two parallel experiments were performed several months apart.

7.3.2 Testing several times on the same day.

There was no significant change in the number of marbles buried on testing mice 4 times on the same day (Table 7.3 and Fig. 7.2). The tests were performed with the same mice at 2 hour intervals starting at 1300 hours. Four of the trials were in the light cycle and the last one in the dark cycle.

7.4 Effects of housing mice with marbles.

Mice housed with marbles in their home cage for four days and later tested in the experimental cage buried the same number of marbles as those that were exposed to marbles for the first time (Table 7.4).

7.5 Mice tested with washed marbles.

One group of mice was handled with rubber gloves and tested with marbles which had been washed with water and handled only with rubber gloves. There was no significant difference in the number of marbles buried by these animals and those buried by control animals tested in the usual way (Table 7.5).

7.6 Effect of gender on marble burying behaviour.

There was no significant difference in the number of marbles buried between male and female MFI mice (Table 7.6).

7.7 Effect of diazepam.

Diazepam at 0.05 and 0.1 mg/kg significantly increased marble burying and also produced an increase in locomotor activity. Higher doses, 0.25 to 5.0 mg/kg decreased marble burying without significantly affecting locomotor activity (Table 7.7 and Fig 7.3).

7.8 Effect of anxiogenic compounds.

The anxiogenic compound beta-CCE at 5 and 10 mg/kg had no effects on marble burying (Table 7.7 and Fig 7.4).

Yohimbine at 1 mg/kg did not affect marble burying. Higher doses (5 and 10 mg/kg) reduced both marble burying (Fig 7.4) and locomotor activity (Table 7.7).

7.9 Duration of digging and moving.

There was no difference in the amount of time spent digging sawdust and moving about the cage between mice tested in the presence or absence of marbles (Table 7.8). Diazepam at 0.1 and 2.5 mg/kg did not affect the duration of digging or the surface area of sawdust disturbed (Table 7.8 and Fig. 7.5). This assessment was conducted by observing the mice over a 10 min period.

7.10 Are there consistent high and low buriers of marbles among mice?

Testing mice on the marble burying test for several consecutive days revealed that there was considerable variation in the number of marbles buried by each mouse from day to day (Table 7.9). Mice which buried large numbers of marbles in one day were just as likely to bury a very small number the following day and vice versa (Table 7.9 and Fig 7.6). There was considerable variation in the number of marbles buried by individual mice, with some mice burying as many as 19 out of 20 marbles and some of their cage mates burying none (Table 7.9). This variation however indicated a normal distribution rather than there being a population of high buriers and one of low buriers.

7.11 Two compartment model.

When mice were tested in a cage half of which contained ten marbles and the other half none they spent equal amounts of time in the two compartments. This was true whether the duration of testing was 5, 10 or 30 minutes (Table 7.10). The animals still found time to bury marbles, the number buried increasing with the duration of the test.

The test cage was identical to the ones used in testing marble burying but was partitioned along the middle with cardboard wall with a 5 x 7.5 cm hole in the middle for the mice to pass through. The test was conducted under the usual animal house lighting .

DISCUSSION

Marble burying seems to be a mixture of deliberate action on the part of mice and burying incidental to their digging saw dust. Since it was found that mice buried most marbles when these were spread evenly in the cage, all the other experiments were done with the marbles so spread. The lower burying rates when the marbles were placed to the side or at the centre of the cage is most probably merely an indication that the mice encounter marbles less frequently and that there was less sawdust at the corner or centre to bury the marbles with. The possibility that fewer marbles were buried because mice had more room to avoid the marbles was addressed by 2-compartment box (see below). After establishing that mice will reliably and consistently bury glass marbles housed with them it was necessary to attempt to establish why mice do bury marbles.

Neophobia (fear of novel objects) is unlikely to be the reason mice bury marbles. There are no signs that mice tried to avoid coming into contact with the marbles. Mice housed with marbles for 4 days would be expected to have become familiar with marbles and lost whatever fear they might have had. On the test day however, they buried marbles to the same extent as those mice that were been exposed to marbles for the first time. This and repeated testing with marbles, both several times on the same day and on successive days has shown that marble burying does not habituate, show extinction or diminution. Neither the approach and manipulatory behaviour appear to be curiosity driven.

If marble burying has no intrinsic value to mice then why does it continue to evoke a response? Generally if a

stimulus is intrinsically neutral the initial avoidance or approach is followed by disinterest (Corey 1978).

The finding that time of day did not affect the extent of marble burying shows that the chronological change in the basic activity of mice does not much affect the extent of marble burying. In particular the finding that the extent of marble burying in the dark phase of the light cycle was no different from that in the bright phase is remarkable. Mice are nocturnal animals and are more active in the dark (Brown 1980), and that they did not bury more marbles then indicates the behaviour is not directly associated with the animals activity status.

Since the bulk of this work was done using female mice, the finding that males have the same propensity to bury marbles shows that the behaviour is not peculiar to female mice.

While there is considerable variation in the number of marbles buried by individual mice in a group, repeated testing also shows that there is also considerable variation in the number of marbles buried from day to day by the same mouse. Repeated testing also showed that marble burying in mice follows normal distribution . This is in contrast to the findings of Broekkamp et al (1986) who found that they could divide their mice into two populations, one burying high numbers and the other low numbers of marbles.

The two compartment model showed that mice neither find marbles aversive nor warranting excessive attention. The presence or absence of marbles made no difference to the amount of time they spent in either compartment. Nevertheless they buried the marbles where present. This

indicates that marble burying was evoked by unconditioned stimuli that have no appreciable aversive component.

That washed marbles handled with gloves were buried to the same extent as hand handled marbles eliminate the possibility that marbles were buried because they had acquired aversive gustatory or olfactory properties from contact with the experimenter's hands. This finding concurs with that of Poling et al (1981).

Is marble burying a model of anxiety? Willner (1984, 1985) proposed face, predictive and construct validity as criteria for validating animal models of human mental disorders. Face validity is the phenomenological similarity between the model and the condition being modelled while predictive validity involves judging the model on the success of predictions based on it in so far as it can identify current and putative treatments without errors of omission and commission. Construct validity is based on the feature being modelled having an empirical or theoretical relation to the human condition and there being a similarity between the behaviour in the model and features of the condition being modelled.

Other criteria have also been suggested: McKinney and Bunney (1969) proposed the similarity of the aetiology, biochemistry, symptomatology and drug effects between the animal model and the human disorder as criteria for validating an animal model. For anxiety the first two are unsuitable as validating criteria as they are themselves still largely subjects of intense research and speculation. The other two criteria assess face validity.

On the face and construct validity criteria, marble burying is not a valid anxiety model as mice do not find

marbles aversive and is therefore unlikely to induce anxiety.

Validation of marble burying as an anxiety test on the predictive validity criterion requires a consideration of the effects of diazepam, beta-CCE and yohimbine on marble burying. The anxiolytic diazepam had biphasic effects increasing marble burying at low doses and decreasing it at high doses. The effects at low doses might be accounted for by the stimulation of exploratory activity which is produced by low doses of benzodiazepines (Christmas and Maxwell 1970). The effects at higher doses appear specific since there was no consistent change in locomotor activity. This finding was consistent with that of Broekkamp et al (1986) and suggests that anxiolytics decrease marble burying. Such a conclusion can not be supported solely by the effects of one drug. We found buspirone, which is anxiolytic in humans (Taylor et al 1985) at 1 to 20 mg/kg to be inactive on this test (Chapter 8).

Anxiogenic compounds beta-CCE and yohimbine however have no effects on marble burying. Even if the test had accurately picked anxiolytic compounds it would be limited by the failure to detect anxiogenic compounds.

This question is further addressed in Chapter 8.

Table 7.1: Effect of spread of marbles in cage.
Mean marbles buried (s.e.m.) N=6

Location of marbles	marbles buried
Spread evenly	12.4(1.9)
On one side of cage	6.3(1.8)*
Centre of cage	6.8(2.8)*

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to results of the test with the marbles evenly spread.

Table 7.2: Effect of testing the same mice on consecutive days.

Mean marbles buried (s.e.m.) N=6

TEST DAY	MARBLES BURIED	
	Expt. 1	Expt. 2
1	9.0(1.6)	10.8(1.4)
2	8.5(1.8)	11.9(1.4)
3	7.3(1.7)	11.4(1.6)
4	7.9(1.5)	11.6(3.0)
5	7.3(1.2)	12.2(1.4)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to results of the first run.

TABLE 7.3: Effect of repeated marble burying on same day (2 hour intervals).

Mean marbles buried (s.e.m.) N=6

TRIAL	MARBLES BURIED
1	6.5(1.3)
2	5.1(1.1)
3	5.2(1.5)
4	7.8(1.6)
5 (dark cycle)	4.4(1.4)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to results of the first run.

TABLE 7.4: Effect of housing mice with marbles.

Mean marbles buried (s.e.m.) N=6

	Marbles buried
Mice housed with marbles 4 days	7.4(1.6)
Mice tested with marbles for the first time	7.3(1.5)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to each other.

TABLE 7.5: Effect of washing marbles before testing.

Mean marbles buried (s.e.m.) N = 6

	Marbles buried
Tested with hand held marbles	8.0(2.3)
Tested with washed marbles	5.3(1.9)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to each other.

TABLE 7.6: Effect of gender on marble burying.

	Marbles buried (s.e.m.) N = 6
Female MFI	8.0 (2.3)
Male MFI	6.3 (1.6)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to each other.

TABLE 7.7: Effect of diazepam, beta-CCE and yohimbine on marble burying.

Mean marbles buried (s.e.m.) N = 6

Drug/dose	Marbles buried	Animex counts
Diazepam		
0.05	8.7(2.3) [6.5(1.4)]	344(18) [382(24)]
0.1	11.8(1.5)* [5.7(1.9)]	476(23)* [393(29)]
0.25	5.8 (2.7) [5.7(1.9)]	
1.0	2.5(1.4)* [5.7(1.9)]	
2.5	0.17(0.17)** [5.7(1.9)]	512(29)* [393(29)]
5.0	2.0(0.8)* [6.5(1.4)]	418(36) [382(24)]
Beta-CCE		
1.0	7.2(2.9) [6.5(1.4)]	
5.0	5.2(1.6) [6.5(1.4)]	
Yohimbine		
1	9.3(2.7) [8.0(2.1)]	
5	2.0(0.4) [8.0(2.1)]	421(9)* [558(24)]
10	0 [8.0(2.1)]	

Statistical comparisons were made on raw data using a t-test. * = $p<0.05$ ** = $p<0.01$ relative to concurrently run vehicle controls.

TABLE 7.8: Effect of diazepam and marbles on duration and extent of digging.

Mean (s.e.m.) N = 6

Drug/dose mg/kg	Time spent digging (sec)	Marbles buried	S. Area disturbed
MARBLES PRESENT			
Saline	77(12)	5.2(1.0)	44(7)
Diazepam 0.1	105(31)	7.3(2.8)	38(12)
Diazepam 2.5	76(17)	4.0(1.6)	45(12)
MARBLES ABSENT			
Saline	79(9)	-	54(9)
Diazepam 0.1	88(11)	-	71(11)
2.5	42(13)	-	24(10)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 7.9: Are there consistent high and low buriers of marbles among mice?

Number of Marbles buried. N = 10

DAY	1	2	3	4	5	Mean(s.e.m) Over 5 days
Mouse						
1	11	9	19	7	8	10.8(2.2)
2	1	16	8	3	4	6.4(2.7)
3	2	6	13	13	7	8.2(2.1)
4	12	3	7	6	5	6.6(1.5)
5	12	17	9	16	17	14.2(1.6)*
6	15	2	0	3	8	5.6(2.7)
7	4	3	4	5	4	4.0(0.3)*
8	7	8	4	11	8	7.6(1.1)
9	15	6	2	12	8	8.6(2.3)
10	11	15	7	3	4	8.0(0.4)
Group Mean	9.0	8.5	7.3	7.9	7.3	8.0(0.3)
s.e.m.	(1.6)	(1.8)	(1.7)	(1.5)	(1.2)	

s.e.m. - standard error of the mean

The marble burying test was performed on the same mice for 5 consecutive days. The mean number of marbles buried by each mouse over that period was compared with the group mean to establish if some mice bury consistently more marbles than the others. Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to the group mean.

TABLE 7.10: Behaviour of mice in a two compartment model.

N=10 Mean (s.e.m.)

Total test time (sec)	Marbles buried	Time (sec) in non-marble side	% Time
1800	8.4(0.4)	916(45)	51 %
600	7.3(0.9)	292(30)	49 %
300	4.2(2.0)	142(14)	47 %

Mice were placed in a two compartment cage, one compartment containing 10 marbles and the other none. Different mice were tested for 30 min (1800 sec), 10 min (600 sec) and 5 min (300 sec). The time spent in the side without marbles was recorded.

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to time spent on the side with marbles.

FIG. 7.1 EFFECT OF DAILY TESTING ON MARBLE BURYING

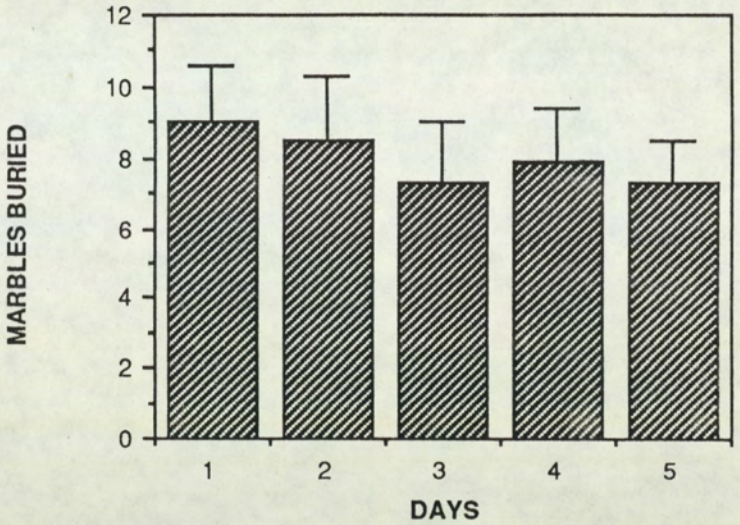
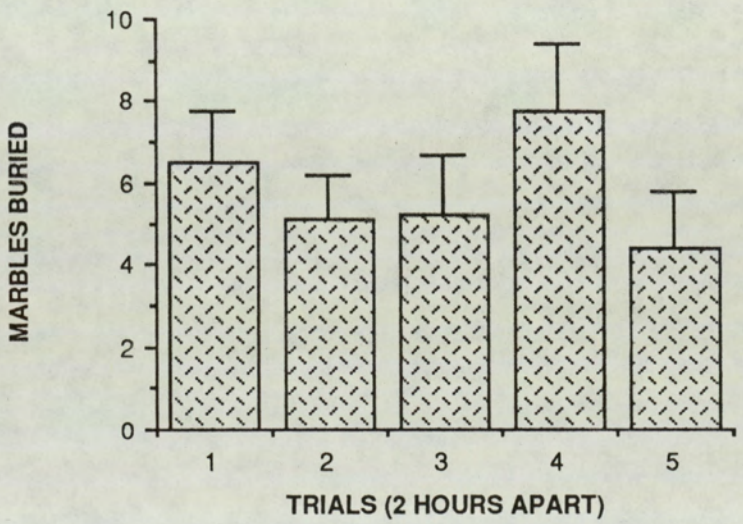
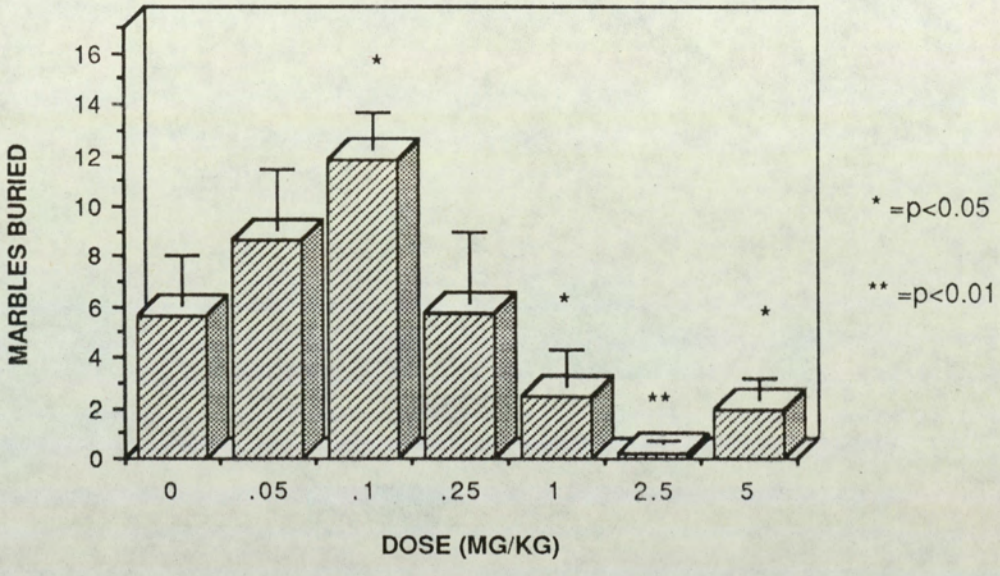


FIG. 7.2 EFFECT OF 2 HOURLY TESTING ON MARBLE BURYING



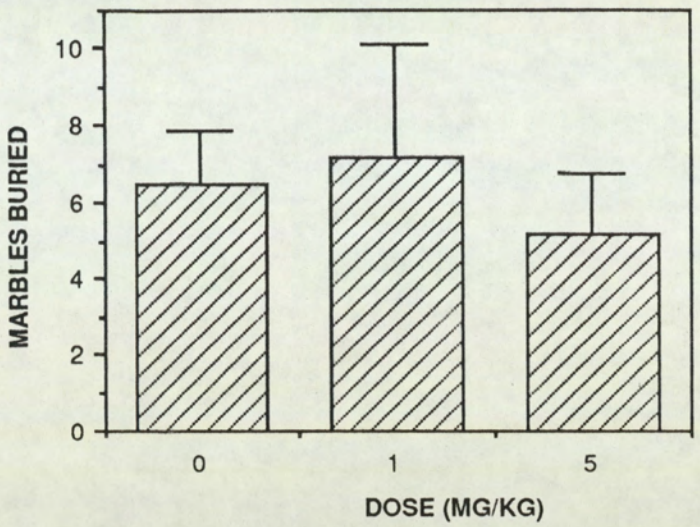
Marbles buried over a 30 min period. Statistical comparisons relative to the first trial were made on raw data using a t-test.

FIG. 7.3 EFFECT OF DIAZEPAM ON MARBLE BURYING

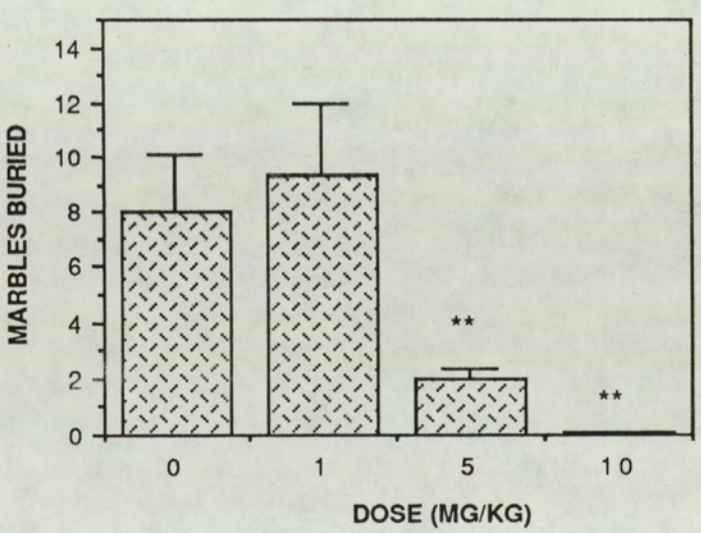


Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 7.4 EFFECT OF BETA-CCE AND YOHIMBINE ON MARBLE BURYING.



BETA-CCE

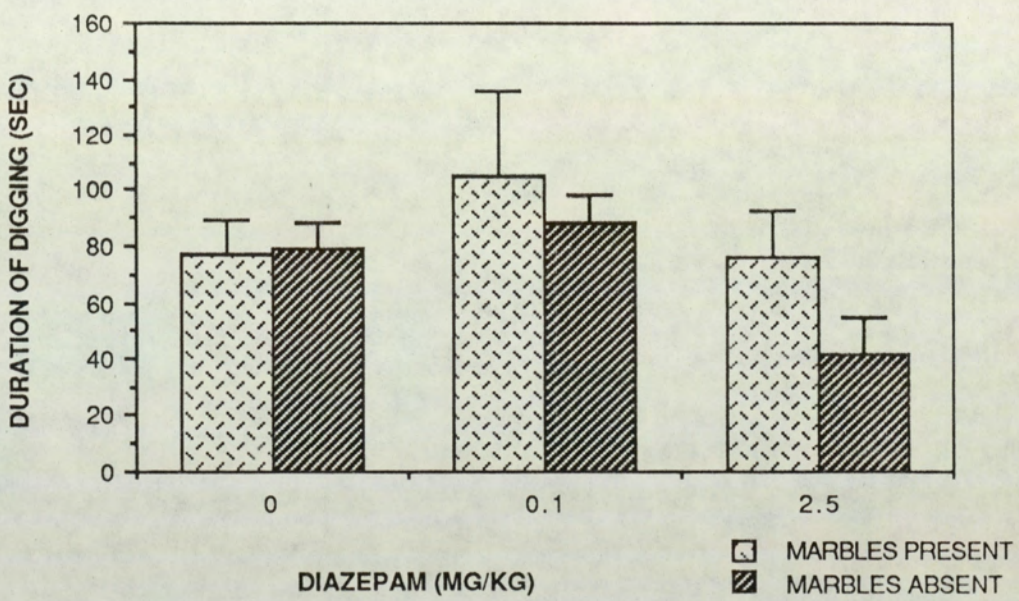


YOHIMBINE

** =p <0.01

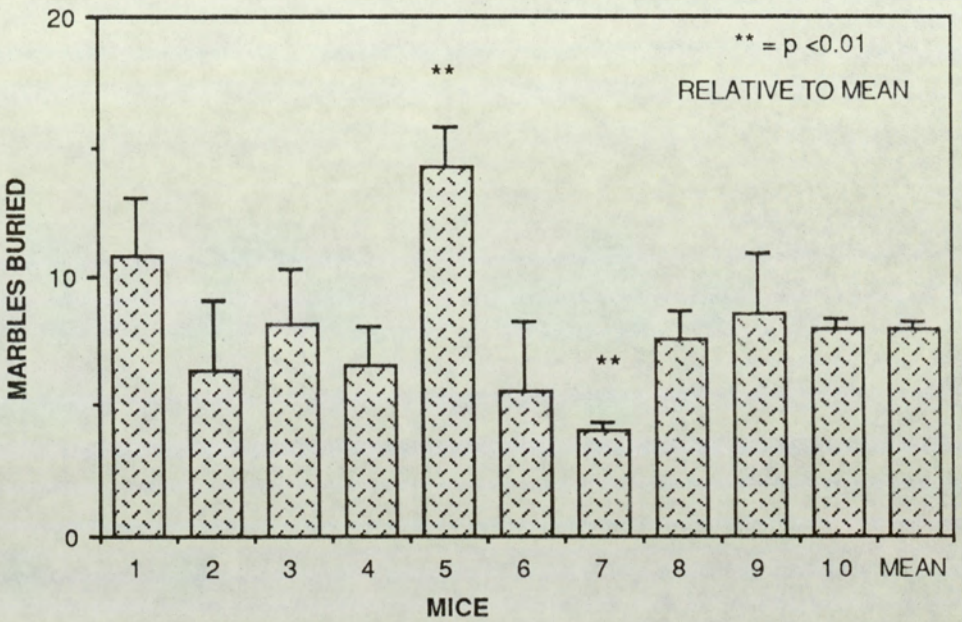
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 7.5 EFFECT OF DIAZEPAM ON DIGGING



Duration of digging of sawdust during a 10 minute observation period both in the presence and in the absence of marbles. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 7.6 ARE THERE HIGH AND LOW MARBLE BURYING MICE?



The mean number of marbles buried over a 5 day period by each mouse. Statistical comparisons relative to the group mean were made on raw data using a t-test.

CHAPTER 8

EFFECT OF 5-HT LIGANDS ON MARBLE BURYING BEHAVIOUR.

Introduction

Results

8.1 Effects of 5-HT1A agonists on marble burying.

8.2 Effects of 5-HT1B/1C agonists on marble burying.

8.3 Effects of the 5-HT2 receptor agonist DOI on marble burying.

8.4 Effects of 5-HT1 antagonists on marble burying.

8.5 Effects of 5-HT2 antagonists on marble burying.

8.6 Effects of 5-HT3 antagonists on marble burying.

8.7 Effect of non-selective 5-HT antagonists on marble burying.

8.8 Effects of 5-HT reuptake inhibitors on marble burying.

8.9 Effects of the 5-HT releaser fenfluramine on marble burying.

8.10 Effects of the 5-HT precursor 5-HTP on marble burying.

8.11 Effects of chronic zimeldine on marble burying.

8.12 Effects of 5-HT2 antagonists on DOI inhibition on marble burying.

8.13 Effects of 5-HT antagonists on zimeldine inhibition of marble burying.

8.14 Effects of ritanserin on the fenfluramine and fluvoxamine inhibition of marble burying.

8.15 Effect of 5-HT antagonists on mCPP inhibition

INTRODUCTION

Marble burying has been reported to be an animal model of anxiety (Broekkamp et al 1986). To date however there has been only one report where 5-HT modulating agents have been tested on this model; 5-HT re-uptake inhibitors were reported to reduce marble burying in mice while other 5-HT ligands were reported to be inactive (Broekkamp and Jenck 1987).

This project set out to establish the effects of 5-HT ligands on this model and to establish what 5-HT receptor subtypes modulate marble burying. Since 5-HT reuptake inhibitors were reported to reduce marble burying (Broekkamp and Jenck 1987) we also wanted to establish which 5-HT receptor(s) were involved.

In addition to acute injection, zimeldine was also administered chronically to mice, on the first day i.p. then orally in water for 21 days. After cessation of zimeldine treatment, marble burying was tested for 2 days during the withdrawal phase. 48 hours after zimeldine withdrawal some of these animals were injected with DOI and tested for marble burying.

Another group of mice were pre-treated for three days with the 5-HT depletor PCPA at 300 mg/kg subcutaneously and 24 hours after the last PCPA injection were tested for marble burying after acute injections of 8-OH-DPAT, zimeldine, DOI and mCPP.

It was necessary to establish that reduction in marble burying was not due to a drug's sedative effects. Specificity of the drug effects was determined by testing the effects of the drugs on locomotor activity. This was done using an Animex counter (see Chapter 2 for details).

RESULTS

8.1 Effects of 5-HT1A agonists on marble burying.

Ipsapirone at 5 to 20 mg/kg produced no effects on marble burying (Table 8.1 and Fig 8.1). Ipsapirone had no effects on locomotor activity up to 20 mg/kg (Table 8.1).

Gepirone 5 mg/kg had no effects on marble burying. At 10 and 20 mg/kg however gepirone reduced both marble burying and locomotor activity (Table 8.1 and Fig 8.2).

Buspirone at 1 to 10 mg/kg produced no effects on marble burying (Table 8.1 and Fig 8.3). At 20 mg/kg buspirone significantly reduced marble burying but this dose also reduced locomotor activity.

8-OH-DPAT at 0.3 to 10 mg/kg reduced marble burying, but only at the highest dose tested (10mg/kg) was this reduction statistically significant (Table 8.2 and Fig 8.4). However, even at 3 mg/kg 8-OH-DPAT significantly reduced locomotor activity so its effect was not specific to marble burying.

5-MeODMT at 0.25 and 1.0 mg/kg produced an increase in marble burying (Table 8.2 and Fig 8.5) but in both cases the increase was not statistically significant. At higher doses, 2.5 and 5.0 mg/kg there was a significant reduction in marble burying but this was accompanied by a significant reduction in locomotor activity (Table 8.2 and Fig 8.5).

8.2 Effects of 5-HT1B/1C agonists on marble burying.

RU 24969 at 0.1 to 1 mg/kg did not have any appreciable

effect on marble burying. At doses of 2.5 to 10 mg/kg there was a significant reduction in marble burying (Table 8.3 and Fig 8.6). At the lowest dose where this decrease in marble burying was observed (2.5 mg/kg), there was a dramatic increase in locomotor activity.

MCPP (1-(3-chlorophenyl)piperazine) at 1 mg/kg produced a slight increase in marble burying while 2.5 mg/kg significantly reduced marble burying. Higher doses, 5 at 20 mg/kg, produced very marked and significant reductions in marble burying (Table 8.4 and Fig 8.7). MCPP at 2.5 and 10 mg/kg increased locomotor activity though the increase was not statistically significant. At 20 mg/kg however, the mice appeared sedated and locomotor activity was significantly reduced (Table 8.4).

TFMPP (1-(m-trifluoromethylphenyl)piperazine) at 1 mg/kg had no effects on marble burying. At higher doses, 5 at 20 mg/kg, it sharply reduced marble burying. At the highest dose, 20 mg/kg there was a sedative effect indicated by a reduction in locomotor activity (Table 8.4 and Fig 8.8).

8.3 Effects of the 5-HT2 receptor agonist DOI on marble burying.

At doses of 0.01 to 0.05 mg/kg, DOI (1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane) had no effects on marble burying. DOI at 0.1 to 10 mg/kg produced a dose dependent reduction in marble burying without producing any changes in locomotor activity (Table 8.5 and Fig 8.9).

8.4 Effects of 5-HT1 antagonists on marble burying.

Neither of the two 5-HT1/beta-adrenoceptor antagonists tested, pindolol and timolol 5 and 10 mg/kg had any

effects on marble burying (Table 8.6 and Fig 8.10).

8.5 Effects of 5-HT2 antagonists on marble burying.

The 5-HT2 antagonists ritanserin at 0.1 to 10 mg/kg and ICI 169,369 at 1 to 10 mg/kg had no effects on either marble burying or locomotor activity. At 20 mg/kg ritanserin reduced both marble burying and locomotor activity (Table 8.7 and Fig. 8.11).

Ketanserin at 1 and 10 mg/kg reduced both marble burying and locomotor activity (Table 8.7 and Fig 8.11).

8.6 Effects of 5-HT3 antagonists on marble burying.

ICS 205-930 at 0.1 to 10 mg/kg and GR 38032 at 0.01 to 1.0 mg/kg had no effects on marble burying (Table 8.8 and Fig 8.12).

8.7 Effect of non-selective 5-HT antagonists on marble burying.

Methysergide at 1 mg/kg had no effects on marble burying but at 5 and 10 mg/kg there was a significant reduction in marble burying. There was a significant reduction in Animex counts at 5 mg/kg (Table 8.9 and Fig. 8.13.1).

Cyproheptadine at 1 mg/kg had no effects on marble burying At 2 mg/kg there was a reduction (statistically insignificant) in marble burying while at 5 mg/kg there was a significant reduction in marble burying accompanied by a reduction in locomotor activity (Table 8.9 and Fig. 8.13.2).

8.8 Effects of 5-HT reuptake inhibitors on marble

burying.

Zimeldine at 1 mg/kg produced no effects on marble burying. Higher doses, 3 to 30 mg/kg, reduced marble burying without affecting locomotor activity (Table 8.10 and Fig 8.14).

Citalopram at 1 to 10 mg/kg did not affect marble burying. The vehicle controls were however very low. At 20 mg/kg the compound reduced marble burying without affecting locomotor activity (Table 8.10 and Fig 8.15).

Fluvoxamine at 5 to 20 mg/kg reduced marble burying without affecting locomotor activity (Table 8.10 and Fig 8.16).

Indalpine at 5 to 20 mg/kg reduced marble burying without affecting locomotor activity (Table 8.10 and Fig 8.17).

8.9 Effects of the 5-HT releaser fenfluramine on marble burying.

Fenfluramine at 1 mg/kg did not affect marble burying. Fenfluramine at doses from 2.5 to 10 mg/kg reduced marble burying without affecting locomotor activity (Table 8.12 and Fig 8.18).

8.10 Effects of the 5-HT precursor 5-HTP on marble burying.

The 5-HT precursor 5-HTP (5-hydroxytryptophan) was co-administered with the decarboxylase inhibitor, carbidopa at 9 mg/kg. Compared to saline controls carbidopa produced a substantial though statistically insignificant reduction in marble burying and reduced

locomotor activity (Table 8.13 and Fig 8.19). 5-HTP at doses of 5 to 50 mg/kg reduced marble burying compared to carbidopa treated animals (Table 8.13 and Fig 8.19). Even at the lowest dose used (5 mg/kg), 5-HTP reduced locomotor activity compared to carbidopa treated animals (Table 8.13).

8.11 Effects of chronic zimeldine on marble burying.

Initially, zimeldine at 10 mg/kg daily reduced marble burying compared to vehicle control animals. However, by the fourteenth day the zimeldine treated group buried the same number of marbles as the control group, and this persisted until zimeldine was withdrawn after 21 days and for two days during the withdrawal phase (Table 8.14 and Fig 8.20).

DOI at 0.1 mg/kg significantly inhibited marble burying in mice tested on the second day after the the withdrawal of zimeldine. The degree of inhibition of marble burying (ca. 50%) was the same as in control animals treated with the same dose of DOI (Table 8.15 and Fig 8.21).

8.12 Effects of 5-HT2 antagonists on DOI inhibition on marble burying.

Ritanserin at 0.2, 0.5 and 1 mg/kg produced dose-dependent reductions in DOI (0.1 mg/kg) inhibition of marble burying (Table 8.16 and Fig 8.22).

ICI 169,369 at 1 and 5 mg/kg did not affect the DOI (0.1 mg/kg) inhibition of marble burying. However, ICI 169,369 at 10 mg/kg reversed the DOI effect (Table 8.17 and Fig 8.22).

Pindolol at 5 mg/kg had no effects on the DOI (0.1 mg/kg) inhibition of marble burying (Table 8.18 and Fig 8.23).

8.13 Effects of 5-HT antagonists on zimeldine inhibition of marble burying.

The 5-HT2/5-HT1C antagonist ritanserin at 1 and 3 mg/kg potentiated the zimeldine (3 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.19 and Fig 8.24.1).

The 5-HT2 antagonist ICI 169,369 at 10 mg/kg potentiated the zimeldine (10 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.19 and Fig 8.24.1).

The 5-HT3 antagonist ICS 205-930 at 10 mg/kg potentiated the zimeldine (10 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.19 and Fig 8.24.2).

The 5-HT1A/1B antagonist pindolol at 10 mg/kg potentiated the zimeldine (10 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.19 and Fig 8.24.2).

The non-selective antagonist cyproheptadine at 1 mg/kg had no effects on the zimeldine (10 mg/kg) inhibition of marble burying (Table 8.20 and Fig 8.25). Cyproheptadine at 2 mg/kg reduced marble burying. When this was co-administered with zimeldine there was an additive effect on the later compound's inhibition of marble burying (Table 8.20 and Fig. 8.25).

8.14 Effects of ritanserin on the fenfluramine and

fluvoxamine inhibition of marble burying.

Both fenfluramine and fluvoxamine at 10 mg/kg abolished marble burying in mice. Ritanserin administered 10 minutes before either drug at 5 mg/kg had no effect on this inhibition of marble burying (Table 8.26).

Ritanserin at 5 mg/kg potentiated the fenfluramine (1 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.21 and Fig 8.26).

Ritanserin at 5 mg/kg potentiated the fluvoxamine (10 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.21 and Fig 8.26).

8.15 Effect of 5-HT antagonists on mCPP inhibition of marble burying.

MCPP at 10 mg/kg completely abolished marble burying. Neither the 5-HT1A/1B antagonist pindolol at 10 mg/kg nor the 5-HT2/1C antagonist ritanserin at 10 mg/kg which alone did not affect marble burying, had effects on this inhibition of marble burying (Table 8.22).

Pindolol at 5 mg/kg and ritanserin at 5 mg/kg potentiated the mCPP (2.5 mg/kg) inhibition of marble burying without affecting locomotor activity (Table 8.22, Fig 8.28 and Fig 8.29).

The non-selective 5-HT antagonist cyproheptadine at 1 and 2 mg/kg had no effects on mCPP inhibition of marble burying (Table 8.23 and Fig 8.30).

8.16 Effects of the 5-HT depleter PCPA on marble burying.

Pretreatment of mice with PCPA at 300 mg/kg for 3 days had no effects on marble burying 24 hours after the last PCPA injection (Table 8.24 and Fig 8.31). PCPA pretreatment did not affect the inhibition of marble burying by DOI (0.1 mg/kg), 8-OH-DPAT (10 mg/kg), mCPP (10 mg/kg) or zimeldine (Table 8.24 and Fig 8.31). Brain 5-HT levels fell by 46 % compared to vehicle controls.

Mean 5-HT level in vehicle controls = 416 ± 27 ng/g
Mean 5-HT level after PCPA = 223 ± 16 ng/g
Brain 5-HT expressed in nanograms per gram wet brain weight. N = 6.

DISCUSSION.

5-HT1A agonists had no specific effects on marble burying behaviour in mice. Either, like ipsapirone or buspirone they did not affect marble burying or, like 8-OH-DPAT or gepirone, they reduced both marble burying and locomotor activity at the same doses. This indicates that 5-HT1A receptors do not specifically modulate marble burying.

The 5-HT1A/1B agonist RU 24969 also had non-selective effects: reducing marble burying but drastically increasing locomotor activity. This hyperlocomotion was such that it was unlikely the mice stopped long enough to notice the marbles let alone bury them.

The 5-HT1B/1C agonists MCPP and TFMPP selectively reduced marble burying at doses that did not significantly change locomotor activity. This suggests a role for 5-HT1B and/or 5-HT1C receptors in mediating this behaviour. Both the 5-HT2/1C antagonist ritanserin and the 5-HT1A/1B antagonist pindolol potentiate mCPP inhibition of marble burying which suggests that both 5-HT1B and 5-HT1C receptors are involved in marble burying behaviour and that blockade of either receptor facilitates the effects of the other. The potentiation by pindolol could be due to the compound's presynaptic inhibition of cell body autoreceptors (Middlemiss 1986a) increasing 5-HT release which would have additive effects to the postsynaptic effects of mCPP. Low doses of cyproheptadine, a non-selective 5-HT receptor antagonist, did not block the mCPP inhibition of marble burying. Higher doses of cyproheptadine however could not used as the compound also reduced marble burying.

The 5-HT1 antagonist effects were variable. The 5-HT1A/1B

and beta-adrenoceptor antagonists pindolol and timolol did not affect marble burying. The non selective 5-HT antagonist methysergide reduced both marble burying and locomotor activity as did high doses of cyproheptadine.

The 5-HT2/1C agonist DOI selectively reduced marble burying without affecting locomotor activity. The inhibition of marble burying was abolished by the 5-HT2/1C antagonists ICI 169,369 and ritanserin which themselves had no effects on marble burying. This indicates that marble burying behaviour is modulated by 5-HT2 and/or 5-HT1C receptors. The lack of effects by the antagonists on their own probably indicates that there is very little neurotransmitter tone in the system involved.

The 5-HT1A/1B antagonist pindolol had no effects on the DOI inhibition of marble burying. This is consistent with the fact that pindolol has negligible affinity for 5-HT1C and 5-HT2 receptors (see Table 1.1).

Both ritanserin (Hoyer 1989, Hoyer et al 1989) and ICI 169,369 (Blackburn et al 1988) block both 5-HT2 and 5-HT1C receptor mediated effects. Ketanserin does not block 5-HT1C mediated effects (Hoyer et al 1989) and would have helped elucidate whether DOI marble burying is mediated by 5-HT2 or 5-HT1C receptors. However ketanserin produced gross sedation characterised by palpebral ptosis and reduced marble burying and locomotor activity at low doses, effects presumably mediated by alpha-1 adrenoceptors [Ki at alpha-1 receptors = 11, at 5-HT2 = 0.63, at 5-HT1C = 98] (Leysen, unpublished data), and so could not be used to elucidate this point.

The potent activity of DOI, mCPP and TFMPP in inhibiting burying suggest the possible involvement of 5-HT1C

receptors (Hoyer 1988,Hoyer et al 1989) without eliminating the possible involvement of 5-HT2 and 5-HT1B receptors.

5-HT3 receptors do not appear to modulate marble burying behaviour as indicated by the lack of effects of the 5-HT3 antagonists GR 38032F and ICS 205-930.

The 5-HT reuptake inhibitors zimeldine, indalpine citalopram and fluvoxamine all inhibited marble burying at doses that did not affect locomotor activity. The same was true of the 5-HT releaser fenfluramine. These findings concur with those of Broekkamp and Jenck (1987).

The effects of selective antagonists on this inhibition of marble burying gave a complex picture. The selective 5-HT antagonists, pindolol (5-HT1A and 5-HT1B), ritanserin (5-HT1C and 5-HT2), ICI 169,369 (5-HT2 and 5-HT1C) and ICS 205-930 (5-HT3) potentiated zimeldine inhibition of marble burying. This was a surprising finding. The same potentiation of marble burying was seen when ritanserin was co-adminstered with fenfluramine or fluvoxamine. This removes the possibility that the potentiation is an idiosyncratic effect of zimeldine. It contrasts with the results in the X-maze where ritanserin failed to reverse the reduction in total arm entries by fenfluramine but reversed the anxiogenic effects.

The effects of the non-selective antagonist cyproheptadine were not that clear cut. At 1 mg/kg cyproheptadine did not affect zimeldine inhibition of marble burying while at 2 mg/kg of cyproheptadine, the net result was a summation of both compounds' inhibition of marble burying.

These results seem to indicate that marble burying

behaviour is mediated by more than one 5-HT receptor subtype and blockade of one facilitates the others.

Para-chlorophenylalanine (PCPA) is a selective depletor of brain 5-HT (Koe & Weissman 1966). The failure of PCPA pretreatment to affect both marble burying behaviour and the inhibition of marble burying by DOI, zimeldine and 8-OH-DPAT is presumably because the reduction in brain 5-HT was inadequate to alter marble burying behaviour and its modulation by 5-HT agents. Three day pretreatment with PCPA at 300 mg/kg produced 46% depletion of brain 5-HT in mice. This dose of PCPA produced 79 % depletion of brain 5-HT in rats (Critchley 1988). In any case whole brain concentration of the neurotransmitter does not necessarily reflect the amount available at the receptor (Grahame-Smith 1971).

The lack of effect on marble burying of 5-HT depletion also concurs with inactivity of 5-HT antagonists when given alone. It could mean that the normal 5-HT tone is too low to affect marble burying and so depletion has no effect.

Chronic administration of zimeldine for 14 days resulted in tolerance to the compound's inhibition of marble burying which persisted until the experiment was terminated after 21 days. This is the time course for the down regulation of 5-HT2 receptors by this compound (Ogren and Fuxe 1985). This appears to suggest that zimeldine effects were modulated by 5-HT2 receptors and the inhibition of marble burying ceased when the receptor activity fell below a certain threshold.

The number of marbles buried by control mice on the first day was significantly lower than the number buried on

subsequent days. Although there was a tendency for the number of marbles buried by vehicle treated mice to increase until day 14 these increases were not significant. It must also be borne in mind that each day's reading in Fig 8.20 represents a different sample of mice from the vehicle or zimeldine treated groups (i.e. each mouse was tested only once). Save for the small number of marbles buried on the first day, the within-treatment group differences in this experiment are within the limits of variation encountered with different vehicle control groups in general.

The results obtained with DOI in mice during the zimeldine withdrawal phase do not support the conclusion that chronic zimeldine produced down-regulation of 5-HT2 receptors. Down regulation of 5-HT2 receptors would be expected to reduce DOI inhibition of marble burying behaviour. It is possible that zimeldine did not cause down regulation of 5-HT2 receptors in mice (there are no studies on receptor numbers on mice after chronic zimeldine) or that some of the brain areas involved in the DOI effects escape down-regulation by zimeldine. Alternatively, the DOI effect may involve 5-HT1C receptors. It is not known whether this receptor down-regulates.

The inactivity of the clinically active anxiolytic buspirone and the putative anxiolytics ipsapirone and gepirone in association with the behavioural findings in chapter 7 indicates that marble burying is not a valid test of anxiety. This is a further indication of the limitations of the use of benzodiazepines to validate anxiety models. Broekkamp et al (1986) had used the inhibition of marble burying by doses of benzodiazepines without sedative effects to suggest this behaviour as an

anxiety model. The ethological studies in Chapter 7 however show that the model lacks most of the features anxiety an model should have.

There is however the striking activity observed with 5-HT reuptake inhibitors. These agents are clinically used to treat obsessive compulsive disorders (OCD) (see Chapter 1). The persistence of marble burying behaviour is similar to OCD in the sense that a functionally useless behaviour is performed repeatedly and without extinction. It has been proposed that marble burying may be an animal model of OCD (Broekkamp and Jenck 1987). However, while 5-HT reuptake inhibitors reduce marble burying on acute administration and their effects fall off on chronic administration, in OCD these agents have to be taken for months to become effective. On the other hand mCPP aggravates the symptoms of OCD in patients (Zohar et al 1988). In addition, the results from the two compartment model (Chapter 7) show that mice do not preferentially spend more time in the side of the cage with marbles which would have been expected if mice had a compulsion to bury marbles. These findings lead to the conclusion that marble burying is unlikely to be a model for OCD.

In conclusion, burying of innocuous objects by mice is a behaviour that could be a useful tool in studying 5-HT receptor interactions. It is modulated by 5-HT2 and/or 5-HT1C receptors as indicated by the effects of DOI, mCPP and TFMPP and by 5-HT uptake inhibitors and the 5-HT releaser fenfluramine. It remains to be established which 5-HT receptor subtypes mediate the effects of the uptake blockers. Fruitful approaches would include intracerebral (i.c.v.) injection of the selective 5-HT neurotoxin 5,7-DHT (Breese 1975) and use of higher doses of PCPA which would give greater depletion of brain 5-HT. The availability of

a selective 5-HT1C receptor antagonist and a 5-HT2 receptor antagonist without effects on marble burying on its own would go a long way in helping elucidate this puzzle.

Table 8.1: Effects of 5-HT1 agonists ipsapirone, gepirone and buspirone on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
Ipsapirone		
5	5.0(1.5) [8.5(1.8)]	
10	7.9(1.4) [8.5(1.8)]	
20	6.3 (1.7) [8.5(1.8)]	159(28) [164(10)]
Gepirone		
5	6.6(0.75) [7.3(1.4)]	
10	2.8(1.0)* [6.5(1.4)]	368(16)* [481(35)]
20	1.3(0.97)* [7.3(1.4)]	28(6.1)** [209(8.3)]
Buspirone		
1	9.5 (2.4) [8.8 (1.4)]	
5	6.7 (1.3) [5.9 (1.3)]	
10	9.8 (1.4) [5.9 (1.3)]	
20	2.2 (0.3) [8.8 (1.4)]	223 (22)** [391 (14)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.2: Effects of 5-HT1 agonists 8-OH-DPAT and 5-MeODMT on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
8-OHDPAT		
0.3	3.0(0.8) [5.1(0.8)]	
1.0	4.0(0.9) [5.1(0.8)]	333(64)* [526(23)]
3.0	2.9(0.8) [5.1(0.8)]	
10	0.9(0.45)* [5.1(0.8)]	61.8(20)* [209(8.3)]
5-MeODMT		
0.25	5.9 (1.3) [3.6 (1.4)]	
1.0	5.8 (1.6) [3.6 (1.4)]	
2.5	2.6 (0.9)* [6.4 (1.3)]	421(16)** [500(14)]
5.0	0.1 (0.1)** [6.4 (1.3)]	

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.3: Effect of RU 24969 on marble burying behaviour.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
RU 24969		
0.1	5.1 (1.2) [6.9 (1.9)]	
1.0	3.3 (1.5) [6.9 (1.9)]	
2.5	1.5 (0.6)* [5.5 (1.6)]	843(39)** [481(35)]
5.0	1.2 (0.4)* [5.5 (1.6)]	
10	0** [5.5(1.6)]	

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.4: Effect of mCPP and TFMPP on marble burying behaviour.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
MCPP		
1	12.5(1.8) [8.0(2.3)]	
2.5	3.8(0.7)* [8.0(2.3)]	556(24) [483(25)]
5	2.0 (0.9)* [10.0 (2.1)]	
10	0.7 (0.4)** [10.0 (2.1)]	539 (21) [481 (20)]
20	0 [10.0 (2.1)]	118 (19)* [217 (16)]
TFMPP		
1	5.5(2.3) [8.0(2.3)	
5	1.3(0.5)* [7.0(1.8)]	
10	0.5(0.3)* [7.0(1.8)]	
20	0 [7.0(1.8)]	259(18)** [391(14)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.5: Effects of the 5-HT2 agonist DOI on marble burying behaviour.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	Marbles buried	Animex counts
DOI		
0.01	9.4(1.8) [7.3(1.0)]	
0.02	7.3(2.1) [7.3(1.0)]	
0.05	5.3(1.3) [7.3(1.0)]	
0.1	3.4(1.05)** [10.0(1.5)]	224(27) [209(8.3)]
0.5	1.4(0.6) ** [10.0(1.5)]	
1.0	0.9(0.9)** [8.5(1.8)]	
2.5	0 ** [8.5(1.8)]	
5.0	0.1(0.1)** [8.5(1.8)]	274(27) [240(24)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.6: Effects of 5-HT1 antagonists on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried
Pindolol	
5	8.3(2.2) [8.9(1.6)]
10	9.2(2.2) [10.0(2.5)]
Timolol	
5	10.2 (2.7) [10.0 (2.5)]
10	6.8 (1.6) [10.0 (2.5)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.7: Effects of the 5-HT2 antagonists on marble burying behaviour.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	Marbles buried	Animex counts
Ritanserin		
1	6.3(1.9) [8.1(1.5)]	
5	4.8(2.0) [8.5(1.8)]	455(36) [483(25)]
10	6.8(2.4) [8.5(1.8)]	137(28) [161(16)]
20	2.1(1.2)* [8.5(1.8)]	371(35)** [558(24)]
ICI 169,369		
1	6.1(2.0) [8.3(1.7)]	
5	12.7(2.1) [10.2(1.9)]	
10	6.8(1.7) [6.1(0.8)]	214(19) [209(8.3)]
Ketanserin		
1.0	1.3(0.8)** [10.0(2.5)]	470(25)* [558(24)]
10	0** [10.0(2.5)]	

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.8: Effects of 5-HT3 antagonists on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried
ICS 205-930	
0.1	5.7 (1.6) [7.5 (1.3)]
1.0	9.0 (2.1) [7.5 (1.3)]
10	6.5 (2.1) [7.5 (1.3)]
GR 38032F	
0.01	10.3 (0.8) [8.8 (1.4)]
0.1	8.3 (1.5) [8.8 (1.4)]
1.0	7.7 (1.1) [8.8 (1.4)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.9: Effects of non-selective 5-HT antagonists on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
Methysergide		
1	6.0(2.4) [8.0(2.3)	
5	2.3 (1.1)** [8.8 (1.4)]	471(45)* [550(13)]
10	3.3 (1.5)* [8.8 (1.4)]	
Cyproheptadine		
1	10.7 (2.6) [10.1 (2.1)]	
2	5.2 (2.6) [10.1 (2.1)]	
5	3.2 (2.2)* [10.1 (2.1)]	376(62)* [507(26)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.10: Effects of zimeldine and citalopram on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
Zimeldine		
1	5.6(1.6) [6.0(1.7)]	
3	4.0(1.4) [5.3(1.2)]	197(15) [209(8.3)]
10	3.2(1.4)* [8.9(1.6)]	445(23) [483(25)]
15	3.8(0.8)* [8.7(0.3)]	
30	0.7(0.4)** [8.7(0.4)]	198(33) [240(24)]
Citalopram		
1	9.3(2.6) [4.7(1.5)]	
5	3.5 (1.0) [4.3 (1.3)]	
10	3.7 (1.9) [4.3 (1.3)]	
20	0.7 (0.5)* [4.3 (1.3)]	513(25) [481(20)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.11: Effects of fluvoxamine and indalpine on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
Fluvoxamine		
1	6.8(1.6) [4.7(1.5)]	
5	3.7(1.1)* [8.7(1.2)]	
10	3.6(1.0)* [8.7(1.2)]	
20	1.4(0.9)** [8.7(1.2)]	284(22) [240(24)]
Indalpine		
1	5.0 (1.3) [5.9 (1.3)]	
5	2.2 (0.7) [4.7 (1.5)	
10	2.8 (0.9) [5.9 (1.3)]	
20	0.3 (0.1)** [5.9 (1.3)]	510 (26) [500 (14)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.12: Effect of fenfluramine on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)	marbles buried	Animex counts
Fenfluramine		
1	6.3(1.2) [6.3(2.7)]	
2.5	2.3(1.2)* [6.3(2.7)]	
5	2.9(1.5)* [6.0(1.7)]	
10	0.3(0.2)** [6.0(1.7)]	226(22) [209(8.3)]

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.13: Effect of 5-HTP on marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		Marbles buried	Animex counts
Saline		8.9 (1.6)	481(35)
Carbidopa	9	4.2 (1.4)	418(40)^
Carbidopa 9/ 5-HTP	5	2.1 (1.3)* [4.2 (1.4)]	314(19)** [418(40)]
Carbidopa 9/ 5-HTP	20	2.0 (1.0)* [4.2 (1.4)]	
Carbidopa 9/ 5-HTP	50	0.1 (0.1)** [4.2 (1.4)]	

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to carbidopa mice.

^ =$p<0.05$ relative to saline controls.

Table 8.14: Effect of chronic administration of zimeldine on marble burying.

	Marbles buried in 30 min (s.e.m.)	
Day	Zimeldine	Saline
1	1.6(0.6)*	4.1(0.9)
4	5.3(1.5)*	8.7(1.2)
7	5.2(1.3)*	11.1(1.4)
10	6.4(1.6)*	13.9(1.7)
14	13.2(1.0)	14.1(1.4)
17	8.0(1.8)	8.7(1.6)
21	9.3(1.9)	11.4(1.6)
Withdrawal phase		
22	8.0(1.4)	12.0(1.7)
23	8.0(1.4)	10.1(1.8)

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.15: Effect of DOI in mice two days after zimeldine withdrawal.

	Marbles buried in 30 min (s.e.m.) Previous drug treatment (21 days)	
Drug/dose (mg/kg)	Zimeldine	Vehicle
DOI 0.1	3.9(1.0)*	5.4(1.2)*
Vehicle	[8.0(1.4)]	[10.1(1.8)]

Statistical comparisons were made on raw data using a t-test. *=$p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.16: Effect of ritanserin on DOI inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		Marbles buried
Ritanserin	0.2	10.1(1.9)
DOI	0.1	3.0(0.7)*
Rit 0.2/DOI	0.1	4.0(0.4)*
Saline		10.0(2.5)
Ritanserin	0.5	10.5(2.2)
DOI	0.1	3.0(0.7)*
Rit 0.5/DOI	0.1	5.8(2.2)
Saline		10.0(2.5)
Ritanserin	0.1	6.3(1.9)
DOI	0.1	2.0(0.8)*
Rit 1.0/DOI	0.1	7.0(1.6)
Saline		8.1(1.7)

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.17: Effect of ICI 169,369 on DOI inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		Marbles buried
ICI 169,369	1.0	6.1(2.0)
DOI 0.1		2.7(0.4)*
DOI 0.1 +ICI	1.0	1.9(0.7) *
Saline		8.3(1.7)
ICI 169,369	5.0	12.7(2.1)
DOI	0.1	4.8(1.4)*
ICI 5.0/DOI	0.1	4.0(0.95)*
Saline		10.2(1.9)
ICI 169,369	10	6.8(1.7)
DOI	0.1	3.0(1.0)*
ICI 10/DOI	0.1	6.1(2.3)
Saline		6.1(0.8)^

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls. ^ = $p<0.05$ relative to DOI.

Table 8.18: Effect of pindolol on DOI inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		Marbles buried
Pindolol	5	5.3(1.8)
DOI	0.1	2.5(1.4)*
Pind 5/DOI	0.1	1.5(0.6)*
Saline		4.7(1.5)

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.19 Effect of selective 5-HT antagonists on zimeldine inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		marbles buried	Animex counts
Saline		5.3(1.2)	
Zimeldine	3	4.0(1.4) *	
Ritanserin	1	12.0(0.4)	
Rit 1/zim	3	1.9(1.1) **	
Saline		5.3(1.2)	240(24)
Ritanserin	5	8.7(2.3)	235(29)
Rit 5/zim	3	1.3(0.4)**	211(33)
zimeldine	3	4.0(1.4) *	197(15)
Saline		6.4(1.9)	483(25)
ICI 169,369	10	6.8(1.7)	490(26)
Zim	10	1.5(0.8)*	445(23)
ICI 10/ Zim	10	0 **	398(22)*
Saline		10.0(2.5)	483(25)
ICS 205-930	10	11.3(1.9)	467(22)
zimeldine	10	2.3(0.9)*	445(23)
ICS 10/Zimeldine	10	0.2(0.2)**	550(33)
Zimeldine	10	3.2(1.4)*	445(23)
Saline		8.9(1.6)	483(25)
Pindolol	5	8.3(2.2)	489(18)
Zim 10/Pind	5	0.25(0.16)*	418(26)

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.20 Effect of the non-selective 5-HT antagonist cyproheptadine on zimeldine inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		marbles buried
Saline		11.6(2.8)
Cyproheptadine 1		10.7(2.1)
Zimeldine	10	1.3(0.7)**
Cypr 1/Zim	10	0.8(0.5)**
Saline		11.6(2.8)
Cyproheptadine 2		5.2(2.6)
Zimeldine	10	1.3(0.7)**
Cypr 2/Zim	10	0**

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.21 Effects ritanserin on fenfluramine and fluvoxamine inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/dose (mg/kg)		Marbles buried	Animex counts
Saline		10.0(2.5)	
Rit	5	4.8(2.0)	
Fenfluramine	10	0**	
Rit 5/Fen	10	0**	
Saline		10.0(2.5)	
Ritanserin	5	4.8(2.0)	
Fluvoxamine	10	0**	
Rit 5/Fluv	10	0**	
Ritanserin	5	8.0(2.5)	455(36)
Saline		6.3(2.7)	483(25)
Fenfluramine	1	6.3(1.2)	488(28)
Rit 5/Fen	1	0.2(0.2)**	398(47)
Saline		6.3(2.7)	483(25)
Ritanserin	5	8.0(2.5)	455(36)
Fluvoxamine	5	3.3(2.2)*	513(14)
Rit 5/Fluv	5	0 **	472(13)

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.22 Effect of pindolol and ritanserin on mCPP inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/Dose (mg/kg)		Marbles buried	Animex counts
Saline		10.0(2.5)	
Pindolol	10	9.2(2.2)	
mCPP	10	0 **	
Pind 10/mCPP	10	0.2(0.2)**	
Saline		8.0(2.3)	483(25)
Pindolol	5	8.3(2.2)	489(18)
mCPP	2.5	3.8(0.7)*	556(24)
Pind 5/mCPP	2.5	0.5(0.3)**	485(21)
Ritanserin	10	3.2(1.1)	
mCPP	10	0**	
Rit 10/mCPP	10	0**	
Saline		10.0(2.5)	
Ritanserin	2	8.0(2.5)	455(36)
Rit 5/mCPP	2.5	2.3(1.7)*	520(33)
MCPP	2.5	3.8(0.7)*	556(24)
Saline		8.0(2.3)	483(25)

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukey-U test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.23 Effect of cyproheptadine on mCCP inhibition of marble burying.

[vehicle control values] (s.e.m)

Drug/Dose (mg/kg)		Marbles buried
Saline		10.1(2.1)
Cyproheptadine 1		10.7(2.1)
mCPP	2.5	3.0(1.1)*
Cypr 1/mCPP	2.5	2.5(1.4)*
Saline		10.1(2.1)
Cyproheptadine 2		5.2(2.6)
mCPP	2.5	3.0(1.1)*
Cypr 2/mCPP	2.5	3.3(2.2)*

Statistical comparisons were made on raw data using a two-way ANOVA followed by Tukeys test for unconfounded means. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

Table 8.24: Effect of 3 day pre-treatment with PCPA 300 mg/kg on the inhibition of marble burying by zimeldine, mCPP, 8-OH-DPAT and DOI.

Mean (s.e.m) N = 6

3 day pretreatment	Acute drugs (mg/kg)	Marbles buried
Saline	Saline	7.0(1.2)
PCPA	Saline	6.8(2.1)
Saline	Zimeldine 10	2.7(2.0)*
PCPA	Zimeldine 10	2.3(2.1)*
Saline	mCPP 10	1.2(0.4)*
PCPA	mCPP 10	0.7(0.7)*
Saline	8-OHDPAT 10	0*
PCPA	8-OHDPAT 10	0*
Saline	DOI 0.1	4.3(1.7)*
PCPA	DOI 0.1	4.8(2.5)*

Statistical comparisons were made on raw data using a t-test. *= $p<0.05$ **= $p<0.01$ relative to vehicle controls.

FIG. 8.1 EFFECT OF IPSAPIRONE ON MARBLE BURYING.

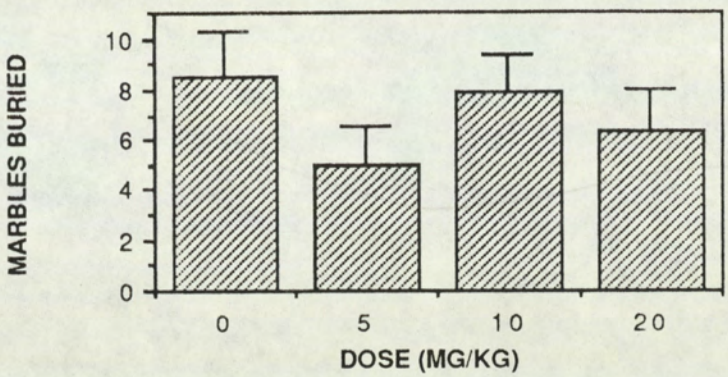
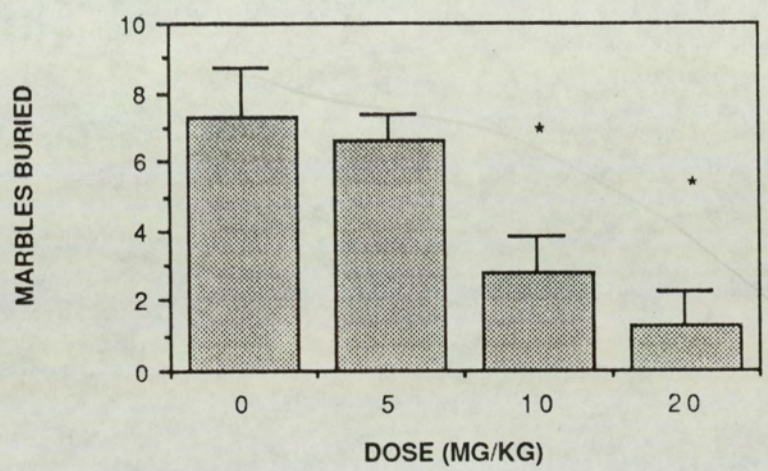


FIG 8.2 EFFECT OF GEPIRONE ON MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.3 EFFECT OF BUSPIRONE ON MARBLE BURYING.

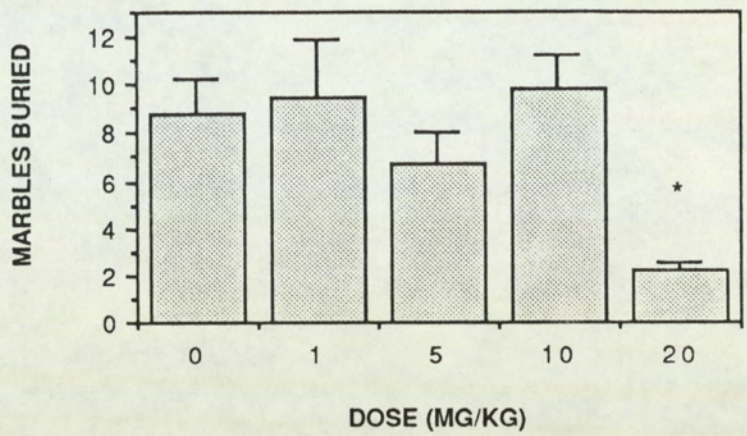
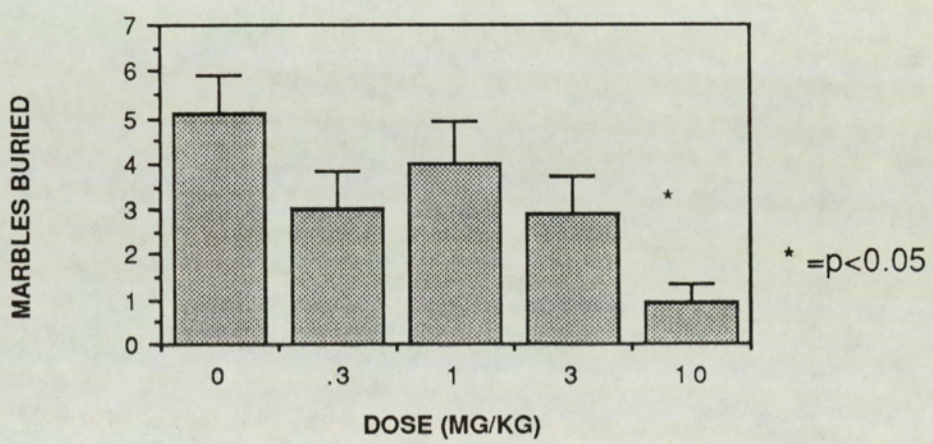


FIG. 8.4 EFFECT OF 8-OHDPAT ON MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.5 EFFECT OF 5-MeODMT ON MARBLE BURYING

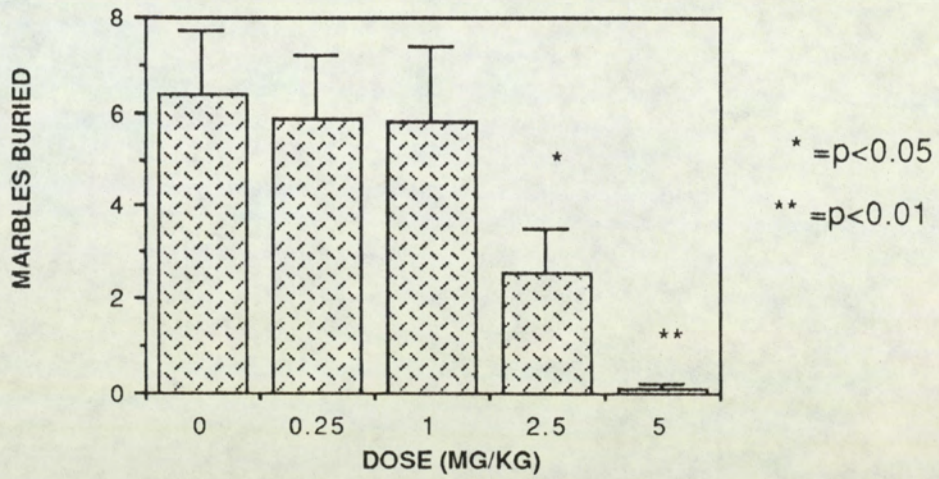
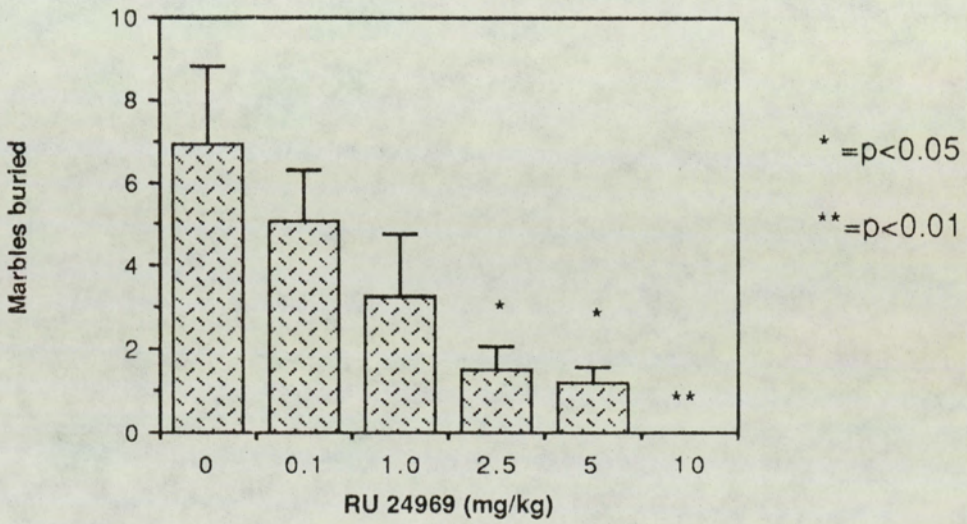


Fig 8.6 Effect of RU 24969 on marble burying



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

Fig 8.7: Effect of mCPP on marble burying

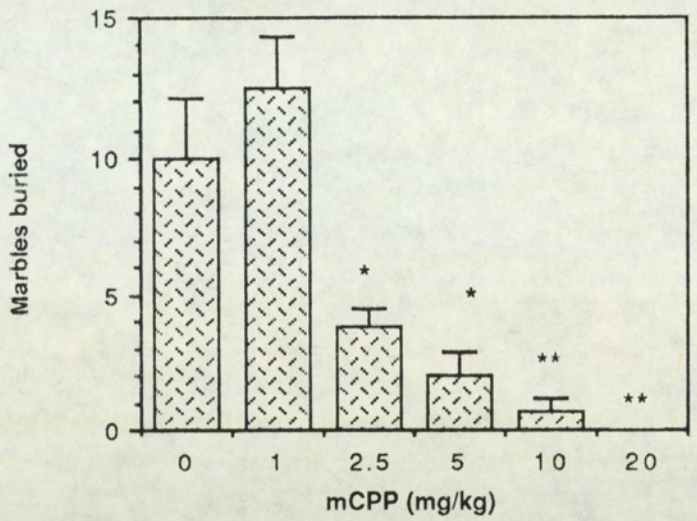
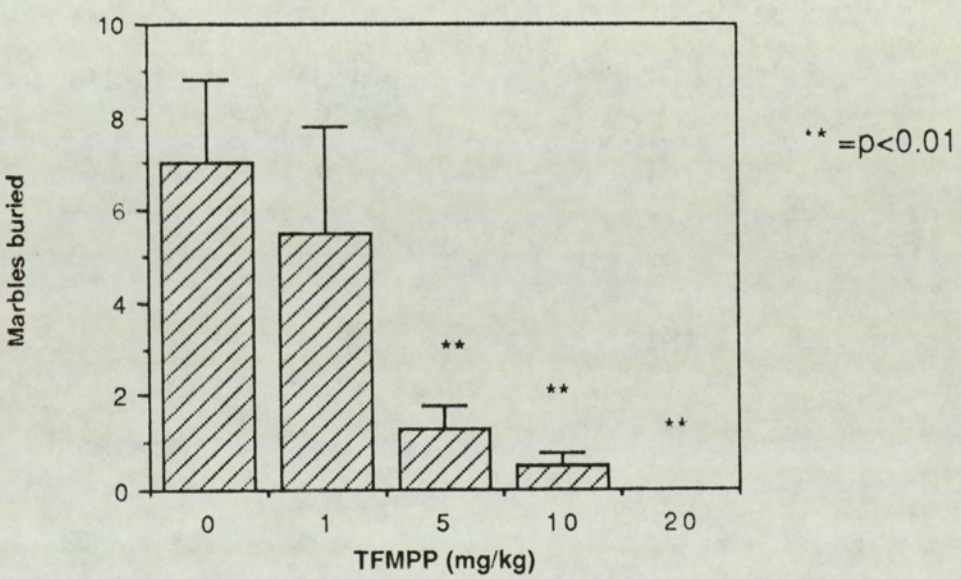
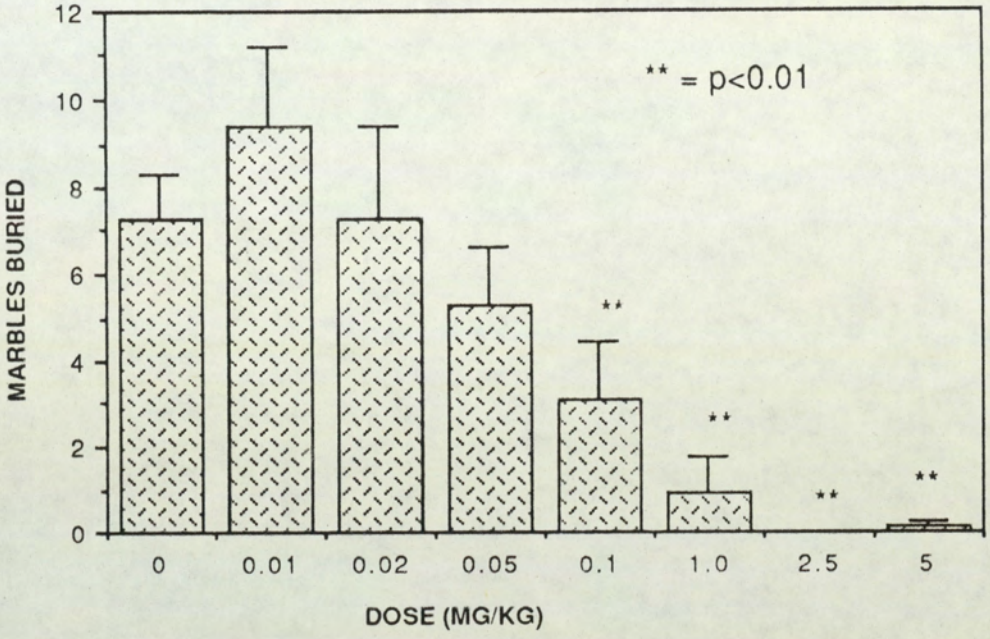


Fig 8.8 Effect of TFMPP on marble burying



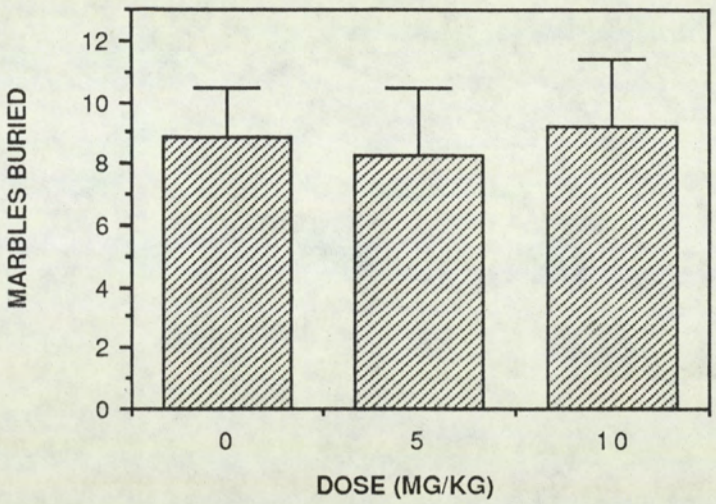
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.9 EFFECT OF DOI ON MARBLE BURYING

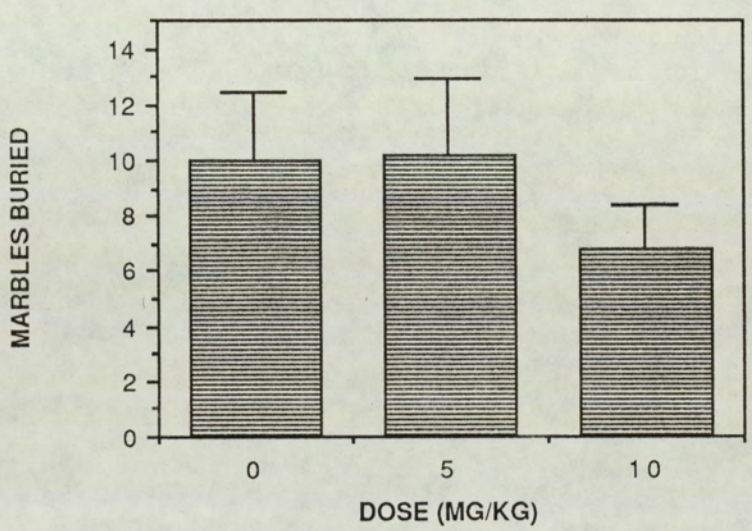


Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.10 EFFECT OF PINDOLOL AND TIMOLOL ON MARBLE BURYING.



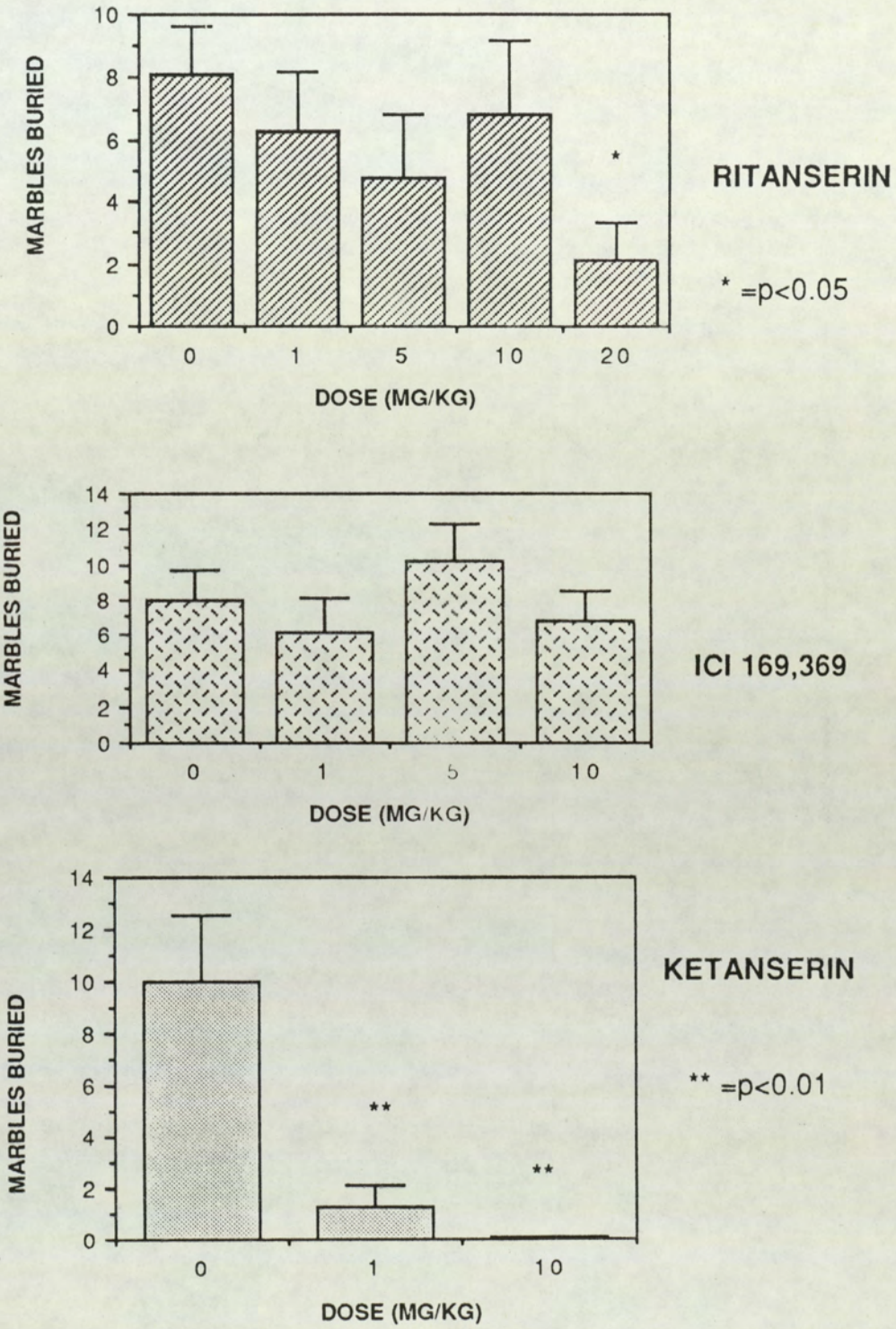
PINDOLOL



TIMOLOL

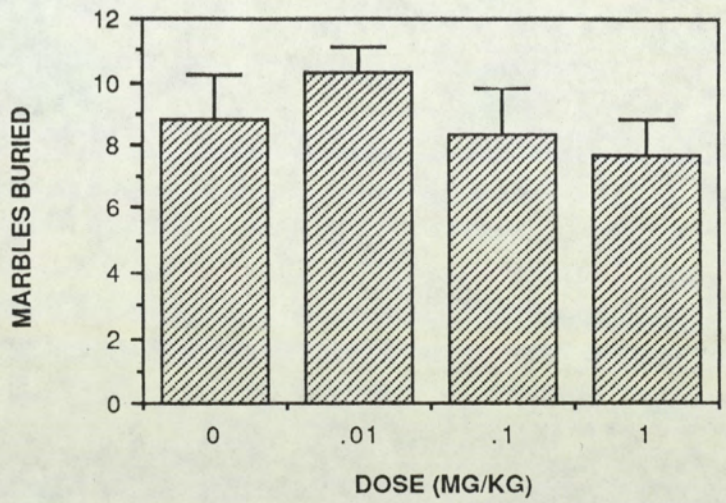
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.11 EFFECT OF 5-HT2 ANTAGONISTS ON MARBLE BURYING.

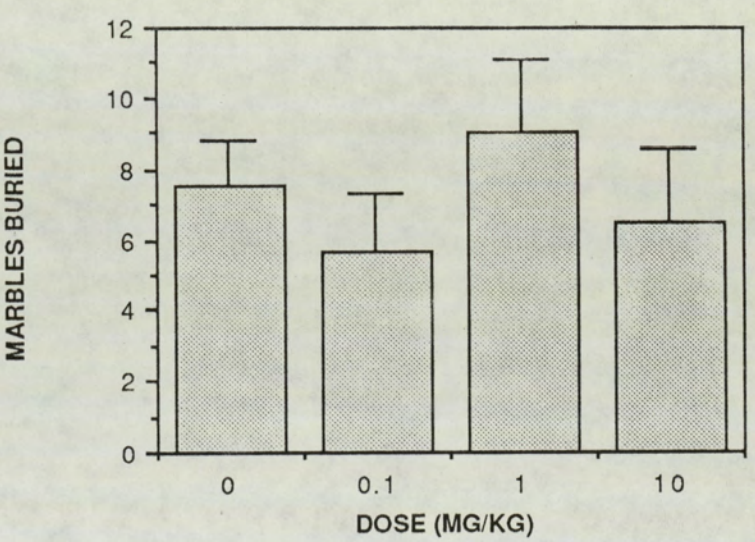


Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.12 EFFECT OF GR 38032F AND ICS 205-930 ON MARBLE BURYING.



GR 38032F



ICS 205-930

Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 8.13.1 EFFECT OF METHYSERGIDE ON MARBLE BURYING.

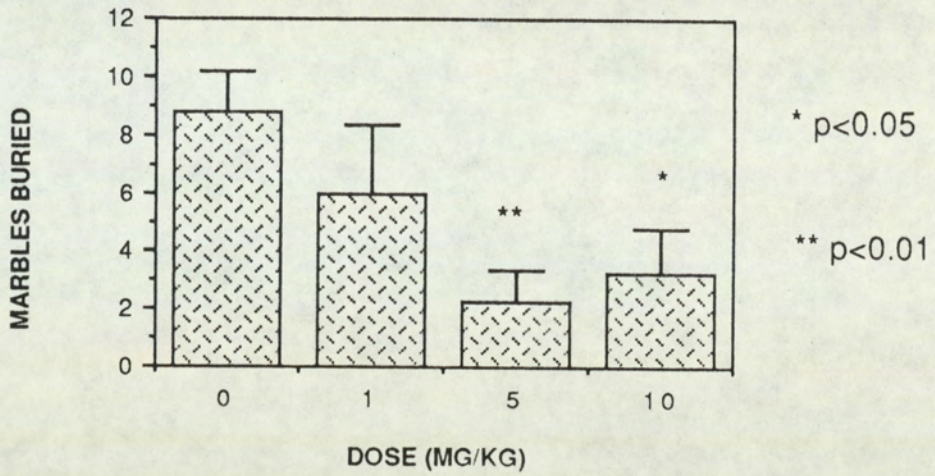
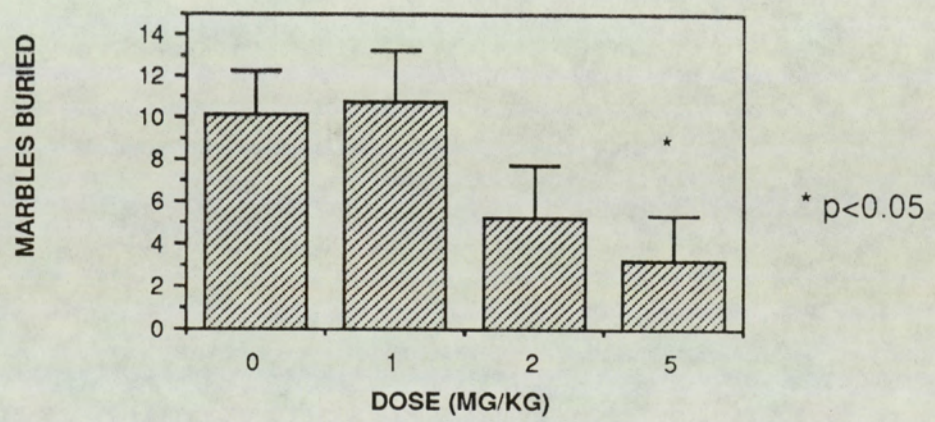


FIG 8.13.2 EFFECT OF CYPROHEPTADINE ON MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.14 EFFECT OF ZIMELDINE ON MARBLE BURYING.

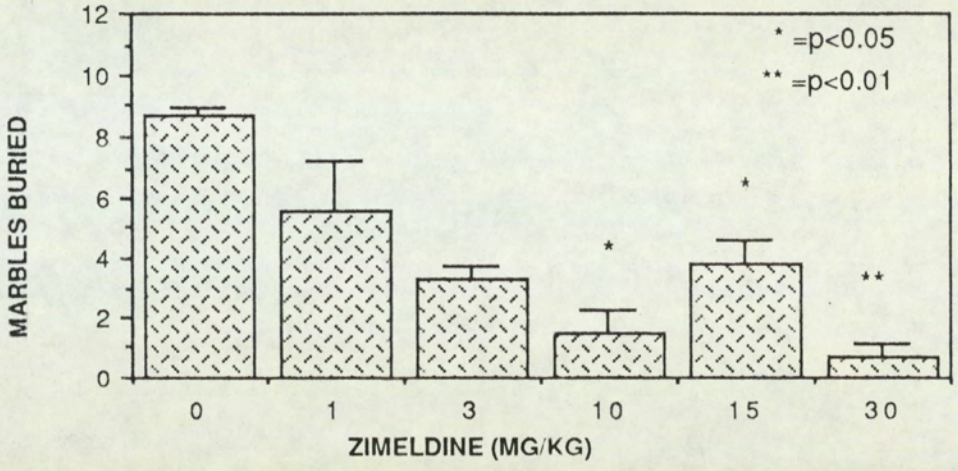
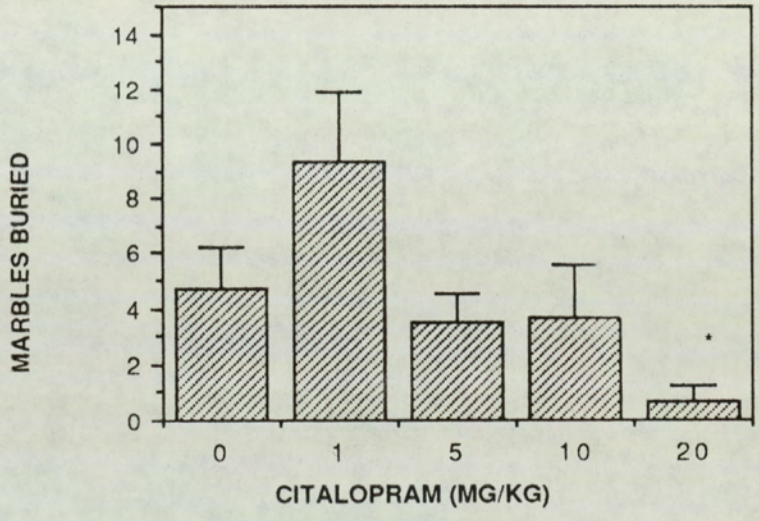


FIG 8.15 EFFECT OF CITALOPRAM ON MARBLE BURYING



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG 8.16 EFFECT OF FLUVOXAMINE ON MARBLE BURYING.

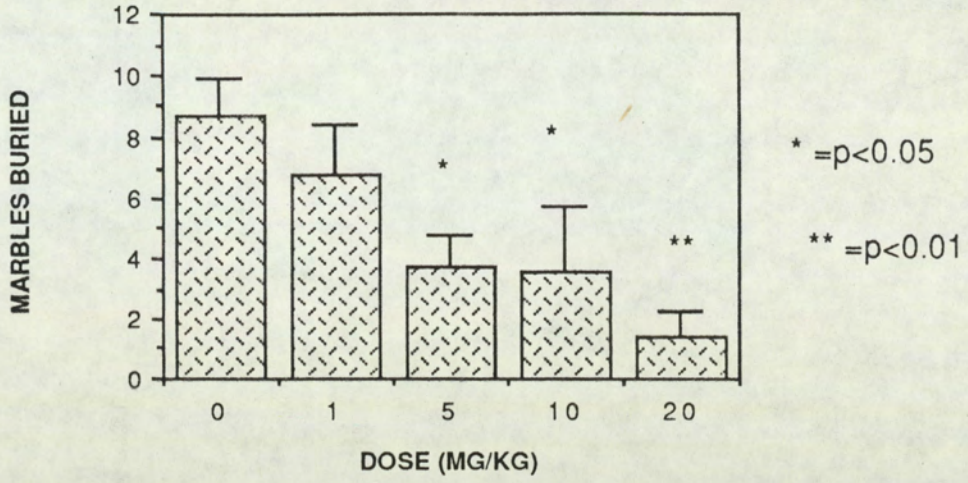
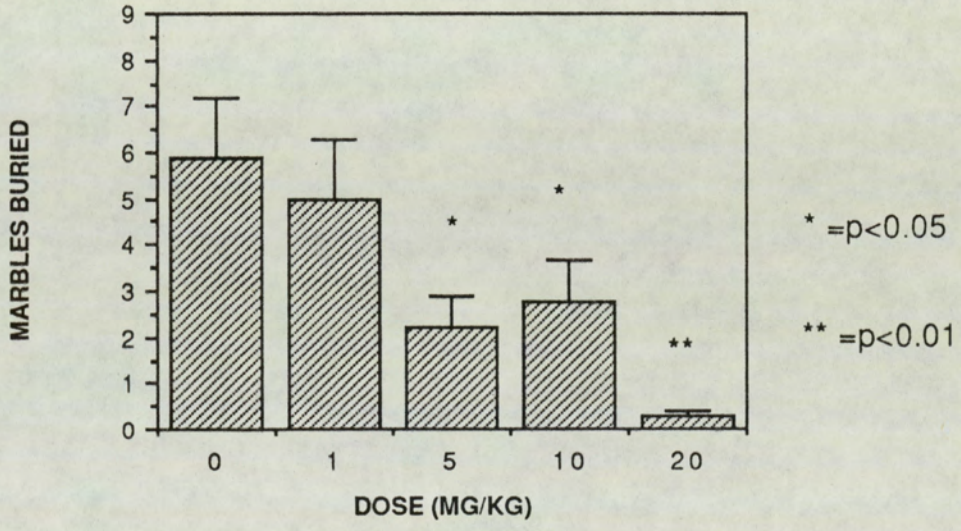


FIG 8.17 EFFECT OF INDALPINE ON MARBLE BURYING



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 8.18 EFFECT OF FENFLURAMINE IN MARBLE BURYING

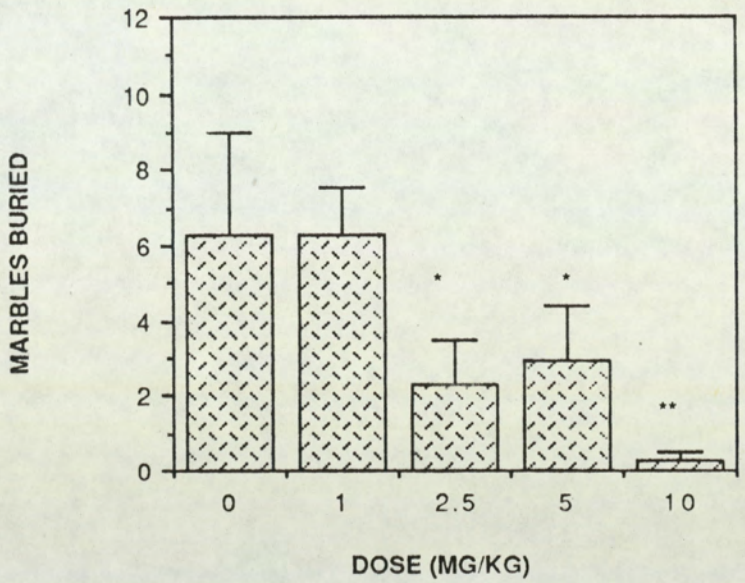
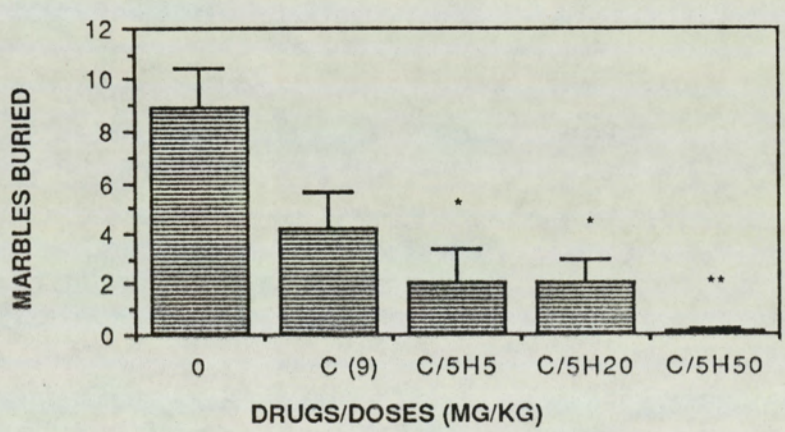


FIG. 8.19 EFFECT OF 5-HTP ON MARBLE BURYING.



C = CARBIDOPA

5H = 5 -HTP

* =p< 0.05

**=P<0.01 relative to Carbidopa

Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

FIG. 8.20 EFFECT OF CHRONIC ZIMELDINE ON MARBLE BURYING.

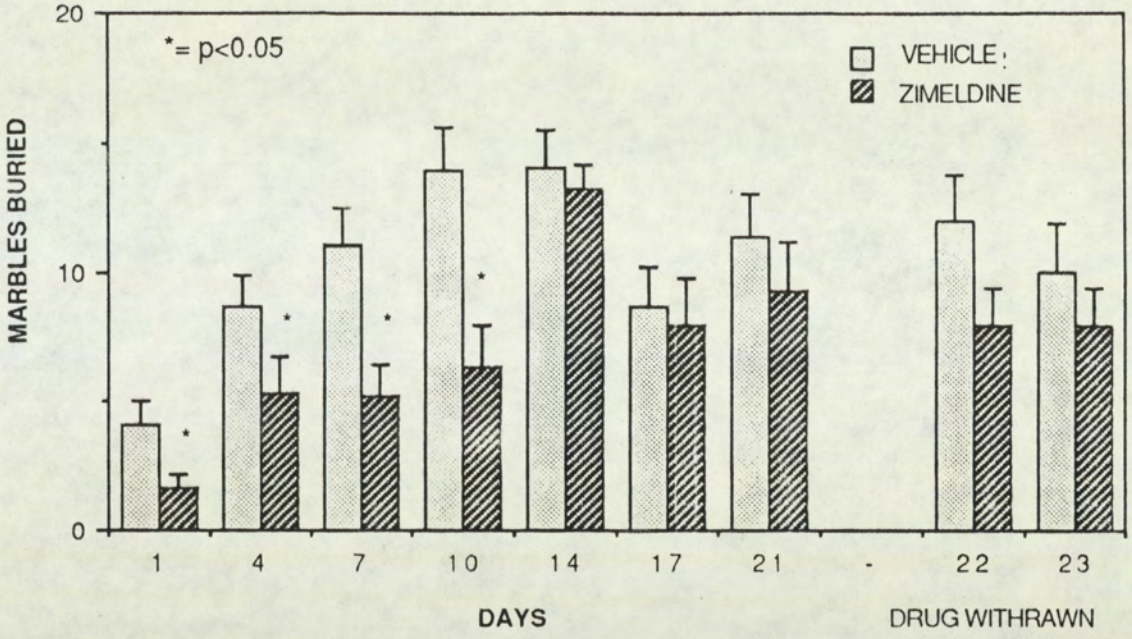
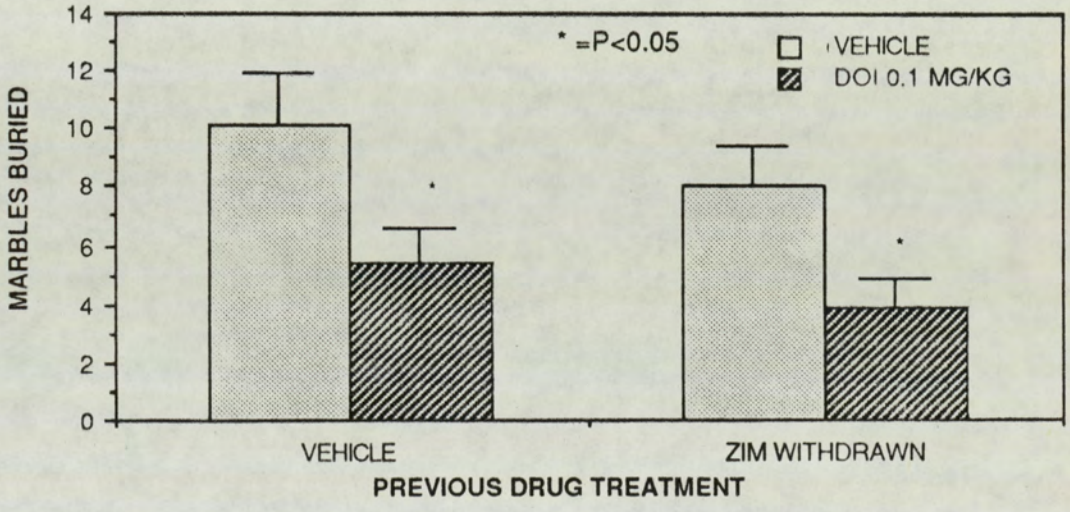
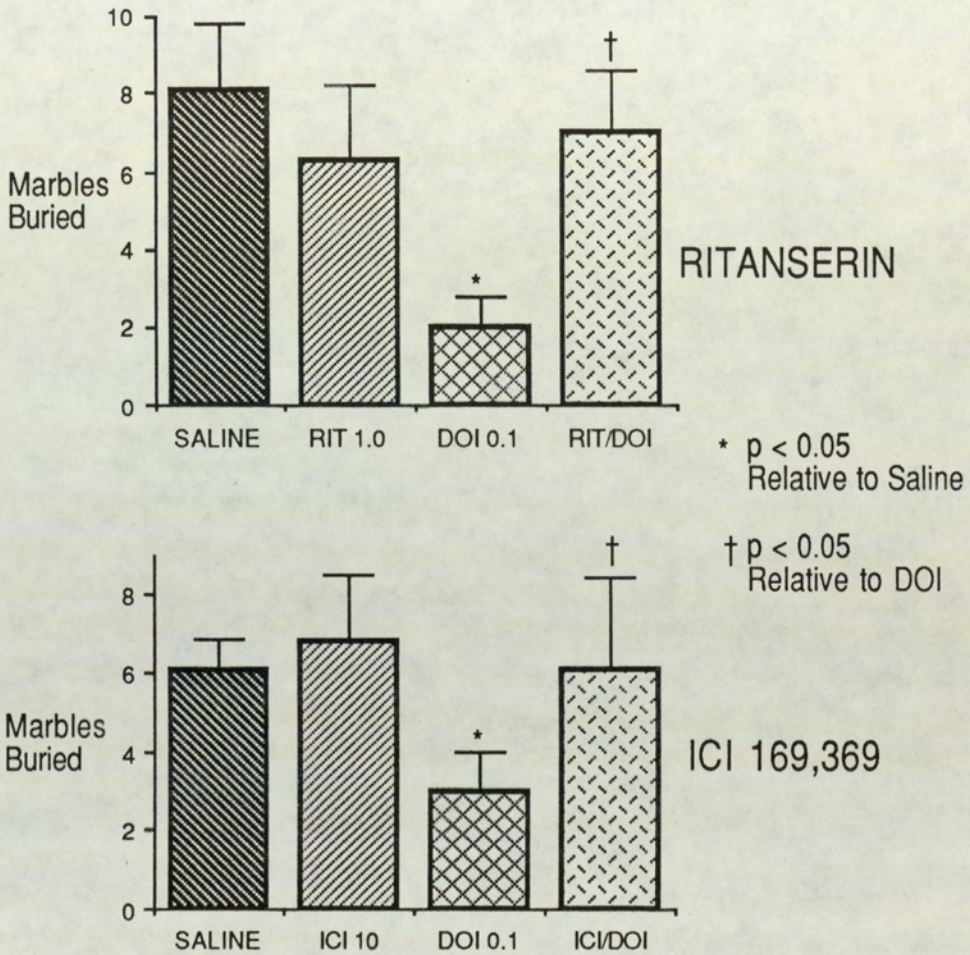


FIG. 8.21 EFFECT OF DOI ON MARBLE BURYING 2 DAYS AFTER ZIMELDINE WITHDRAWAL.



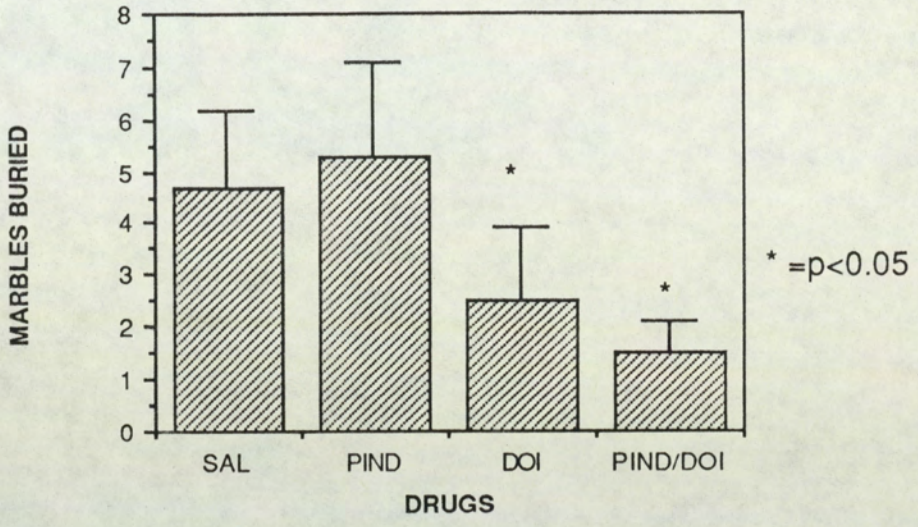
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a t-test.

Fig. 8.22: Effect of $5HT_2$ Antagonists on DOI inhibition of Marble Burying



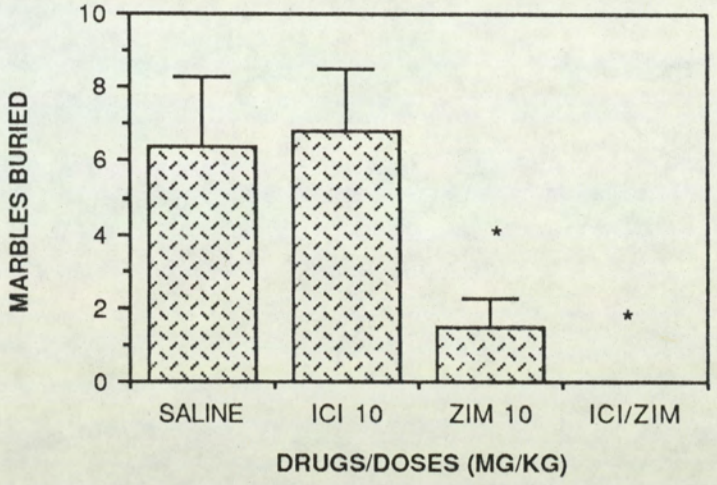
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

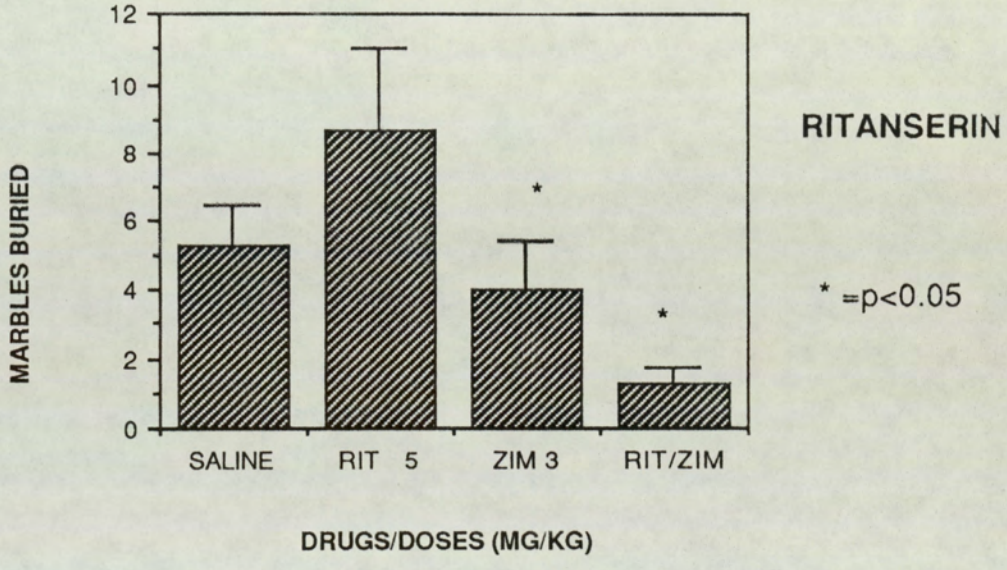
FIG 8.23 EFFECT OF PINDOLOL ON DOI INHIBITION OF MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

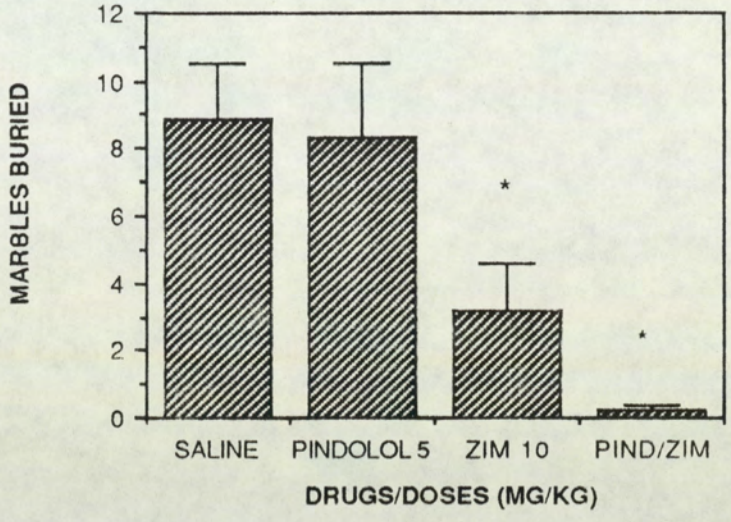
FIG 8.24.1 EFFECT OF ICI 169,369 AND RITANSERIN ON ZIMELDINE INHIBITION OF MARBLE BURYING





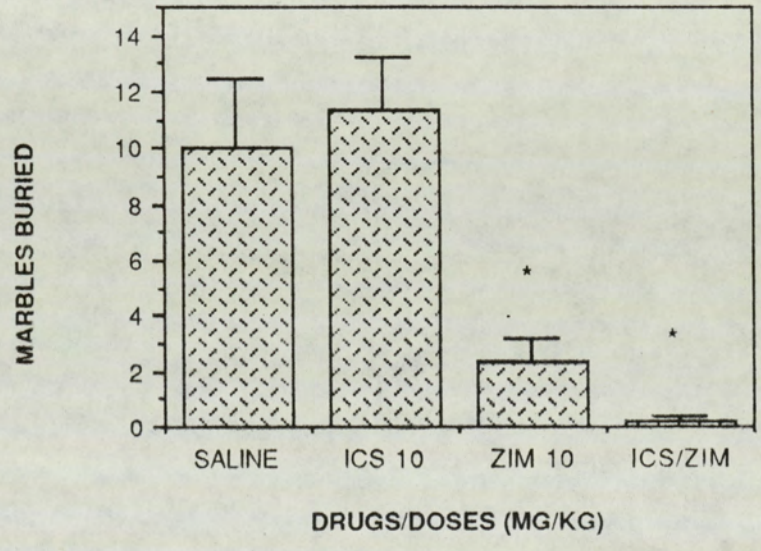
Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 8.24.2 EFFECT OF PINDOLOL AND ICS 205-930 ON ZIMELDINE INHIBITION OF MARBLE BURYING.



PINDOLOL

* =p<0.05



ICS 205-930

* =p<0.05

Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG. 8.25 EFFECT OF CYPROHEPTADINE ON ZIMELDINE INHIBITION OF MARBLE BURYING.

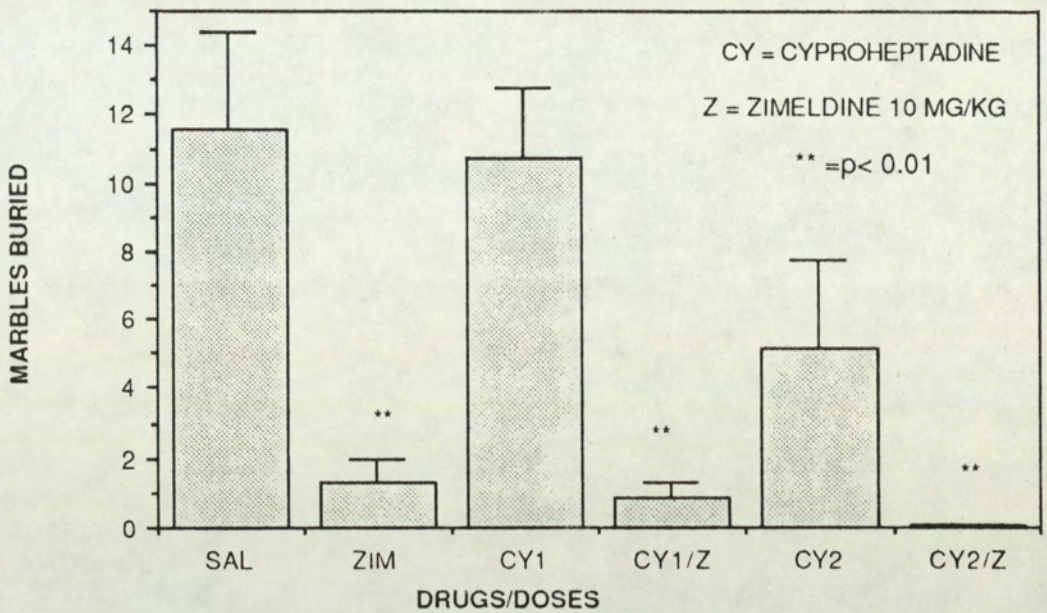
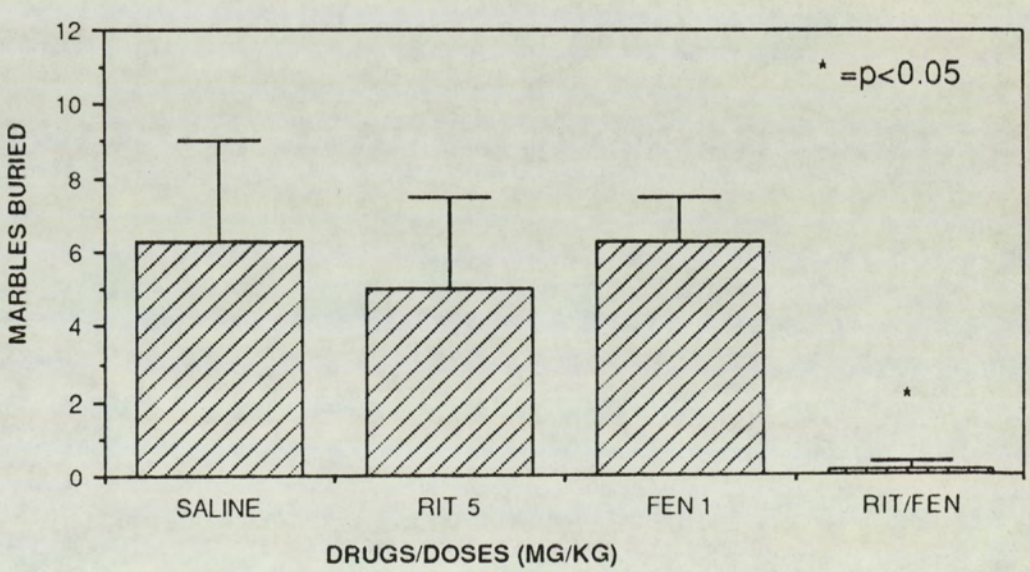


FIG 8.26 EFFECT OF RITANSERIN ON FENFLURAMINE INHIBITION OF MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 8.27 EFFECT OF RITANSERIN ON FLUVOXAMINE INHIBITION OF MARBLE BURYING.

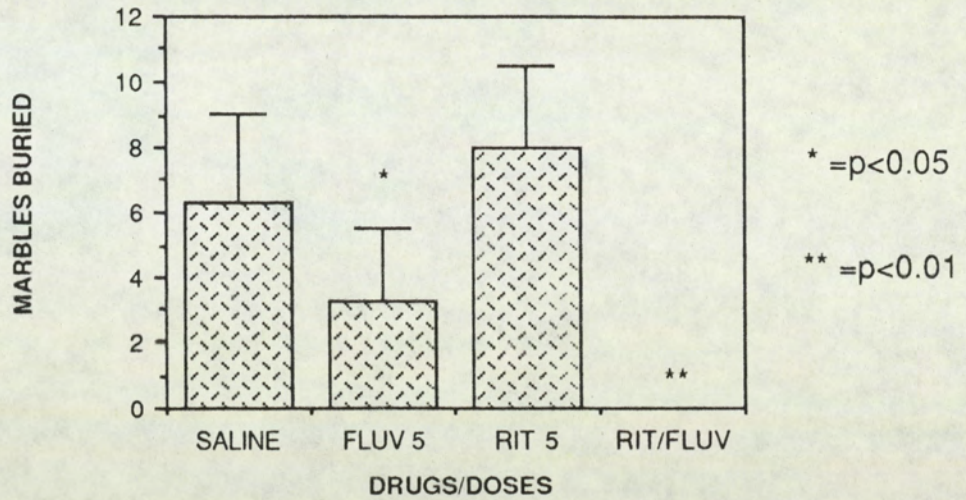
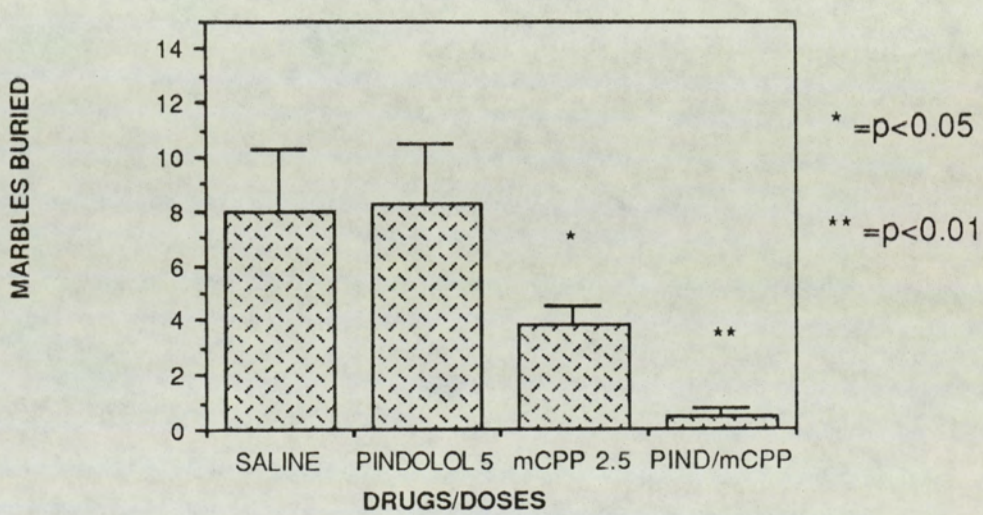


FIG 8.28 EFFECT OF PINDOLOL ON mCPP INHIBITION OF MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG 8.29 EFFECT OF RITANSERIN ON mCPP INHIBITION OF MARBLE BURYING.

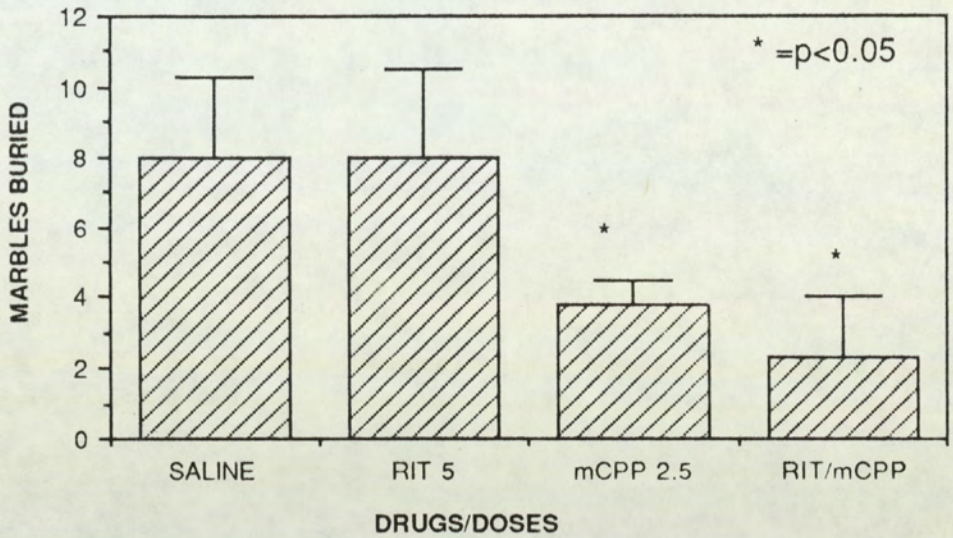
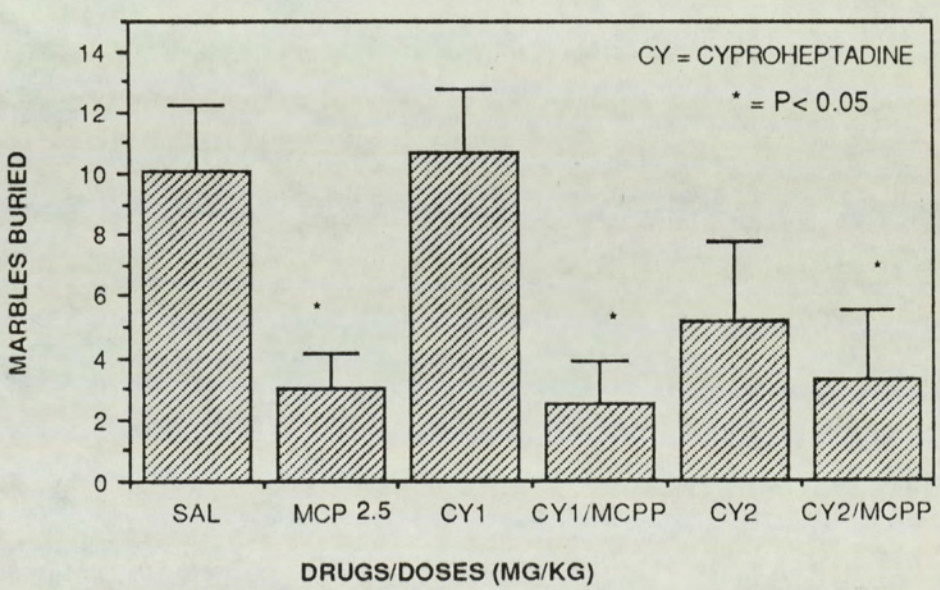
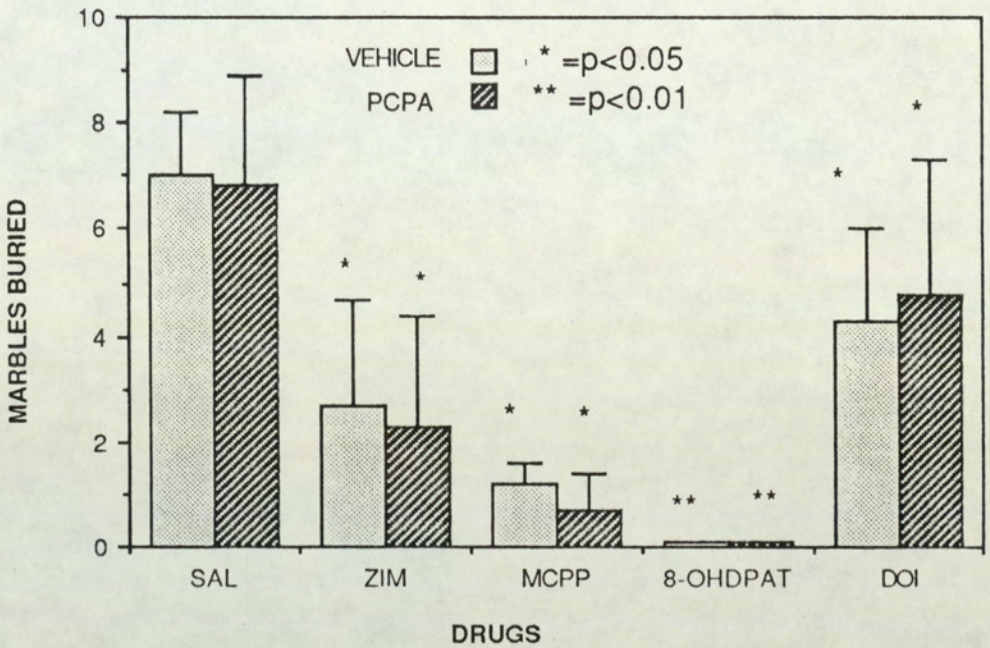


FIG. 8.30 EFFECT OF CYPROHEPTADINE ON MCPP INHIBITION OF MARBLE BURYING.



Marbles buried over a 30 min period. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

FIG. 8.31 EFFECT OF PCPA PRETREATMENT ON MARBLE BURYING.



All the mice were pretreated with PCPA at 300 mg/kg s.c. for three days and drug effects on marble burying tested 24 hours after the last PCPA injection. Statistical comparisons relative to vehicle controls were made on raw data using a t-test. Drug doses: zimeldine 10 mg/kg, mCPP 10 mg/kg, 8-OH-DPAT 10 mg/kg and DOI 0.1 mg/kg.

CHAPTER 9

EFFECT OF DORSAL RAPHE LESIONS ON BEHAVIOUR OF RATS IN THE ELEVATED X-MAZE.

Introduction

Results

9.1 Effect of dorsal raphe lesions on exploration in the elevated X-maze.

9.2 Effect of dorsal raphe lesions on the 8-OH-DPAT anxiogenic effect.

9.3 Effect of dorsal raphe lesions on the RU 24969 anxiogenic effect.

Discussion

Tables and Figures

INTRODUCTION

The bulk of 5-HT neurones in the brain occur in the nine raphe nuclei (see chapter 1). Of these, the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) contain 80 % of the forebrain 5-HT (Azmitia 1978, Dahlstrom and Fuxe 1964). The effects of lesions of 5-HT pathways in these nuclei on animal models of anxiety are varied. Lesions of the DRN were found to produce anxiolytic effects in the social interaction test (File et al 1979) and in the Geller Seifter test (Green and Hodges 1986), but to produce no effects on anxiety in the Geller Seifter test (Thiebot et al 1984) and the elevated X-maze tests (Critchley and Handley 1989). Combined DRN/MRN lesions produced an anxiogenic response in the social interaction test (File and Deakin 1980) while selective MRN lesions were without effect (File et al 1979). Electrical stimulation of the MRN produced signs of fear in the rat while stimulation of the DRN had no effect on anxiety (Graeff & Silviera Filho 1978).

Critchley and Handley (1987) reported that PCPA abolished the anxiogenic effect of 8-OH-DPAT in the X-maze. In contrast, PCPA blocked the anxiolytic effect of 8-OH-DPAT in punished drinking (Engel et al 1984). In both studies interpretation was made difficult by the anxiolytic effect of PCPA itself. The use of PCPA is also uninformative as to what 5-HT neuronal systems are involved. Critchley and Handley (1989) therefore selectively lesioned the DRN and found that DRN lesions reversed the anxiogenic effect of 8-OH-DPAT in the X-maze. They did not however block uptake of the neurotoxin into noradrenaline neurones or assay noradrenaline levels subsequently.

The present study therefore had the purpose of determining whether the results of Critchley and Handley (1989) could

be replicated while remedying these defects in experimental design. The opportunity was also taken to examine the effect of DRN lesions on X-maze responses to RU 24969. The lesions were performed by injection of 5,7-hydroxytryptamine which is a selective neurotoxin for 5-HT neurones (Breese 1975). Desipramine was administered before the neurotoxin to prevent the destruction of noradrenergic neurones (for details see Chapter 2). Behavioural studies were performed two to four weeks after the lesions were performed.

RESULTS

9.1 Effect of dorsal raphe lesions on exploration in the elevated X-maze.

Compared to sham operated animals, DRN lesions had no effects on either the open/total arm entry ratio or the total number of arm entries (Table 9.1 and Fig 9.1).

9.2 Effect of dorsal raphe lesions on the 8-OH-DPAT anxiogenic effect.

In dorsal raphe lesioned rats 8-OH-DPAT at 0.2 mg/kg significantly increased the open/total arm entry ratio without changing the total number of arm entries (Table 9.1 and Fig 9.1). In the sham operated rats 8-OH-DPAT at 0.2 mg/kg significantly reduced the open/total arm entry ratio without affecting the total number of arm entries (Table 9.1 and Fig 9.1).

9.3 Effect of dorsal raphe lesions on the RU 24969 anxiogenic effect.

In the sham operated rats RU 24969 at 1.0 mg/kg significantly reduced the open/total arm entry ratio and increased the total number of arm entries (Table 9.2 and Fig 9.2). In dorsal raphe lesioned rats RU 24969 at 1.0 mg/kg also produced a significant reduction in the open/total arm entry ratio and an increase in the total number of arm entries that was not significantly different from the effect in the sham operated animals (Table 9.2 and Fig 9.2).

DISCUSSION

Dorsal raphe lesions produced no changes in the exploratory behaviour of rats in the X-maze. This concurs with the findings of Critchley and Handley (1989) in the same model. This indicates that, in the absence of extraneous modulation of 5-HT neurotransmission, lesions of the DRN do not have effects on anxiety detectable in this model, two to four weeks after the lesion. Similar findings have been reported by Thiebot et al (1984) in the Geller-Seifter model but other workers found similar lesions to be anxiolytic on the social interaction model (File et al 1979) and on punished responding (Green & Hodges 1986). However the DRN lesions in File et al (1979) were accompanied by a partial lesion of the MRN.

On DRN lesioned rats 8-OH-DPAT was anxiolytic, rather than anxiogenic. This result parallels that obtained in the same conditions by Critchley & Handley (1989). The reversal of the anxiogenic effects of 8-OH-DPAT suggests that the compounds anxiogenic effects are mediated via the DRN or pathways originating in the DRN. The anxiolytic effects produced by 8-OH-DPAT after the destruction of the DRN are probably mediated post-synaptically or through the other raphe nuclei. Of these nuclei, the MRN is the most likely candidate. Infusions of 8-OH-DPAT (Carli & Samanin 1988) and buspirone (Carli et al 1989) into the MRN but not the DRN produced anxiolytic effects in the light/dark aversion and punished drinking models in rats. On the other hand, infusion of 8-OH-DPAT into the DRN produced an anxiogenic response, albeit at a dose which also produced the flat body posture, in the light-dark aversion test (Carli and Samanin (1988). This would suggest that the compound has different effects on the two nuclei with the DRN mediating anxiogenic effects and the MRN anxiolytic effects. It appears therefore that in intact rats it is the DRN effects

that prevail in the X-maze model.

Not all findings however, are consistent with this conclusion. Engel et al (1984) reported that 8-OH-DPAT injected systemically was anxiolytic in the punished drinking anxiety model and that depletion of 5-HT with PCPA reversed this effect. In addition, Higgins et al (1987) reported that 8-OH-DPAT injected into the DRN produced anxiolytic effects in the same model. It is difficult to reconcile their findings with ours.

Unlike 8-OH-DPAT, the effects of the 5-HT1A/1B agonist RU 24969 were not affected by DRN lesions. This would suggest that unlike 8-OH-DPAT, the anxiogenic effects of RU 24969 are mediated through nuclei other than the DRN (or in addition to the DRN). It is possible that the MRN is one of these. Scatton et al (1989) have found that 8-OH-DPAT potently reduced extracellular 5-hydroxy-indole acetic acid (5-HIAA) levels in the DRN and frontal cortex but not in the MRN and the dentate gyrus {which is innervated exclusively by the MRN (Ibid.)}. On the other hand RU 24969 reduced 5-HIAA levels to the same extent in both the 5-HT systems. The differential effect of the two compounds in the two nuclei could account for the persistence of the RU 24969, but not the 8-OH-DPAT, anxiogenic effect after DRN lesion. Further work, including lesions of the MRN would be necessary to establish this.

At the time of writing, measures of 5-HT and noradrenaline depletion in the rats used for this study are still awaited, and it is hoped that they will be available shortly. However parallels with the work of Critchley and Handley (1989) as far as effects of DRN lesions and 8-OH-DPAT on DRN lesioned rats suggest the likelihood of selective dorsal raphe lesions.

Table 9.1: Effect of 8-OH-DPAT in dorsal raphe lesioned rats.

Mean number of arm entries in 10 minutes (s.e.m)
[vehicle control values]

Drug/dose	Open arms	Total entries	Open/total
DRN lesions/			
8-OH-DPAT	7.7(0.6)*	11.9(2.6)	0.53(.04)*
Saline	3.2(1.0)	10.2(1.7)	0.28(.08)
Sham operated/			
8-OH-DPAT	1.5(0.7)	8.7(2.9)	0.20(.06)^
Saline	2.8(0.8)	7.5(1.2)	0.36(.06)

F(A X B) = 10.6 DF = 1/20 $p<0.01$

Statistical comparisons were made on raw data using a 2-way ANOVA. This was followed by a t-test to establish if the 8-OH-DPAT treated animals were different from saline controls. * = $p<0.05$ relative to DRN saline controls; ^ = $p<0.05$ relative to sham saline controls (t-test).

Table 9.2: Effect of RU 24969 in dorsal raphe lesioned rats.

Mean number of arm entries in 10 minutes (s.e.m)
[vehicle control values]

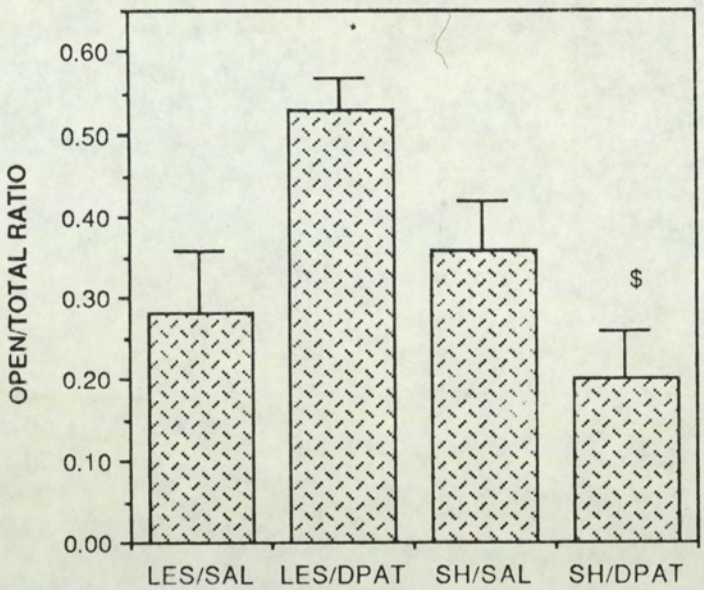
Drug/dose	Open arms	Total entries	Open/total
DRN lesions/			
RU 24969	3.5(2.0)	24.8(2.9)	0.14(.08)**
Saline	3.8(0.7)	11.0(1.3)	0.33(.03)
Sham operated/			
RU 24969	2.0(0.3)	18.8(2.1)	0.11(.01)**
Saline	4.2(1.1)	12.0(2.5)	0.35(.03)

F(A) = 19.6 DF = 1/20 $p<0.01$

F(A X B) = 0.293 Non significant.

Statistical comparisons were made on raw data using a 2-way ANOVA followed by Tukeys test for unconfounded means. ** = $p<0.01$ relative to saline controls.

FIG. 9.1 EFFECT OF 8-OHDPAT ON DRN LESIONED RATS

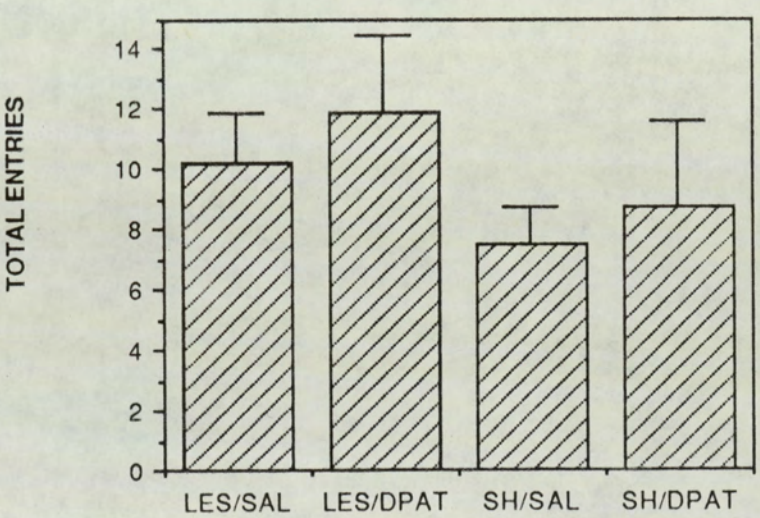


* =p <0.05
RELATIVE TO LES/SAL

$ = p< 0.05
RELATIVE TO SH/SAL

LES = LESIONED

SH =SHAM OPERATED

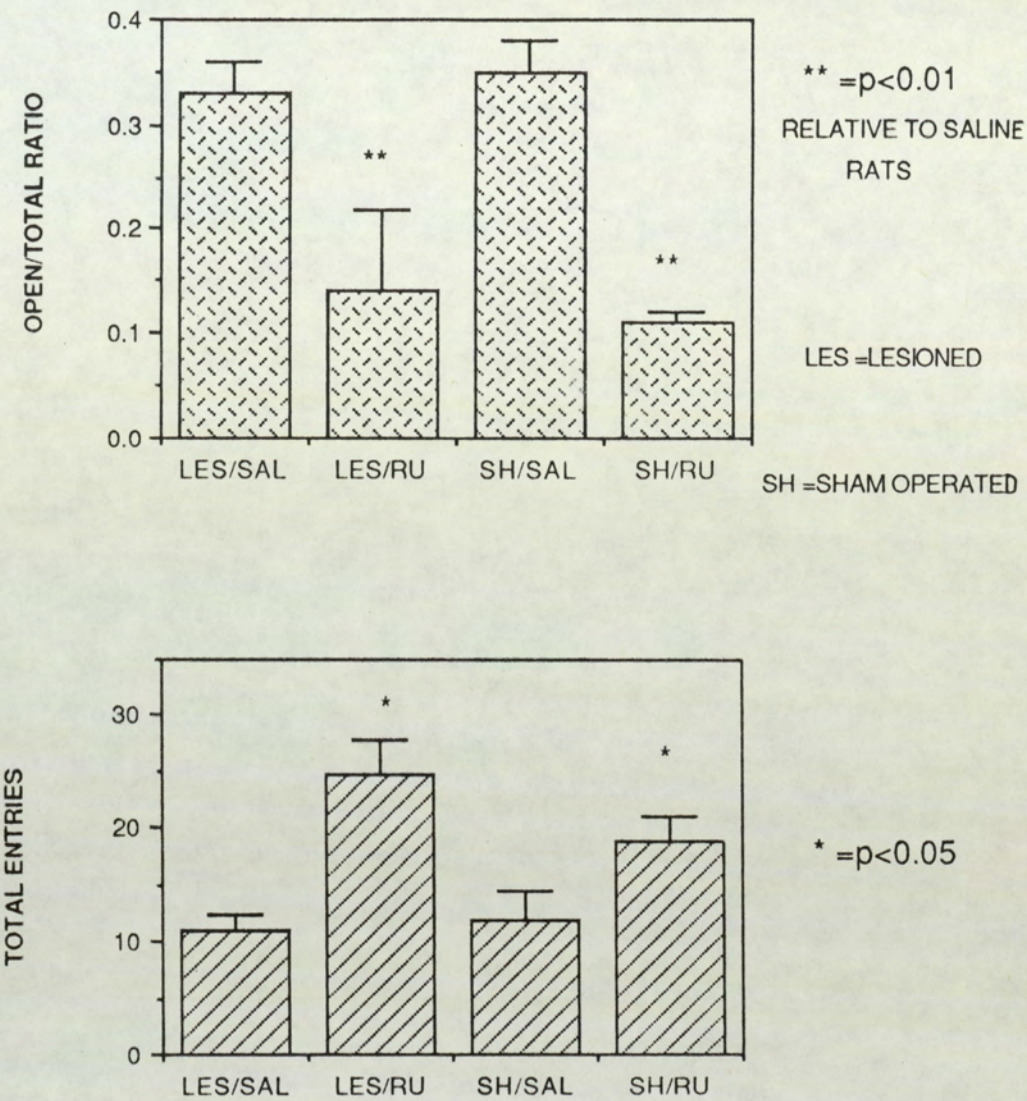


SAL = SALINE

DPAT = 8-OHDPAT 0.2 MG/KG

Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA. In addition a t-test was performed comparing the 8-OH-DPAT treated rats against their respective saline controls.

FIG. 9.2 EFFECT OF RU 24969 ON DORSAL RAPHE LESIONED RATS



Effect RU 24969 on the total/open entry ratio and on the number of total entries in DRN lesioned and sham operated rats. Statistical comparisons relative to vehicle controls were made on raw data using a 2-way ANOVA.

GENERAL DISCUSSION

CHAPTER 10
GENERAL DISCUSSION.

This work set out to establish the effect of various 5-HT ligands on different animal models of anxiety. The models chosen were the elevated X-maze in rats, social interaction in rats and marble burying in mice. In all three models the standard anxiolytic diazepam, was anxiolytic, increasing the open/total arm entry ratio in the X-maze, the duration of social interaction in the social interaction model and reducing the number of marbles buried in the marble burying test. The standard anxiogenic beta-CCE had opposite effects to diazepam in the elevated X-maze and social interaction models but no effects in the marble burying test.

The results obtained with the X-maze model are summarised below.

8-OH-DPAT, BAY R 1531	Anxiogenic
Buspirone, Gepirone	Inactive
Ipsapirone	Anxiolytic
RU 24969	Anxiogenic
mCPP, TFMPP	Anxiogenic
DOI, Ritanserin	Anxiolytic
ICI 169,369	Inactive
5-HT3 antagonists	
GR 38032F, ICS 205-930	
MDL 72222, BRL 24924	Inactive
Zimeldine, fenfluramine	Anxiogenic

In the social interaction model the 5-HT agonists, 8-OH-DPAT, 5-MeODMT and RU 24969 were anxiogenic while ipsapirone and the antagonist pindolol were anxiolytic. The

5-HT2 agonist DOI was anxiolytic while the 5-HT2 antagonists ritanserin and ketanserin were without effect. The 5-HT3 antagonist GR 38032F was inactive.

In the marble burying test, the 5-HT1A agonists and all the 5-HT antagonists were without effect. Only the 5-HT reuptake inhibitors, the 5-HT2/5-HT1C agonist DOI and the 5-HT1B/ 5-HT1C agonists mCPP and TFMPP had any effects, reducing marble burying without affecting locomotor activity.

Taken together, the results obtained in the elevated X-maze and the social interaction models concur for most of the drugs. The results for the marble burying test indicate that this test is modelling a different condition from the other two.

The behavioural analysis of the X-maze model of anxiety indicated that the use of the open/total arm entry ratio is a more appropriate measure of anxiety than the time spent in either set of arms. In the open arms the rats spent virtually all the time in exploratory activity while behaviour in the enclosed arms also included a large amount of home cage activity and peering into the open arm and the central square.

To establish the mechanism of action of 5-HT agents in modulating anxiety in the elevated X-maze and social interaction models it is useful to summarise the results that have been obtained with these drugs. Depleting brain 5-HT levels with PCPA was anxiolytic in the elevated X-maze (Critchley and Handley 1987) while increasing synaptic 5-HT with fenfluramine and zimeldine was anxiogenic (Chapter 4). This indicates that generalised increases in brain 5-HT are anxiogenic and vice versa for decreases. In the

elevated X-maze 8-OH-DPAT was anxiogenic (Chapter 4, Critchley and Handley 1987, Moser 1989), while buspirone was inactive (Chapter 4, Critchley and Handley 1987, Pellow et al 1987, Johnston and File 1988), and ipsapirone was anxiolytic (Chapter 4, Critchley and Handley 1987, Soderpalm et al 1988). The 5-HT1A/1B agonist RU 24969 was anxiogenic in the elevated X-maze (Chapter 4, Critchley and Handley 1987). Depleting brain 5-HT with PCPA abolished the anxiogenic effects of 8-OH-DPAT (Critchley and Handley 1987). In social interaction 8-OH-DPAT was anxiogenic and ipsapirone anxiolytic (Chapter 6) and buspirone inactive (File 1985).

On the other hand, in the punished drinking model of anxiety, 8-OH-DPAT had anxiolytic effects in water deprived rats (Engel et al 1984). These effects were reversed by PCPA (Engel et al 1984). These findings indicate that the anxiogenic effects obtained with 8-OH-DPAT in the elevated X-maze and the anxiolytic effects obtained in punished drinking are both likely to be mediated presynaptically. PCPA blocks 5-HT synthesis and would abolish effects of drugs acting at presynaptic autoreceptors (Engel et al 1984, Dourish et al 1986a) and either have no effect or increase effects of postsynaptic 5-HT receptor stimulation (Dourish et al 1986a).

The 5-HT1A agonists, 8-OH-DPAT, buspirone and ipsapirone inhibit dorsal raphe 5-HT neuronal firing after systemic administration (Basse-Tomusk and Rebec 1986, Fallon et al 1983, VanderMaelen et al 1986) and microiontophoretic application (Sprouse and Aghajanian 1985, 1987, VanderMaelen et al 1986). This is presumably via activation of somatodendritic 5-HT1A autoreceptors (Dourish et al 1986a). The doses used in the systemic studies were comparable to those used in anxiety studies in this work.

The administration of 8-OH-DPAT peripherally at doses comparable to those used in this work (Hjorth et al 1982) and centrally (Hjorth et al 1987) reduced synthesis and turnover of 5-HT in various brain regions including the cortex, striatum, spinal cord and the brain stem (Hjorth et al 1987).

These 5-HT1A ligands are full agonists in so far as inhibition of dorsal raphe firing is concerned (Sprouse and Aghajanian 1987) and partial agonists in their efficacy to inhibit forskolin stimulated adenyl cyclase activity with the rank order 8-OH-DPAT> buspirone> gepirone> ipsapirone (De Vivo and Maayani 1986, Traber and Glaser 1987). The results obtained in the elevated X-maze appear to follow this partial agonist efficacy with 8-OH-DPAT anxiogenic, buspirone and gepirone inactive and ipsapirone anxiolytic.

There is evidence that both the dorsal raphe and the median raphe nuclei are involved in mediating anxiety. Electrical stimulation of the median raphe produced fear responses in rats (Graeff and Silviera Filho 1978) while injection of buspirone and 8-OH-DPAT into the median raphe but not into the dorsal raphe was anxiolytic in the light dark discrimination and punished drinking anxiety models in rats (Carli et al 1989, Carli and Samanin 1988). On the other hand 5-HT was anxiogenic and buspirone anxiolytic in the light dark discrimination model in mice when injected into the dorsal raphe (Costall et al 1989).

Lesions of the dorsal raphe had no effects on baseline anxiety but reversed the effects of 8-OH-DPAT in the elevated X-maze to anxiolytic (Chapter 9, Critchley and Handley 1989). This indicates that the anxiogenic effects of 8-OH-DPAT are mediated by the dorsal raphe nucleus and

pathways innervated by this nucleus. 8-OH-DPAT has latent anxiolytic effects in the elevated X-maze, as revealed by DRN lesions, which were overshadowed by DRN-mediated anxiogenic effects in intact animals.

From this, it appears that 8-OH-DPAT produces its anxiogenic effects in the elevated X-maze by acting as an agonist at the dorsal raphe. After the DRN was destroyed the compound produced anxiolytic effects which could have been mediated postsynaptically or by the other raphe nuclei. Postsynaptic involvement however is unlikely since 8-OH-DPAT was not anxiolytic in PCPA depleted rats (Critchley and Handley 1987).

It could therefore be proposed that 8-OH-DPAT exerts its anxiogenic effects in the elevated X-maze and social interaction models by reducing 5-HT neurotransmission via its action on the cell-body autoreceptors in the DRN and anxiolytic effects in punished drinking and light dark discrimination test by reducing neurotransmission in the neurones originating in the MRN.

It has been suggested that there are two functionally opposing systems controlling behavioural effects of manipulation of 5-HT neurotransmission (Gardner 1985). It is possible that these are represented by the DRN and the MRN. Which of these systems is dominant may depend on the basal 5-HT tone. Peripheral injection of 8-OH-DPAT was without effect in unstressed rats in the punished drinking and light dark discrimination models of anxiety but had anxiolytic effects in both models when the rats were stressed by 48 hour water deprivation or two hour immobilisation (Carli and Samanin 1988). This indicates that the degree of stress of the animals is an important factor in determining the effect of these agents. Stress

increases 5-HT turnover (Kantak et al 1978, Joseph and Kennett 1983) and hence the reduction in firing of raphe neurones would then have greater effects. It could also turn partial agonists into antagonists. The suggestion above that the anxiolytic effects seen with 8-OH-DPAT and the anxiogenic effects with RU 24969 after DRN lesion were mediated by the MRN is supported by the greater sensitivity of the DRN compared to the MRN to the inhibitory effects of 8-OH-DPAT on 5-HT turnover and the similar sensitivity of both nuclei to RU 24969 (Scatton et al 1989). That 8-OH-DPAT has different effects in the DRN and in the MRN has been established by other investigators. Local application of 8-OH-DPAT into the MRN of rats produced locomotor stimulation and reduced 5-HT turnover in the forebrain while in the DRN it suppressed locomotion and had only minor effects on 5-HT turnover (Hillegaart 1989, Hillegaart et al 1989, Hillegaart and Hjorth 1989). It appears to be a viable hypothesis that in intact rats the punished drinking model detects the effects of 8-OH-DPAT on the MRN while the elevated X-maze detects the compound's effects on the DRN. Coupled with the fact that 5-HT1A ligands may act as agonists/ partial agonists / or antagonists at the receptor this means the effects obtained in different animal models could vary very widely depending on prevailing tone in the two systems as influenced for example by stress. This could also explain the differences obtained in different laboratories even with the same model as is the case with the X-maze where for example ipsapirone was anxiolytic (Chapter 4, Critchley and Handley 1987, Soderpalm et al 1988), inactive (Pellow et al 1987, Johnston and File 1988) and anxiogenic (Moser 1989). The state of the 5-HT systems in different batches of animals could vary widely depending on housing conditions, diet and differences in experimental factors like handling, lighting levels during tests and so on. All this indicates that the

conditions described as anxiety in animals are very sensitive to the test conditions. Examples of this sensitivity include our inability to detect the anxiogenic effects of 8-OH-DPAT for a period of several months (Chapter 4) and the inability to detect the anxiogenic effects of yohimbine in the X-maze model for an eighteen month period reported by another laboratory (Johnston et al 1988).

RU 24969 acts as an agonist at both 5-HT1A and 5-HT1B receptors (Gardner and Guy 1983). It is not certain which of the two receptor subtypes mediate the compounds anxiogenic effects. Full agonist effects at either receptor would be analogous with the terminal 5-HT1B receptors reducing 5-HT release and 5-HT1A receptors reducing firing of 5-HT neurones. Antagonism of the compound's anxiogenic effects by ipsapirone suggests possible 5-HT1A involvement because ipsapirone has low affinity for 5-HT1B receptors (Peroutka 1987). If this is the case why were the effects of 8-OHDPAT, a very potent 5-HT1A ligand prevented by DRN lesions while those of RU 24969 were not? It is possible that RU 24969 was acting primarily on MRN. However, both MRN and DRN are equally sensitive to RU 24969 while the DRN was more sensitive than the MRN to inhibition by 8-OHDPAT (Scatton et al 1989). This problem needs further investigation.

Ipsapirone had effects opposite to those of 8-OH-DPAT and hence appeared to be an antagonist in the elevated X-maze and social interaction tests. It resembled pindolol in antagonising the anxiogenic effects of 8-OH-DPAT in the elevated X-maze (Critchley and Handley 1987). These effects are likely to be mediated by the DRN since the anxiolytic effects of ipsapirone are blocked by DRN lesions (Critchley and Handley 1989). The 5-HT1 antagonist effects of

ipsapirone have been reported in another behavioural test, the compound inhibits 8-OH-DPAT induced hypothermia (Goodwin et al 1986). How a compound that reduced firing at the DRN in electrophysiological studies i.e. an agonist-like effect, can act as an antagonist in behavioural studies can only be speculated. Since the compound is a partial agonist it is conceivable that with the prevailing state of the 5-HT tone the compound facilitated the firing of DRN neurones during X-maze exposure. A similar situation where a 5-HT1A partial agonist acted as an antagonist has been reported with buspirone. Costall et al (1989) found that 5-HT administered to the dorsal raphe was anxiogenic in the light/dark discrimination test in mice while intra-raphe injection of buspirone had the opposite effect, being anxiolytic. This indicates that the electrophysiological effects observed on a brain nucleus do not, in isolation, predict overall behavioural effects.

Other than acting as a partial agonist at 5-HT receptors there is an alternative explanation for the anxiolytic effects of ipsapirone. Ipsapirone has high affinity for alpha-1 adrenoceptors [Ki: alpha-1 =23 nM, 5-HT1A =47 nM (Leysen, unpublished data)]. Alpha-1 antagonists were anxiolytic in the X-maze model of anxiety (Handley and Mithani 1984a, Mithani 1984). It is possible that ipsapirone acts as an antagonist at these receptors hence producing anxiolytic effects which could override the anxiogenic effects of RU 24969. These alpha-1 effects could also explain why alone among the 5-HT1A ligands, ipsapirone was anxiolytic. Further work will be needed to establish if this is how ipsapirone is acting in anxiety models.

The anxiolytic effects of the 5-HT2 agonist DOI in the X-maze and social interaction models and the anxiolytic effects of ritanserin in the X-maze indicate a role of

5-HT2 receptors in anxiety. That both an agonist and an antagonist at 5-HT2 receptors were anxiolytic is puzzling. It is conceivable that the anxiolytic effects seen with DOI are secondary to the compounds hallucinogenic effects (Titeler et al 1988) and that the primary anxiolytic effects are those due to blockade of 5-HT2 receptors. It is uncertain why the anxiolytic effects of ritanserin were not evident in the social interaction test. The role of 5-HT1C receptors in these effects is unclear. Data indicating that ritanserin and DOI have effects on 5-HT1C receptors (Hoyer et al 1989) become available too late for the appropriate experiments to be done.

The 5-HT3 antagonists were inactive in all three tests. These compounds have been reported to be anxiolytic in some animal models of anxiety in some laboratories (Costall et al 1987, Piper et al 1988, Tyers et al 1987) and inactive in other laboratories (Johnston and File 1988, Molewijk et al 1987, Morinan 1989). This variability would suggest that the conditions necessary to detect their anxiolytic effects are very exacting.

Since buspirone is clinically effective in the treatment of anxiety (Goldberg and Finnerty 1979, Taylor et al 1985), and gepirone is also anxiolytic in humans (Cott et al 1985), the failure of the elevated X-maze anxiety model to detect their anxiolytic effects raises questions as to the predictive value of this model. It is worth pointing out however, that unlike the benzodiazepines, buspirone has to be administered chronically to produce anxiolytic effects in humans (Wettstein 1988). This could explain the inactivity of acute buspirone but not why even after subchronic administration buspirone was still inactive in the X-maze model. No data on the pharmacokinetics of buspirone in the rat was available but on a different

dosing regimen, twice daily chronic buspirone has also been reported to be ineffective in the elevated X-maze (Moser 1989).

Studies with beta-adrenoceptor agonists and antagonists indicated that these receptors do not modulate anxiety as measured in the X-maze model. Beta-adrenoceptor antagonists with 5-HT antagonist activity attenuated the anxiogenic effect of 8-OH-DPAT. This 5-HT antagonist activity is restricted to the mixed beta-1 and beta-2 antagonists (Costain and Green 1978, Middlemiss et al 1977). Selective beta-1 antagonists like metoprolol and beta-2 antagonists like ICI 118,551 had no effects on the 8-OH-DPAT anxiogenic-like activity hence discounting beta-adrenoceptors involvement. The beta-adrenoceptor agonists did not produce specific effects on anxiety in the elevated X-maze. The beta-2-adrenoceptor agonists reduced the number of total entries and this confounded the interpretation of the reduction of the open/total arm entry ratio seen with clenbuterol. The clinical effects of beta-adrenoceptor antagonists are considered to be modulated by peripheral mechanisms (Noyes 1985, Peet 1984) and from these results it appears that the peripheral aspect of anxiety is not detectable by the X-maze test.

From the ethological analysis the marble burying test does not appear to be a model of anxiety since mice do not find marbles aversive (Chapter 7). However the continued burying of marbles even after repeated exposure seems to support the proposal of Broekkamp and Jenck (1987) that the test is a model of obsessive compulsive disorders (OCD). The potent activity of 5-HT uptake blockers, which are effective in treatment of OCD, in inhibiting marble burying seems to support this conclusion. Against this is the finding that

mCPP inhibited marble burying but exacerbated OCD in humans (Zohar et al 1988). In addition, the finding that mice did not spend more time in the compartment with marbles (Chapter 7) indicates that mice were not attracted to the marbles either, as might have been the case if the test was a model for OCD. Overall therefore, the evidence that marble burying is a model of OCD is not strong.

The results obtained in this work implicate 5-HT2 and/or 5-HT1C receptors as mediating marble burying behaviour. This conclusion is made on the strength of the potent inhibition of marble burying behaviour by mCPP, TFMPP and DOI and the ability of ritanserin and ICI 169,369 to reverse the DOI effects. The potentiation of the zimeldine and the mCPP inhibition of marble burying by agents selective for 5-HT receptor subtypes however indicates that the receptor involvement is a lot more complex.

Further experiments suggested by this work includes: to establish the effect of median raphe lesions in animal models of anxiety; to establish reasons for the differences between ipsapirone on one hand and buspirone and gepirone on the other, in the elevated X-maze and social interaction models of anxiety; to identify how the interplay of noradrenergic and 5-HT systems affects the effects of 5-HT ligands in anxiety tests; and to identify the receptor subtypes modulating marble burying in mice and to seek to establish what, if any clinical condition it most closely models.

In assessing the role of 5-HT receptors in the modulation of anxiety it is worth remembering that anxiety is a complex emotion that is modulated by several neurotransmitters. Noradrenaline has been associated with anxiety: alpha-2-adrenergic antagonists have been reported

to be anxiolytic in punished drinking (Gower and Tricklebank 1988) and anxiogenic in X-maze and punished responding (Handley and Mithani 1984a, b) and in humans (Uhde et al 1984), alpha-1-adrenergic antagonists have been reported to be anxiolytic in X-maze and Geller Seifter anxiety models (Handley and Mithani 1984a, b). Evidence has also been adduced implicating noradrenaline in the anxiolytic effects of the benzodiazepines (Yang et al 1988).

In conclusion, the most significant findings of this work include establishing (a) the effects on three anxiety models of a large number of 5-HT ligands of varying selectivity for 5-HT receptor subtypes; (b) that marble burying behaviour in mice is not due to novelty or aversion and is modulated by 5-HT2 and/or 5-HT1C receptors; (c) that the 5-HT1A agonists 8-OH-DPAT, RU 24969 and 5-MeODMT produce anxiogenic effects in the social interaction model of anxiety, which parallel those obtained in the elevated X-maze; (d) the effects of beta-adrenoceptor antagonists on the elevated X-maze and in blocking 8-OH-DPAT anxiogenesis are due to their activity at 5-HT receptors; (e) that 5-HT1A effects in the elevated X-maze model appear to be mediated by the dorsal raphe nucleus.

The study has also shown while the marble burying test can be used to study 5-HT receptor interactions it is not, contrary to previous reports (Broekkamp et al 1986), a valid model of anxiety since the mice are indifferent rather than averse to the marbles.

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