DRUG ACTION ON ANXIETY MODELS WITH SPECIAL REFERENCE TO SEROTONERGIC MECHANISMS

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Summary

A study has been made of drugs acting at 5-HT receptors on animal models of anxiety.

An elevated X-maze was used as a model of anxiety for rats and the actions of various ligands for the 5-HT receptor, and its' subtypes, were examined in this model. 5-HT agonists, with varying affinities for the 5-HT receptor subtypes, were demonstrated to have anxiogenic-like activity. The 5-HT_2 receptor antagonists ritanserin and ketanserin exhibited an anxiolytic-like profile.
The new putative anxiolytics ipsapirone and buspirone, which are believed to be selective for 5-HT$_1$ receptors, were also examined. The former had an anxiolytic profile whilst the latter was without effect.

Antagonism studies showed the anxiogenic response to 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) to be antagonised by ipsapirone, pindolol, alprenolol and para-chlorophenylalanine, but not by diazepam, ritanserin, metoprolol, ICI118,551 or buspirone.

To confirm some of the results obtained in the elevated X-maze the Social Interaction Test of anxiety was used. Results in this test mirrored the effects seen with the 5-HT agonists (8-OH-DPAT, 5MeODMT, RU24969), ipsapirone and pindolol, whilst the 5-HT_2 receptor antagonists were without effect.

Studies using operant conflict models of anxiety produced marginal and varying results which appear to be in agreement with recent criticisms of such models.

Finally, lesions of the dorsal raphé nucleus (DRN) were performed in order to investigate the mechanisms involved in the production of the anxiogenic response to 8-OH-DPAT.

Overall the results lend support to the involvement of 5-HT, and more precisely 5-HT_1, receptors in the manifestation of anxiety in such animal models.

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

Dedicated to My Family

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List of Abbreviations.

ANOVA	analysis of variance
βCCE	ethyl-β-carboline-3-carboxylate
BIS	behavioural inhibition system
CER	conditioned emotional response
CRF	continuous reinforcement schedule
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
DRN	dorsal raphé nucleus
GABA	γ-amino-butyric-acid
5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan
ICV	intra-cerebroventricular
IP	intra-peritoneal
LSD	lysergic acid diethylamide
5MeODMT	5-methoxy-N,N-dimethyltryptamine
MRN	median raphé nucleus
NA	noradrenaline
NMDA	N-methyl-2-aspartate
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
OPT	opthalaldehyde
PCPA	para-chlorophenylalanine
VI	variable interval

INTRODUCTION

1. General Introduction.

Anxiety is a word used often in today's society which is reflected by the fact that the anti-anxiety or anxiolytic drugs are among the most heavily prescribed drugs in the western world (*Usdin, 1983*). By far the most used anxiolytic agents are the benzodiazepines which in terms of alleviation of anxiety symptoms are extremely efficacious compounds. However, the benzodiazepines also possess several other actions; most notably those of muscle relaxation, sedation and anti-convulsant properties. They have also been reported to increase irritability and hostility, to cause vivid and disturbing dreams and a paradoxical increase in anxiety when treatment is discontinued (rebound anxiety), resulting in the desire to re-commence treatment and hence a form of psychological dependence. A schematic review of the adverse effects of benzodiazepines is presented by Laux and Puryear (1984). So, whilst overall these drugs are safe, much time and effort is being expended in searching for a major replacement for the treatment of anxiety. New classes of compounds are being studied in an attempt to produce anxiolytics with a more specific action on anxiety thus avoiding numerous secondary effects. Many classes of transmitter have, at one time or another, been implicated in anxiety but recently 5-hydroxytryptamine (5-HT, serotonin) has appeared at the forefront of such research. This is coupled with the great advances made recently concerning 5-HT receptor pharmacology. The work reported in this thesis examines the role of 5-HT in anxiety using a variety of different animal models.

2. Distribution of 5-HT Neurones in the Brain.

Significant amounts of 5-HT have been found in the mammalian brain (*Twarog and Page, 1953*), especially in the midbrain, thalamus and hypothalamus. Highest concentrations are found in the hypothalamus and the caudate nucleus, and lowest concentrations in the cerebellum (*Amin et al., 1954; Bertler and Rosengren, 1959*).

With the advent of fluorescence histochemistry (*Falck et al., 1962*) mapping of 5-HT-containing neurones was possible and together with other techniques it has been possible to visualise much of the fine structure and organisation of 5-HT neurones in the CNS. (*See Steinbusch, 1981*). Thus the central serotonergic system is a diverse system which can best be described as consisting of nine clusters of cells on or around the midbrain or raphé regions of the pons and upper brainstem (*Breese, 1975*). The caudal groups B1 to B3 project to the medulla and pons (*Dahlstrom and Fuxe,1964*). B1-*raphé pallidus nucleus*, B2-*raphé obscurus nucleus*, B3-*raphé magnus nucleus*. The rostral groups, B7 to B9, project to the neostriatum, cortex and thalamus and to the limbic system. B7-*dorsal raphé nucleus*, B8-*median raphé nucleus*. Pathways from B9 do not appear at present to be well defined (*Breese, 1975*). Lesioning studies (*Geyer, 1976*) show two distinct 5-HT systems; 1. the mesolimbic pathway and 2. the mesostriatal pathway.

3. 5-HT Receptors.

After more than two decades of virtual stagnation concerning the understanding of the pharmacology and function of 5-HT receptors,

since Gaddum and Picarelli's paper in 1957, rapid progress has recently been made and several receptor subtypes have been identified. The true classification and organisation is still a matter of controversy and is a field which changes rapidly. As is pointed out by J.P.Green (1987) 5-HT receptors still lack a standardized nomenclature and classification which serves only to add to the confusion.

3.1 Binding Studies

Binding studies with [^{3}H]-5-HT have indicated two types of binding sites for 5-HT (*Whitaker & Seaman, 1978*). Peroutka & Snyder (1979) reported that one population of receptors (termed 5-HT$_1$ receptors) is preferentially labelled by [^{3}H]-5-HT with non-specific binding being measured by displacement with 5-HT; whilst the other population (termed 5-HT$_2$ receptors) is preferentially labelled with [^{3}H]-spiroperidol (a neuroleptic used in dopamine receptor studies) with lysergic acid diethylamide (LSD) as the displacing agent for the measurement of non-specific binding. Both populations are labelled equally with [^{3}H]-LSD. Evidence suggests that both 5-HT$_1$ (*Pedigo et al., 1981; Monroe and Smith, 1983*) and 5-HT$_2$ (*Kendall and Nahorski, 1983*) binding sites are heterogeneous. Further light was shed on the matter when Cortes et al, (1984) used autoradiographic techniques to reveal sub-populations of the receptors. 5-HT$_2$ sites (as defined by *Peroutka & Snyder, 1979*) were visualised by using four ligands; [^{3}H]-ketanserin, [^{3}H]-mesulergine, [^{3}H]-spiperone and [^{3}H]-LSD. The same distribution was observed with all four ligands (although none labelled 5-HT$_2$ sites exclusively). Three distinct populations of the

5-HT_1 site were observed which were termed; 5-HT_{1a} (hippocampus, cerebral cortex, nucleus raphé dorsalis), 5-HT_{1b} (globus pallidus, substantia nigra) and 5-HT_{1c} (choroid plexus and ventricles). 5-HT_{1a} sites were labelled with nanomolar concentrations of [^{3}H]-5-HT, [^{3}H]-LSD, and [^{3}H]-8-hydroxy-2-(Di-n-propylamino)tetralin (8-OH-DPAT) (*Hjorth et al., 1982*). 5-HT_{1b} sites were labelled with [^{3}H]-5-HT and were sensitive to nanomolar concentrations of the agonist RU24969 (5-methoxy-3-[1,2,3,6,-tetrahydropyridin-4-yl] 1H-indole) (*Euvrard and Boissier, 1980*) and some β-adrenergic blockers (*Middlemiss et al., 1977*). The 5-HT_{1c} sites had nanomolar affinities for [^{3}H]-mesulergine, [^{3}H]-5-HT, and [^{3}H]-LSD. RU24969, 8-OH-DPAT, the β-adrenergic blockers and 5-HT_2 receptor blockers had very low affinities at these sites (*Cortes et al., 1984*).

Of crucial importance here is the fact that functional receptor correlates have now been established for each of these 5-HT_1 subtypes (*Middlemiss, 1986b*; *Buchheit et al., 1986*), as follows; 5-HT_{1a} sites mediate the stimulant action of 5-HT on neuronal adenylate cyclase, 5-HT_{1b} sites mediate the inhibition of evoked 5-HT and noradrenaline from nerve terminals, and 5-HT_{1c} sites mediate the contractile actions of 5-HT in the rat fundus.

More recently, a new framework for the classification and nomenclature for these receptors has been suggested (*Bradley et al., 1986*) with three basic divisions. This proposes that until there are selective ligands available for the various 5-HT_1 receptor subtypes and their functional correlates have been more clearly determined,

such receptors should be classified as '5-HT_1-like'. The other two classes are 5-HT_2 and 5-HT_3 (see later). Such a classification goes some way to uniting the 'D' and 'M' receptors of Gaddum with the classification of Peroutka and Snyder.

3.2. Electrophysiological studies

Ennis & Cox (1982) criticised the work done by Peroutka and Snyder by saying that there was no measurement of pharmacological activity but merely an indication of two binding sites. They subsequently showed that there were two definite populations of receptors which were functionally active. They showed this by demonstrating the different orders of potency of a series of indoleamines on the potassium-evoked release of previously accumulated [^{3}H]-5-HT from slices of rat raphe nuclei.

Similarly, work based on neuronal responses to 5-HT, determined by unit recording, proposed the existence of three distinct 5-HT receptors designated S_1, S_2, and S_3 (*Aghajanian, 1981*).

3.2.1. S_1

The S_1 receptor was characterised from unit recording of facial motoneurones (*McCall and Aghajanian, 1979; 1980*) and spinal motoneurones (*White and Neuman, 1980*). Its activation appears to facilitate the depolarising action of excitatory amino acids; an action blocked by small doses of classical 5-HT antagonists e.g. methysergide or metergoline.

3.2.2. S_2

5-HT neurone firing is modified by local availability of 5-HT (*Aghajanian, 1978*), an action which appears to be mediated by the S_2 receptor (*Aghajanian, 1981*). The activation of such receptors (usually termed autoreceptors) results in a decrease in firing of the neurone involved. Ennis and Cox (1982) also suggested that this receptor was involved in modulating 5-HT release.

3.2.3. S_3

Microiontophoretic application of 5-HT or its agonists to rat forebrain reduces firing in most neurones. The receptor mediating this response appears distinct from S_1 and S_2 receptors as indicated by lack of effect of the classical 5-HT antagonists and weak activity of LSD (*Haigler and Aghajanian, 1974; de Montigny and Aghajanian, 1977; Aghajanian and Wang, 1978*).

3.3. 5-HT Auto-receptor

5-HT release is modulated by presynaptic autoreceptors believed to be on nerve endings (*Cerrito and Raiteri, 1979a;1979b*). The 5-HT_1 sites have been suggested to be involved in this process (*Martin and Sanders-Bush, 1982a; 1982b; Engel et al., 1983; Ennis et al., 1981*). There is an observed correlation between binding affinities for 5-HT_1 sites and potency to inhibit the potassium stimulated release of [^{3}H]-5-HT from hypothalamic and cortical tissues (*Martin and Sanders-Bush, 1982a; 1982b; Engel et al., 1983*). There are some discrepancies here since the relative binding affinities for some 5-HT receptor antagonists did not correspond to their potency in releasing 5-HT

(*Martin and Sanders-Bush, 1982a; 1982b*), and the binding of 5-HT is not altered by lesions of 5-HT or catecholamine neurones (*Whitaker and Deakin, 1981*). 8-OH-DPAT has been shown to bind to pre-synaptic sites in the striatum but to post-synaptic sites in the hippocampus and brainstem (*Gozlan et al., 1983*). These authors suggested that the presynaptic striatal 8-OH-DPAT site represents the 5-HT autoreceptor in this region. This was questioned on the strength of evidence showing that 5-methoxytryptamine blocked release of 5-HT from striatal brain slices but failed to displace 8-OH-DPAT in this region (*Middlemiss, 1985*). The possibility exists that different types of 5-HT autoreceptors exist in different CNS regions. It is thought, though, that the terminal autoreceptor is in fact of the 5-HT_{1b} subtype (*Middlemiss, 1984; Engel et al., 1986; Maura et al., 1986; Feuerstein et al., 1987*).

3.4. 5-HT_3 receptors.

Of the receptors described so far the 5-HT_2 receptor appears to correspond to the D-receptor described several decades ago (*Gaddum and Picarelli, 1957*). The M-receptors also described appear not to correspond to the 5-HT_1 or the 5-HT_2 receptor since neither can be blocked by methiothepin or ketanserin (see *Bradley et al., 1986*). Thus a third type of receptor exists (*Pedigo et al., 1981*) being responsible for mediating certain excitatory actions of 5-HT on certain elements of the peripheral nervous system. These receptors have been designated 5-HT_3 receptors (*Bradley et al., 1986*). Several potent competitive antagonists for these receptors have recently been identified. The most notable to date being ICS 205-930, being derived

from the 5-HT molecule, and MDL 72222, being derived from the cocaine molecule. (see *Richardson and Engel, 1986*). Several more are in varying stages of development including the Glaxo compound GR38032F (*Brittain et al., 1987*). Unfortunately these developments came too late to be investigated in any great detail in this thesis. Only preliminary reports on these compounds were therefore possible, but they should make exciting future investigations particularly since GR38032F has been reported to have anxiolytic effects (*Jones et al., 1987; Costall et al., 1987*).

3.5. Discrepancies between Binding Studies and Electrophysiologically Characterised 5-HT Receptors.

There are many discrepancies between the receptors characterised by the described methods which are still not understood. The classical 5-HT antagonists, having high affinities for 5-HT$_1$ and 5-HT$_2$ receptors, fail to antagonise S$_3$-mediated responses. Methiothepin has a high affinity for both 5-HT$_1$ and 5-HT$_2$ sites (*Leysen et al., 1981*) but it fails to block S$_1$-mediated facial motoneurone responses (*McCall and Aghajanian, 1980*), suggesting that neither site corresponds to the S$_1$-receptor.

A possible reason for such discrepancies lies in the fact that [^{3}H]-spiperone, used to characterise 5-HT binding sites, has a thousand times greater affinity than the neurotransmitter itself, and high concentrations of 5-HT may have non-specific effects (*Middlemiss et al., 1980*). Also, because of the few functional correlates and limited number of chemical congeners binding with high affinity to the 5-HT$_1$

site (*Leysen, 1984*), its nature as a receptor, as opposed to simply a binding site, can be questioned. However, there are an increasing number of more specific compounds for 5-HT$_1$ sites becoming available. The most widely known are 8-OH-DPAT (*Arvidsson et al., 1981*), the putative anxiolytic TVXQ7821 (ipsapirone)(*Glaser and Traber, 1985*), and the putative anxiolytic buspirone (*Goldberg, 1979; Peroutka, 1985*).

4. 5-HT Binding Sites and Their Second Messengers.

4.1. 5-HT$_1$ Sites.

Initial evidence suggested that these sites were linked to adenylate cyclase in the brain, being stimulated by nanomolar concentrations of 5-HT (*Fillion et al., 1979a; 1979b*). Whilst this hypothesis is still correct, it is now clear that 5-HT$_1$ receptors transduce signals via a number of different pathways as well (see *Roth and Chuang, 1987* for review). In addition to adenylate cyclase linkage, phosphoinositide hydrolysis may also be involved, specifically in the case of 5-HT$_{1c}$ subtypes (*Conn and Sanders-Bush, 1986; 1987*). Modulation of K^+ channel activity may also be involved, either consequent on adenylate cyclase activation or in a direct link with 5-HT$_{1a}$ receptors in the rat hippocampus (see *Roth and Chuang, 1987*).

4.2.5-HT$_2$ Sites.

5-HT$_2$ binding sites are linked to phosphatidyl inositol (PI) metabolism. PI metabolism induced by 5-HT is blocked by drugs such as pizotifen and ketanserin in the rat cerebral cortex (*Berridge et al., 1982*; *Conn and Sanders-Bush, 1984*). PI hydrolysis has been

proposed to be a multifunctional transducing mechanism for generating a number of important intracellular signals, including calcium fluxes, prostaglandin synthesis, production of cyclic guanosine monophosphate and control of protein kinase activity (*Downes, 1983*). The possibility remains, however, that the above-mentioned mechanisms are not dependent on activation of a single biochemical pathway i.e. PI hydrolysis, but are themselves directly linked to 5-HT_2 receptors. Thus 5-HT_2 receptors may be coupled directly or indirectly to PI metabolism, voltage gated calcium channels or prostaglandin synthesis. (see *Roth and Chuang, 1987*).

4.3. 5-HT_3 Sites.

At present little is known about any second messenger system involved with 5-HT_3 sites, except that they do not appear to be linked to phosphatidyl inositol metabolism (*Cory et al., 1987*).

5. Possible Central Roles of 5-HT

The central serotonergic system is a diverse and functionally important system. It has many possible roles in the control of physiological and behavioural mechanisms.

5-HT is involved with the regulation of body temperature (*Feldberg & Myers, 1964*), with transmission of nociceptive information (*Evans, 1961*) with sleep regulation (*Jouvet, 1973*), with feeding behaviour (*Blundell, 1979*) and also with sexual behaviour (*Gessa & Tagliamonte, 1979*). Injection of 5-HT into mice produces a characteristic behavioural syndrome involving hyperactivity, tremor, rigidity, head-

twitching etc. (*Graheme-Smith, 1971a*). This syndrome can be used as a direct measure of 5-HT activity in the fore-brain. There have been suggestions that 5-HT systems are involved in depression (*Abrams, 1978*) with tryptophan being reported as being just as effective as imipramine (*Coppen et al, 1972*). Anti-depressant-like actions of 5-HT_{1A} agonists have also been suggested in animal models of depression (*Kennett et al., 1987*). The so-called monoamine hypothesis of affective disorders suggests that a decrease in monoamines is responsible for depression. However, the possibility is now being considered that increased 5-HT activity may be responsible (*Aprison, et al., 1978*). There is also evidence that 5-HT may impair learning and memory (*Essman, 1978*). In stress situations e.g. restraint stress in mice, 5-HT turnover has been shown to increase (*Bliss, 1978*), although many other studies have shown stress to have no effect on 5-HT turnover (see *Anisman, 1978*).

6. Psychology of Anxiety

There is much inconsistency when trying to find a definition of anxiety for it is a very subjective emotion and can be produced by an infinite variety of situations depending on the subject involved. Whatever the definition of anxiety, it plays a major part in everyday life and is a far more serious ailment than would at first appear. To quote *Davison and Neale (1978)* from their Textbook of Abnormal Psychology, "There is perhaps no other single topic in abnormal psychology that is as important or controversial as anxiety. This emotional state is considered as a symptom of almost all psychopathologies and in particular of the neurotic disorders.

Furthermore, anxiety plays an important role in the study of psychology of normal people as well, for very few of us go through a week of our lives without experiencing it in at least some measure what we would all agree is the emotion of anxiety."

There are many theories concerning this subject which cannot be discussed at length here. There are, however, several recurring themes apparent. Perhaps the most important of these is that anxiety is associated with the inability to express impulses or desires, and with a disruption of behaviour patterns. The two crucial points here, as regards the selecting of a model to study anxiety, are that the inability to express impulses or desires can be equated to a state of conflict and that the inhibition of behaviour occurs as a consequence of experience which can provide the basis for operant conditioning experiments.

It would appear that there are several forms of the anxious state, or more correctly several subtypes of the all-encompassing term anxiety. Thus the following subtypes have all been proposed; situational, free floating, phobic, anticipatory, anxiety with depression, anxiety secondary to medical conditions and medication, psychotic terror, and traumatic anxiety. (*Shader, et al. 1981*). Whether these all originate from the same neuro-biological mechanism or whether they have seperate mechanisms is not clear, though it is unlikely that each one has its own mechanism. There is evidence, however, that panic anxiety is distinctly noradrenergic in its origin and is not

susceptible to treatment with benzodiazepines (*Charney et al., 1984; see also Hamilton, 1982*).

The terms anxiety, fear, nervousness and tension seem to be used interchangeably in the research literature. Psychoanalysts reserve the term anxiety for fear that is experienced in the absence of external danger, which is probably one of the most succinct definitions of anxiety. Fear and anxiety have been said to be identical as a felt experience i.e. there is nothing in one's awareness to distinguish one discomfort from the other. Fear, however, if present as a significant factor, is unequivocal whereas anxiety is seldom clearly represented as such. Fear is said to be related to some definable situation and is roughly the same for everyone but situations arousing anxiety are often obscure. Anxiety can be infinitely varied among people and shows little habituation (see *Sullivan, 1964*).

7. Animal Models of Anxiety

To enable anxiety to be studied requires the use of animal models since studies in humans are problematic because of the ethical considerations of using anxiety-inducing drugs. Problems also arise in the reporting of drug effects since anxiety is a subjective emotion and may manifest itself differently between subjects. The techniques to be employed in this study vary from the simple exploratory-type behavioural model to the more complex techniques of operant conditioning.

7.1. Operant Conditioning.

The pioneer of this field was Skinner, who in the early 1950's, described how *operant* conditioning was fundamentally different from *classical* (or Pavlovian) conditioning. In classical conditioning a physiological response e.g. salivation, is elicited in response to a natural stimulus such as food. The animal can then be conditioned to respond in the same way to an associative stimulus, such as a bell. In operant conditioning the response by the animal is not *elicited* in response to a stimulus but is *emitted* by the animal as a conscious action. Operant responses are not responses in the true sense of the word as referred to in classical conditioning since the animal is not responding to a stimulus or event. The animal learns that a particular action brings about a reward or punishment and is therefore conditioned by these two events to either respond or not respond. Signals are used to indicate to the animal whether rewards or punishment are imminent (see *Blackman, 1974*). These signals are crucial in that they are the link which triggers the realisation of imminent punishment and therefore it is the onset of these signals or cues which trigger the anxious state.

7.1.1. Conflict Tests

Conflict tests are useful models of anxiety in animals and rely on the fact that conflict plays an important role in anxiety. As mentioned earlier anxiety is associated with inability to express impulses or desires. This can lead to states of conflict. In operant conditioning and conflict tests, food reinforcement is usually alternated with a schedule combining punishment, usually in the form

of footshock, with the food reward. The footshock produces partial suppression of responding during the punished component. Performance during this period is designated as "conflict behaviour". Clinically effective anti-anxiety agents are able to restore the suppressed responding. The terms *'anxiolytic'* and *'anti-conflict'* are therefore interchangeable in this context. In summary therefore, in all conflict tests behaviour suppressed by punishment is reinstated by anxiolytic (anti-conflict) agents (*Sepinwall & Cook, 1978, Stein et al, 1973*). Conflict models therefore have considerable value in predicting clinical anxiolytic efficacy (*Cook and Davidson, 1973*) and in studying the behavioural and biochemical mechanisms of action of putative and known anxiolytics (*Cook and Sepinwall, 1975a; 1975b; Stein et al., 1975*).

a) Geller-Seifter Test

This test was designed by *Geller & Seifter (1960)* and was the forerunner of all the presently used conflict tests, and is still used itself. Rats are trained to lever press for a food reward on two different alternating schedules. The first component provides rewards on an intermittent basis, on a variable interval-two minute schedule (VI-2). This schedule produces a high constant rate of lever pressing due to the fact that rewards cannot be predicted, and is present to detect sedation. This component lasts for 10 minutes. The second component delivers a reward for each lever press, (continuous reinforcement, CRF) but also delivers a small electric shock to the grid floor of the cage. This component is signalled by the house lights in the operant boxes. A complete experimental run lasts for

49 minutes, consisting of four 10-minute components alternating with three 3-minute punished components.

One of the problems with this test is that since the unpunished and punished components are based on different schedules it is not possible to directly compare the components to determine the level of supression caused by the footshock. Another problem is that the two components may not be equally sensitive to drug effects. However, the anxiolytic profile with the benzodiazepines is observed both acutely and chronically yet there is rapid tolerance to the sedative effect. This distinction is found clinically (*Warner, 1965*) - a possible reason why this test is used widely to establish anxiolytic drug profiles (*Cook & Davidson, 1973*).

b) Variable Interval Punished Schedule

As a result of the criticisms of the Geller-Seifter schedule, a wholly variable interval schedule was designed (e.g. *Mithani, 1984*) to enable direct comparisons to be made between punished and unpunished components. The schedule consists of alternating 5-minute periods of variable-interval-30 seconds (VI30 secs.). Punished components deliver footshock with each reward and are signalled by flashing houselights in the experimental chamber. An additional 5-minute period of *extinction* (or time-out) is included where no rewards are obtained at all. This is signalled by switching the lights on constantly throughout the 5 minutes. Thus since the two components of the schedule are the same, one can titrate the shock intensity and duration to produce the desired level of suppression.

c) Continuous Reinforcement Schedule (CRF).

This test also consists of alternating identical components as above. Here the 5-minute components consist of continuous rewards with and without shock. Punished components again deliver footshock to the grid floor and are signalled by flashing the houselights.
(see *Mithani, 1984*).

d) Conditioned Emotional Response (CER)

This test was developed by Estes and Skinner (1941). Rats trained to lever press are presented regularly with a tone every few minutes which is followed by an unavoidable footshock. The procedure results in a disruption of operant responding during the pre-shock tone, which is attributed to anxiety (*Estes & Skinner, 1941*). The test appears, however, to show inconsistant results with anxiolytics (*Millenson & Leslie, 1974;* also see *Gray, 1981a*).

e) Punished Drinking

In this test, conflict is induced in thirsty naive rats by periodically administering shocks for licking water (*Vögel et al, 1971*). Conflict is established between thirst (after 24 hours water deprivation) and drinking contingent footshock. Benzodiazepines appear to increase the number of shocks accepted *ibid*). This is also a widely used animal model of anxiety since it is easy to perform and requires no training of the experimental animals.

7.2. Non-consummatory Tests.

a) Exploratory Activity

These are simple tests involving exploratory activity in novel environments. The tests vary e.g. exploratory activity in a 'Y'-maze (*Marriott & Spencer, 1965*), a circular enclosure (open field) (*Christmas & Maxwell, 1970*), the 'hole board' (*Nolan & Parkes, 1973*) and the 'staircase' test (*Boissier et al, 1968*). The model employed in this study is the **elevated 'X'-maze** (*Handley & Mithani, 1984a*). This model was developed in this laboratory and is based upon the ideas of Montgomery (1953 & 1955) and his elevated 'Y'-maze. The model measures differential exploration of equal numbers of 'open' and 'enclosed' arms of a raised 'X'-shaped maze. Two drives operate on the 'X'-maze; the 'fear drive', manifested in the fear of entering an exposed open arm as opposed to a 'safe' enclosed arm, and an 'exploratory drive' compelling the rat to explore this novel environment. Thus the ratio of open arm entries to total entries is used as an index of anxiety with the total entries value indicating any secondary effects on motor activity. Thus, compounds exhibiting anxiolytic-like activity will increase the entry ratio whilst anxiogenic-like compounds will produce a decrease of the ratio. Diazepam and other classic anxiolytic compounds increase the entry ratio whilst putative anxiogenics decrease the ratio (see *Pellow, 1986* for review). A more detailed explanation of the 'X'-maze follows in a later section.

b) Punished Locomotor Activity.

In this test animals are punished with footshock when passing across electrifiable plates on the floor in order to reduce locomotor activity. Diazepam, meprobamate and phenobarbitone all reinstate the behaviour suppressed by punishment (*Boissier et al, 1968*).

c) Social Interaction Test

File and Hyde (1978) proposed that uncertainty as to source, nature and timing of events is a potent cause of anxiety. They applied this principle to the development of the Social Interaction model of anxiety which measures the time spent in active social interaction between pairs of male rats measured under various conditions. Social interaction is highest in familiar surroundings under low illumination, and decreases in unfamiliar surroundings and as illumination increases. Specific behaviours are scored e.g. grooming, sniffing, licking, boxing, wrestling etc. Benzodiazepines increase the time spent in active social interaction compared with control animals (*File et al, 1976*). However, acute effects of the benzodiazepines are not detected. Therefore the Social Interaction method used in this study is as modified by *Gardner and Guy (1984)* using low light and familiar conditions with rats being pre-exposed to the test arena. Rats were also housed in pairs and tested with unfamiliar partners. The result of these modifications is that the model is now sensitive to acutely administered benzodiazepines (*Gardner and Guy, 1984; Guy and Gardner, 1985*).

d) Potentiated Startle Paradigm.

This involves the association of a cue (light) with an inescapable shock, and pairing of this cue with a tone. This produces a potentiation of the startle produced by the tone. Anxiolytic drugs decrease the potentiated startle amplitude (*Davis, 1979*).

e) Light/Dark Box.

The light/dark box is a model of anxiety using an arena divided into a brighly illuminated section and a dark section, being separated by a partition with a 'doorway'. It relies on a differential exploration of the two areas. Animals are aversive to the light area and anxiolytic drugs increase the amount of exploration and normal behaviour patterns (e,g, grooming, rearing) in the light section (*Crawley and Goodwin, 1980; Crawley, 1985; Costall et al., 1987*). The model has been used mainly for mice but a larger version has been reported active using rats (*Colpaert et al, 1985*).

7.3 **Validation of Anxiety Models.**

Consummatory tests can be criticised because drugs may affect food or water intake and the tests may then not give a true representation of the anxious state. With the Geller-Seifter test, however, anxiolytics have a selective effect on punished behaviour (*Stein et al, 1973*), and therefore the feeding behaviour may override the effects of anxiety.

Geller et al, (1963) dispensed with the criticism that drugs may change the animals sensitivity to pain, by showing that morphine at

analgesic doses does not reinstate punished responding. Another major criticism is that changes in punished responding may reflect general stimulatory or depressant effects. However, amphetamines and chlorpromazine do not significantly alter punished responding (*Iversen, 1980*). Neuroleptics in general are ineffective in conflict tests except at high dose when both punished and unpunished behaviour is suppressed. Antidepressants show a similar profile to the neuroleptics (see *Geller, 1962; Hanson et al., 1967; McMillan, 1975; Cook and Davidson, 1973*). Antihistaminics appear not to have any anti-punishment effect (*Goldberg and Ciofalo, 1969*), neither do hypnotic sedatives such as chloral hydrate (*McMillan, 1973*). Thus the conflict tests appear to be relatively specific for antianxiety agents and such agents tend to selectively affect punished responding, unless given in high doses.

However, it has been pointed out recently that all the various conflict models of anxiety are based on the fact that the benzodiazepines are active in them. Could there be, therefore, some facet other than anxiety here, which is responsible for the behavioural effects seen with the benzodiazepines? A possible answer could lie in the fact that benzodiazepines appear to reduce the capacity of an animal to tolerate a delay before having access to a reward (*Thiébot et al., 1985; Thiébot, 1986*). It is conceivable then that the benzodiazepine-induced release of punishment-suppressed behaviour, is due partly to a lessened ability of the operant rat to withhold responding (lever pressing) when normally the punishment would cause the rat to check its responding (*Soubrié, 1986a; 1986b*).

It is notable that during 'time out' responding (extinction) lever presses are increased in a similar manner to punished responding with diazepam (*Mithani, 1984*). Taken to its conclusion could this mean that the conflict tests which have been in use since the 1960's, are not actually measuring anxiety at all? This is clearly a problem which must be considered when conducting experiments on putative anxiolytics using solely conflict models.

Because of the criticisms outlined above, a test was sought which is more biologically relevant to normal animal behaviour since it can be argued that such punishments as electric shock are alien to an animal and indeed to a human for whom, ultimately the compounds screened in anxiety tests are intended. Therefore the exploratory models of anxiety may produce a more relevant measure of anxiety. The 'X'-maze utilises two of the rat's most basic instincts. Firstly rats are curious, inquisitive animals and therefore have a natural tendency to explore a novel environment. This facet of their behaviour is the basis of all the exploratory models. Secondly, the animals have a natural tendency to avoid open unprotected spaces where unknown dangers may be present. The X-maze combines both of these behavioural facets in the form of open arms and enclosed arms of the maze which is elevated from the floor to increase the aversiveness of the open arms. Following work by Montgomery (1955), Handley and Mithani (1984a) in this laboratory suggested that the open arms are more aversive than the enclosed which would be expressed as an increased proportion of enclosed entries from rats allowed to freely explore the maze. Thus the ratio of open to total entries over a

fixed period is deemed to be a relatively pure index of anxious state. This measure should be free from contamination by variables having no differential effect on type of arm entered but could theoretically be affected by drugs or procedures which modify 'curiosity' without affecting 'caution'. Total arms entered is a measure of overall total exploratory activity which would indicate any motor depressant or stimulant or sedative effects.

Since the first work on the 'X'-maze (*Handley and Mithani, 1984a*) the model has been extensively validated by several workers (*Pellow et al., 1985; Briley et al., 1985; 1986; Pellow and File, 1986*) and again in this thesis. The anti-anxiety agents chlordiazepoxide, diazepam, alprazolam, adinazolam, phenobarbitone and amylobarbitone have all been shown to have an anxiolytic profile (*Pellow et al., 1985; Handley and Mithani, 1984*a).

Standard anxiogenic compounds are scarce. The compound FG7142 which has been shown to be anxiogenic in man (*Dorow et al., 1983*) and animals (*File and Pellow, 1984*) and CGS8216 which is also an inverse agonist at benzodiazepine receptors and is anxiogenic in animals (*Pellow and File, 1984*), have an anxiogenic profile in the 'X'-maze (*Pellow et al., 1986*). The convulsant pentylenetetrazole (PTZ) which has anxiogenic effects in humans (*Rodin and Calhoun, 1970*) reduced time spent in the open arms (but also reduced total entries). Overall, however, it has anxiogenic effects (*Pellow et al., 1985*). Also the α_2-adrenoceptor antagonist yohimbine and the psychostimulant caffeine, both of which have been found to have anxiogenic properties in man

(*Charney et al., 1984; Uhde et al., 1984*) and in animals (*Handley and Mithani, 1984b; File and Hyde, 1979*), have an anxiogenic profile on the 'X'-maze decreasing both the ratio and time spent on open arms (*Pellow et al., 1985*).

The observed changes in the maze behaviour seem to be confined to anxiolytic and anxiogenic compounds since a whole range of antidepressants and the neuroleptic haloperidol have no significant effects in this test (*Pellow et al., 1985; Briley et al., 1986*). Amphetamine has no effect on overall activity and shows slight anxiogenic-like actions which is consistent with reports that it can produce anxiety (*File and Hyde, 1979*).

7.4. Rate Dependency

There is evidence that in operant procedures some drug-induced changes in response rate may depend on the pre-existing rates (*Robbins, 1981*). This phenomenon is known as *rate dependency*. As an example, Harris et al, (1978) demonstrated that clonidine, fenfluramine and quipazine increased low response rates whilst decreasing high response rates on certain schedules of behaviour. This is clearly a factor which must be taken into account when analysing results from operant conflict procedures.

7.5. Choice of Model

The present study will principally examine the 'X'-maze model of anxiety since it appears to be a sensitive, biologically relevant model which examines anxiety via a different approach from the

classical models and may well indicate activity with compounds which have little or no activity in the other models.

The study will also examine the operant conflict models of anxiety. Most work in the past has used the Geller-Seifter model (*Geller & Seifter, 1960*) which has proved a reliable test. It has the advantage that trained animals with stable baseline responses can be used as their own controls and can participate in successive experiments over many months. It can detect sensitive drug-effects and its unpunished components can be used to evaluate non-specific effects. The wholly variable-interval schedule would appear to have the above advantages in addition to the advantage of being able to compare punished with unpunished responding. One major advantage with operant conditioning procedures is that they are easily automated and controlled using real-time microprocessor techniques.

Finally, as another approach, the social interaction test was used in order that overall results can be compared in three quite different tests of anxiety.

8. Involvement of 5-HT in Anxiety.

Early studies of pharmacological models of anxiety implicated central serotonergic mechanisms in the mediation of aversive responses (*Cook and Sepinwall, 1973; Stein et al., 1977*) and led to suggestions that the anxiolytic effects of drugs such as the benzodiazepines resulted from reduced activity of these systems (*Stein et al., 1973*).

8.1.Manipulation of Central 5-HT Levels via Lesions.

Initial evidence for a role for 5-HT in anxiety was provided by Robichaud & Sledge (1969). They showed that parachlorophenylalanine (pCPA), a 5-HT depletor, had anti-conflict activity in the Geller-Seifter conflict test. This work was quickly corroborated by other workers (*Stein et al., 1973; Cook and Sepinwall, 1975a; Shephard et al., 1982*). Although pCPA will deplete catecholamines to some extent, it appears that its antipunishment effects are due to depletion of 5-HT since the 5-HT precursor 5-hydroxytryptophan (5-HTP) reversed the effect (*Robichaud and Sledge, 1969; Geller and Blum, 1970; Tye et al., 1979*), whilst the catecholamine precursor L-Dopa did not (*Tye et al., 1979*).

Selective degenerative lesions of central 5-HT pathways provided further evidence for a role for 5-HT in anxiety. The neurotoxins 5,7-dihydroxytryptamine (5,7-DHT) and 5,6-dihydroxytryptamine (5,6-DHT) injected into the ventral tegmentum deplete the hypothalamus, limbic structures and the cortex and have been reported to result in a marked release of punished responding on the Geller-Seifter paradigm (*Tye et al., 1977*). Tye et al, (1975) showed that animals with 5,7-DHT lesions of forebrain pathways accepted more shocks than sham operated controls and lesions of the lateral septum produced a decrease in dominant behaviour in the social interaction test (*Clarke and File, 1981*). Such lesions also appear to impair the acquisition of response suppression (*Iversen, 1983*). In dorsal raphé lesioned rats the attenuation of suppressed responding produced by intra-raphé injections of chlordiazepoxide is abolished (*Thiébot et al., 1982;*

Soubrié et al., 1981). However, other work has shown that chlordiazepoxide injected into the dorsal raphé has no effect on punished behaviour (*Green and Hodges, 1986*), whilst dorsal raphé lesions with 5,6-DHT significantly increased punished responding (*Green and Hodges, 1986*) and showed an anxiolytic-like profile in the social interaction test of anxiety (*File et al., 1979*). Again, however, contradictory results have appeared. Thiébot et al, (1984) reported that 5,7-DHT lesions of the raphé dorsalis did not affect behavioural inhibition in control rats nor did they modify the ability of diazepam to release punished responding.

8.2. Stimulation of central serotonin systems.

Direct intraventricular injection of 5-HT produces a complex response in operant conflict (*Stein et al., 1977*) consisting of an initial behavioural depression followed by a release of punished responding. Intraventricular 5-HT has also been shown to antagonise the effects of oxazepam on punished responding and produce an overall depression of responding (*Wise et al., 1972*). This tends to contradict findings from lesioning studies suggesting that activation of ascending 5-HT pathways would lead to an increase in locomotor activity (*Hole et al., 1976*). Problems will inevitably arise here since 5-HT pathways in the brain and spinal cord are able to both increase and decrease motor function independently (*Gerson and Baldessarini, 1980*) from their actions concerned with behavioural suppression. Placed in the vicinity of the dorsal raphé, 5-HT has been found to produce a release of suppressed responding in the CER (*Thiébot et al., 1984*), whilst iontophoretically applied to the amygdala 5-HT inhibits neuronal

firing (*Wang and Aghajanian, 1977*). Several lines of evidence indicate that 5-HT and 5-HT agonists, when applied iontophoretically to the raphé nuclei, inhibit firing of dorsal raphé neurones (*de Montigny and Aghajanian, 1977; Aghajanian, 1978*). Such injections subsequently produced a release of punishment suppressed responding in rats (*Thiébot et al., 1982*).

Various serotoninomimetics have been studied with differing results. The precursor 5-hydroxytryptophan (5-HTP) suppresses food reward behaviour in the pigeon (*Aprison and Ferster, 1961*) whilst the 5-HT agonist α-methyltryptamine reduces punished and unpunished responding in both the rat (*Winter, 1972*) and the pigeon (*Graeff and Schoenfeld, 1970*). The agonist N,N-dimethyltryptamine shows similar activity (*Winter, 1972*), whilst 5-MeODMT appears to decrease unpunished but not punished responding (*Shephard et al., 1982*), and quipazine was shown to have no effect in punished responding (*Commisaris and Rech, 1982*). It is not possible to make any specific conclusions as regards anxiety here since it could be said that severe anxiety would depress all responding anyway, and the effect of these direct and indirect agonists on thirst and hunger must be taken into consideration. For example it is known that the putative 5-HT_{1a} agonist 8-OH-DPAT, will increase food intake in rats (*Dourish et al, 1985*) and in general serotonin agonists decrease food and water intake (*Samanin et al., 1979; Soulairac and Soulairac, 1970*). Such actions could therefore contribute to the effects seen in food or liquid-motivated behaviour.

There appears to be little clinical evidence to support the concept of increased anxiety associated with increased central 5-HT neuronal activity. The precursors 5-HTP and L-tryptophan have been investigated and do not seem to show any indication of an anxiety-inducing effect (*Trimble et al., 1975*), in fact one study even investigated a possible anxiolytic effect of L-tryptophan (*Wilbur and Kulik, 1981*). Serotonin re-uptake inhibitors, however, do appear to have some consistent effects on anxiety. Such agents would be expected to enhance transmission over the short term and have been demonstrated to be effective in alleviating phobic anxiety. Zimeldine appears to be particularly effective in doing this (*Evans and Moore, 1981*). However, as mentioned earlier, phobic anxiety is possibly pathologically different from anticipatory or 'free floating' anxiety.

8.3. 5-HT Antagonists.

Initial studies with 5-HT antagonists in food-motivated conflict paradigms indicated an anxiolytic-like release of punished responding. Cinanserin, cyproheptadine, methysergide, lysergic acid diethylamide (LSD), and metergoline have all shown an anxiolytic-like increase in punishment suppressed responding (*Sepinwall and Cook, 1978; Schoenfeld, 1976; Stein et al., 1973; Graeff and Schoenfeld, 1970; Graeff, 1974*).

Other models have also shown some anxiolytic activity with 5-HT antagonists. Metergoline reduces stress-induced ultrasounds from rat pups (*Gardner, 1985*) and ritanserin, a 5-HT_2 receptor antagonist,

shows anxiolytic activity in a light/dark box model (*Colpaert et al., 1985*).

However, more recent data has shown inconsistent activity with 5-HT antagonists. Kilts et al., (1982) used the punished drinking model and examined the effects of the antagonists methysergide, cinanserin, cyproheptadine and metergoline. They declared that these agents failed to produce any large and reliable increases in punished responding when compared with diazepam in the same test. Metergoline showed no activity in the social interaction test of anxiety (*Gardner and Guy, 1984*) and ritanserin showed little activity in either social interaction, licking conflict or food-motivated conflict (*Gardner, 1986; Colpaert et al., 1985*).

Very little clinical data on the effects of 5-HT antagonists on anxiety exists. Ritanserin however, has shown possible anxiolytic properties in preliminary clinical trials, which may be qualitatively different from the anxiolytic properties exhibited by the benzodiazepines (*Arriaga et al., 1984; Ceulemans et al., 1984*).

With the recent availability of selective 5-HT_3 receptor antagonists has come suggestions that they too may possess anxiolytic activity (*Jones et al., 1987; Costall et al., 1987*).

Thus, whilst 5-HT antagonists show considerable activity in various animal models of anxiety, there is an equally large and convincing body of evidence suggesting little or no activity for such compounds.

8.4. β-Adrenoceptor Antagonists.

Antianxiety effects of some β-blockers are well known and are considered to be due to peripheral autonomic effects on some of the symptoms of anxiety (*Peet, 1984; Noyes, 1985*). Propranolol can be therapeutically useful in anxiety states, due to dentistry or stage fright for example (*Brantigan et al., 1982*). This effect was proved to be peripheral and independent of 5-HT receptor blockade since practolol, a β-blocker with poor brain penetration, is equally effective (*Bonn et al., 1972*).

The β-adrenoceptor antagonists deserve mention here, however, since recently some of them have been shown to interact with the 5-HT receptor (*Middlemiss et al., 1977; Nahorski and Willcocks, 1983*). Indeed some have been described as the most specific antagonists of 5-HT$_1$ receptors yet available in the absence of true, specific antagonists for these receptors (*Middlemiss, 1984; Schlicker et al., 1985; Middlemiss, 1986a*).

8.5. Biochemical Evidence.

Biochemical evidence showed that the benzodiazepines reduced turnover of catecholamines in the brain as well as indoleamines (*Corrodi et al., 1971*). This has more recently been confirmed by other workers (*Collinge et al., 1982; 1983*). The effect on noradrenaline (NA) showed tolerance whereas no tolerance was seen with 5-HT (*Stein et al., 1975*). This correlates well with clinical observations that tolerance develops quickly to sedation but not to the anxiolytic action of benzodiazepines. More recent biochemical evidence has

confirmed the fact that benzodiazepines reduce 5-HT turnover and release (*Saner & Pletscher, 1979*). In the study of Wise et al., (1972), where i.c.v. 5-HT antagonised the effects of oxazepam, parallel biochemical studies showed that oxazepam given acutely reduced the turnover of both NA and 5-HT whereas given chronically it only reduced 5-HT turnover.

Other studies have failed to replicate such results however. Cook and Sepinwall (1975b) found a reduction of 5-HT turnover after chronic dosage but not after acute dosage and, whilst this may correspond to their demonstration of only weak anti-conflict effects acutely, the maximum anti-conflict effects did not correspond with the maximum reduction of 5-HT turnover. No changes were observed after a single dose of chlordiazepoxide, whilst 5 days of treatment produced a reduction in turnover with accompanied anxiolytic effects (*File and Vellucci, 1978*). Another study reported completely the opposite effects (*Lister and File, 1983*). Thus, on the whole, biochemical evidence suggests a role for 5-HT in anxiety although the effects are far from clearcut.

8.6. Benzodiazepines and 5-HT.

The fact that oxazepam and diazepam reduce 5-HT turnover provided initial evidence that the neurotransmitter may be involved in the manifestation of the anxiolytic effects of benzodiazepines. Electrophysiological findings also support the suggested role for 5-HT since in the rat clonazepam depresses firing in 5-HT neurones

(*Pratt et al., 1979*) and both chlordiazepoxide and midazolam depress neuronal firing in the dorsal raphé nuclei (*Laurent et al., 1983*).

One could argue that, if the anti-conflict actions of the benzodiazepines are mediated via an inhibition of 5-HT pathways, then they would be without effect in animals whose 5-HT pathways had been lesioned. However, the anti-conflict effect seen with i.c.v. 5,6-DHT lesions of serotonergic pathways, was increased by diazepam (*Lippa et al., 1979*), as was the anti-conflict effect of pCPA with chlordiazepoxide (*Shephard et al., 1982*). Thus it seems that depletion of central 5-HT levels does not decrease the activity of benzodiazepines in conflict models. It must be noted though that despite the lesions there could still be some functional 5-HT pathways remaining to be inhibited further.

It must also be considered that the benzodiazepine receptor is part of an oligomeric receptor complex which includes a $GABA_A$ receptor (see *Bowery et al., 1984*). Benzodiazepines binding at the receptor complex enhance GABA transmission. When one considers the areas of brain discussed with reference to 5-HT content, it also becomes clear that such areas are also rich in GABA-containing neurones. Iontophoretically applied GABA in the dorsal raphé, amygdala and hippocampus inhibits the firing of 5-HT neurones (*Gallager, 1978*), and infused into the dorsal raphé, GABA produces a release of suppressed responding (*Thiébot and Soubrié, 1983*). Thus although data is sparse there appears to be a body of evidence implicating benzodiazepine-GABA interactions with 5-HT pathways in the anti-punishment effects

of benzodiazepines, although the local circuitry has yet to be established.

Thus, although convincing evidence exists that 5-HT pathways, possibly arising from the dorsal raphé and sensitive to benzodiazepines and GABA-mimetics, are crucialy involved in the manifestation of anxiety, a close survey of the literature reveals many discrepancies. Such discrepancies necessitate further studies before associating modulation of central 5-HT systems directly with anxiety.

9. The Behavioural Inhibition System (BIS)

This is a model of the neuropsychology of anxiety proposed by Gray (1981a; 1981b) on the basis of the analysis of behavioural effects of anti-anxiety agents in animals. Consequently, according to Gray (1981a; 1981b), it is the activity of the BIS which constitutes anxiety and anti-anxiety drugs reduce this activity. In summary, he suggests that anxiety is a central state consisting of activity in the hypothetical BIS and that this system is activated by punishing stimuli, frustrative stimuli and novel stimuli. The behavioural results of activating this system are an inhibition of ongoing behaviour and increased arousal and attention. He also suggests the most likely sites of specific anti-anxiety effects of drugs to be the ascending noradrenergic and serotonergic pathways, and possibly the ascending dopaminergic pathway to the pre-frontal cortex, and, much less certainly, the ascending cholinergic pathway to the septo-hippocampal system (see *Gray, 1981a*).

The possibility that benzodiazepines may inhibit the waiting capacity of an animal (*Thiébot et al., 1985; Thiébot, 1986; Soubrié, 1986a; 1986b*) may be connected with the above theory. Activation of the hypothetical BIS, thus inhibiting normal ongoing behaviour may in fact be a reflection of the increase in impulsivity produced by anti-anxiety agents such as the benzodiazepines, as suggested by Thiébot, Soubrié and colleagues.

11. Aims of the Project

1. To study the possible involvement of 5-HT in anxiety by establishing the effects of agonists and antagonists varying in selectivity for 5-HT_1 and $5HT_2$ receptors (and their sub-populations) on several models of anxiety.

2. Initially to assemble, connect and learn how to use new computer-controlled operant conditioning apparatus.

3. To further define conditions for the elevated 'X'-maze model of anxiety.

Experimental Methods Page No.

METHODS

1. Animals and Animal Husbandry.

Experiments reported in this thesis were carried out on male hooded PVG rats supplied by Bantin and Kingman Limited, with the exception of a group of hooded Lister rats obtained from Olac for a particular experiment as described in the text. Rats were kept in the animal housing unit at an ambient temperature of 21±1°C and under a 12 hour light/dark cycle (0800-2000h), and were maintained on a conventional 41B cube diet supplied by Pilsbury Limited with tap water ad libitum. Rats used in operant conditioning experiments were fed daily an amount to maintain their body weight at 80% of free feeding weight, and also had access to water ad libitum.

2. General Experimental Conditions.

Animals for use in the X-maze were held in groups of 6 in a quiet room for at least one week prior to experiments. Animals for use in the social interaction test were housed in pairs for at least one week prior to experiments. Animals for use in operant conditioning experiments were housed singly to enable the required dietary manipulations to be made. All experiments were carried out at the same temperature as the housing rooms and as far as possible between 1000 and 1700h unless otherwise stated.

3. Injection Technique.

All drugs were injected intraperitoneally (i.p.). Injections were made by inserting the needle (25G) through the abdominal wall towards the

diaphragm. Care was taken not to penetrate too deeply and thereby damaging the internal organs. If more than one injection was required care was taken to avoid using the same injection site. The injection volume used was 1ml/kg except where otherwise stated. All drug solutions were prepared in saline (0.9% NaCl) unless otherwise stated (in such cases the appropriate vehicle controls were used).

4. Behavioural Assessment.

4.1. Maze Exploration.

The apparatus used was similar to that described by Montgomery (1955), modified as described previously by this laboratory (*Handley & Mithani, 1984a*). It consisted of an X-shaped wooden maze elevated 70cm from the floor and comprising two open arms with no sides or ends, and two opposite enclosed arms with 10cm high sides and ends. All four arms were 45cm long and 10cm wide. The central square formed by the arms was open and the entire floor of the maze was lined with wire mesh to enable the animals to grip.

Animals weighing 180-280g were kept in groups of six either in the experimental room or in a quiet adjacent room and transferred the day prior to an experiment. Entry to the experimental room was then barred to all except the experimenter. Animals were assigned randomly to test or vehicle control groups but remained in their home cage such that each home cage contained a selection of control and test rats. Usually six rats received each treatment and a vehicle control group was included in each experimental run. All data analysis thus compared test data with the concurrently run control. Drugs were

injected the required time prior to placement in the maze depending on the duration of effect of the individual drug. Immediately prior to removal from their home cages each rat was observed and any behavioural changes or sedation were noted. Rats were handled gently (not by their tails) and placed in the centre of the maze always facing the same **enclosed** arm. The number of open and enclosed arm entries were noted over a 10 minute period. Any other behavioural changes were also noted. Observations were made either by an observer sitting quietly and motionlessly at least 1.5m away and equidistant between an open and enclosed arm or by a video camcorder (Fergusson) attached to a television monitor/VCR (Amstrad). Maze exploration could then be observed either directly on the television screen or recorded and played back on a VCR at a later date. The criterion for an arm entry was a full body-length entry (all four limbs) excluding the tail. On removal of the rat from the maze, the maze was cleaned with a brush and a damp cloth soaked in a weak 'Dettol' solution.

Experience during the work described in this thesis and previously (*Mithani, 1984*) has shown that the experimental conditions used are critical for consistent results to be obtained. Particular points are as follows:

1. Rats should be handled with care by the same experienced person all the time.
2. Rats should be allowed at least 1 week after delivery to become accustomed to the environment and should be

handled daily by the experimenter.

3. The maze should have a space clear of obstructions for at least 1.0m all the way round.

4. Conditions in the experimental room should be kept quiet at all times.

5. Source of rats should remain constant if possible.

6. Rats over 280g should not be used.

7. If possible remote observation of the maze should be used.

The number of open and enclosed arm entries were recorded and results expressed as total number of entries and the ratio of open/total entries for each individual animal. Thus the group mean proportion was expressed as (open/total)/n. Statistical comparisons were carried out between raw data for each test condition against the concurrently run control group using a t-test. Where the particular experimental design dictated, analysis of variance (ANOVA) was used e.g. for determining the effect of one drug in the presence of another.

4.2. 5-HT Syndrome.

Male PVG rats were given their pre-treatments (saline for controls) the required time prior to administration of the agent inducing the 'syndrome'. The 'syndrome' was induced by 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (5mg/kg i.p.). Rats were then placed in an open topped cage and their behaviour observed for a period of 20 minutes either by two observers sitting quietly in the room or

monitored via a video camera. Behaviours were scored at 5 minute intervals from the administration of the inducing agent according to the method of *Deakin and Green (1978)*, as follows: 0-absent, 1-just present, 2-definite, 3-severe. Behaviours observed were flat body posture, reciprocal forepaw treading, abducted hindlimbs, head weaving, straub-tail and tremor. Where possible experiments were done blind and each run included a control animal.

4.3. Social Interaction

Social interaction as described by *File and Hyde (1978)* was modified according to *Gardner and Guy (1984)* in order to detect the acute effects of diazepam. Male PVG rats (200g) were kept in pairs for at least 1 week prior to experiments. All rats were paired so that their weights were within 5g of each other, and were tested in a wooden arena (58cm x 58cm x 46cm) under low light and familiar conditions. Rats were therefore pre-exposed to the arena with their cagemates for 5 minutes on the two days prior to the experiments. The arena was illuminated by a 60W bulb approximately 1.5m above the floor of the arena. On the test day each rat was placed in the arena, after its drug pre-treatment, with a rat from a different, and not adjacent, cage for 5 minutes. Each rat therefore only met its test partner under test conditions in the arena. Time spent in active social interaction, number of rears and walks (one body length of continuous ambulation) were scored by two observers via a video camera with monitor in adjacent room. Aggression was not scored seperately but was included in the overall social interaction score. Each group of rats were used in the test twice only with an interval

of at least 1 week between each test and upon the second testing rats were tested with a different test partner than on the first exposure. Both observers scored independently and the mean of the scores used. Between-observer variations of more than 10% were discarded.

5. Operant Conditioning

5.1.Apparatus.

The operant conditioning apparatus consisted of 8 Operant Conditioning Units (Skinner Boxes), dimensions 25x22x20 cm (Operant Conditioning Unit Model 105 - Campden Instruments Limited) with attached shock scrambler. Each box contained two levers 6cm above an electrifiable grid floor, two lights 4cm above each lever, and an automatic dipper feeder between the two levers for the delivery of a liquid food reward (0.2cc of sweetened condensed milk diluted 1 part in 2 parts tap water). In all operant conditioning experiments, only the left lever was active so animals quickly learned to ignore the right lever. Experiments were controlled by an Apple IIe microcomputer running OPN software (Operant Package for Neurosciences, TCOM) via a 'Med Associates' interface. Data was stored on disc or printed out on an Epson FX-80 printer. The computer was housed in an adjacent room, being linked via a wall mounted junction box and 10-core cables to the operant boxes. The duration of electric shock was controlled by an Acorn System 3 microcomputer programmed in OnliBASIC. The intensity of electric shock was monitored by the control box for each individual chamber.

5.2. Experimental Conditions.

Male rats weighing 250-300g initially were used. Animals were caged singly in polypropylene cages (40 x 25 cm) and their weights gradually reduced to 80% of their free feeding weights by limiting the diet. Animals were then weighed at least 3 times per week and the diet altered to maintain weight within limits of ±10g. The operant chambers were kept in a room adjacent to the animal holding room and the computers and printer were in another adjacent room connected by wall mounted junction boxes. Once animals were placed in the chambers the room was vacated until the experimental run had been completed. Rats were then removed to home cages and the grid floor of the chambers wiped down before the next run was started. The number of lever presses were recorded for the various components of the schedule. Animals were injected i.p. the required time prior to the start of the experiment. A facility exists to enable a 'staggered start' of the 8 chambers. A 'stagger' of 30 seconds was therefore used to enable the single operator to place each rat in the chamber at the precise moment that the schedule starts. Data from each experimental day was compared with the previous and following days' control data.

5.3. Experimental Schedules of Conflict Behaviour

5.3.1. Geller-Seifter Schedule.

Rats were first trained to associate left lever presses with sweetened condensed milk rewards by using a continuous reinforcement schedule (CRF), every response resulting in a reward. Once animals were established on this the CRF was changed to a 10-second variable

interval schedule (VI-10 sec) in which rewards were obtained after a mean interval of 10 seconds. Once the animals were established on the variable schedule then it was slowly extended until rewards were given after a mean interval of 2 minutes (VI-2 min). It is necessary to extend the variable interval slowly to avoid extinction of the responding when rewards are not forthcoming. If an extinction (time-out) period is to be included (with its appropriate cue of houselights constantly on) then it is necessary to train the animals on this before the variable intervals are introduced. Otherwise the animals will regard the extinction period simply as a very long variable interval period and training will therefore take considerably longer. Once animals were established on the VI-2 min schedule a light cue was introduced (flashing houselights) every 10 minutes for 3 minutes to signal that every lever press would result in a reward (CRF). When all these contingencies were established the conflict was introduced in the form of electric footshock through the gridfloor at every lever press during the signalled 3 minute CRF period. Total duration of the schedule was 49 minutes. The rate of responding during these punished periods was controlled by varying the intensity and duration of each electric shock. Shock intensities varied from 0.1mA up to a maximum of 0.5mA with the duration being either 10 or 20 centiseconds. Each group of rats numbered eight and unless otherwise stated footshock intensity and duration were titrated to produce approximately 40-60% suppression of responding in order that both increases and decreases in responding could be observed.

5.3.2. Continuous Reinforcement Schedule.

Rats were initially trained to lever-press, as above, and then were given the flashing light cue to signal that responses would be accompanied by shocks, or the constant light cue to signal extinction. The schedule consisted simply of alternating 5-minute periods of CRF and CRF with shock with one extinction period. Total duration of the schedule was 35 minutes.

5.3.3. Variable Interval Schedule.

Rats were trained to lever-press as above and then established on a VI-10 sec. schedule which was then extended to a VI-30 sec. schedule. The cues described were then introduced. The schedule consisted of alternating 5 minute periods of VI-30 sec. with and without shock and one extinction period. Total duration of the schedule was 35 minutes.

6. Stereotaxic Lesioning.

Lesions of the dorsal raphé nucleus were performed on male PVG rats weighing 250 ±10g. Rats were anaesthetised with pentobarbitone sodium (60mg/kg) and were positioned in the stereotaxic frame such that the upper incisor bar was 5.0mm 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:-

-5.6mm 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 and Cushman) to the point prior to differentiation of the dorsal raphé nucleus into two distinct bodies. A uni-lateral lesion was therefore used. Lesions were performed by injecting 4µg in 1.0µl of 5,7-DHT 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.

N.B. It is possible to perform bilateral lesions of the dorsal raphé nucleus entering at an angle of 25° thus avoiding the mid-saggital sinus. However the particular stereotaxic frame employed here was not suitable for accurately measuring the angle of entry and it was discovered that it was possible, with care, to enter exactly on the midline and pass through the sinus with little haemorrhaging occurring and no apparent resultant ill effects on the animal.

Prior to the lesions being performed, a black dye (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.

7. 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 hippocampus/cortex and the pons/medulla were used for the assay after pCPA depletion, and 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 to 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.

8. 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 region 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.).

2.5ml of the supernatant from the acidified n-butanol homogenate was added to 5ml n-heptane and 0.4ml 0.1M hydrochloric acid (containing 2% w/v cysteine hydrochloride in order to prevent oxidation). 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°C for 20 minutes. The solutions were then removed, allowed to cool and the fluorescence was read on an Aminco Bowman Spectrophotofluorimeter at excitation and emission wavelengths of 360 and 470 nm respectively. The excitation and emission slits were 3.0 mm.

The amounts of 5-HT were determined by running known standards and reagent blanks through the assay. A stock solution of 5-HT creatinine sulphate complex (1mg/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 - 89.5% ±3.5. N=10

Amounts of 5-HT in the various brain samples were expressed as ng/g of brain tissue.

9. Drugs and Vehicles used.

DRUG	SOURCE
*ketanserin	Janssen Pharmaceutica
*ritanserin	Janssen Pharmaceutica
*seganserin (R56413)	Janssen Pharmaceutica
5,7-dihydroxytryptamine	Sigma Ltd.
diazepam (Valium)	Roche Products Ltd.
*ethyl-beta-carboline-3-carboxylate (β-CCE)	Roche Products Ltd.
8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT)	Research Biochemicals Inc.
*5-methoxy-3(tetrahydro-pyridin-4-yl)1H-indole (RU24969)	Roussel Uclaf
5-methoxy-N,N-dimethyl-tryptamine (5MeODMT)	Sigma Ltd.
*buspirone	Bristol Myers Ltd.
*TVXQ7821 (ipsapirone)	Troponwerke
±pindolol	Sigma Ltd.

Dl-p-chlorophenylalanine methyl ester (pCPA)	Sigma Ltd.
*(-)alprenolol	Hässle AB
quipazine maleate	Miles Laboratories
idazoxan (RX781094)	Reckitt and Colman
*ICS905930	Sandoz Ltd.
*GR38032F	Glaxo Research Ltd.
*MDL72222 (tropan-3-yl 3,5-dichlorobenzoate)	Merrell Dow Ltd.
*BRL24924	Beecham Research Ltd.
*metoprolol tartrate	Geigy Pharmaceuticals Ltd.
*ICI118,551	ICI Ltd.
*methysergide	Sandoz Ltd.
cyproheptadine	Sigma Ltd.
*propranolol	ICI Ltd.
*timolol	MSD Ltd.
pentobarbitone sodium ('Saggatal')	May & Baker Ltd.

* - Gift gratefully acknowledged.

Drugs were dissolved in saline (0.9% w/v NaCl) with the following exceptions:-

8-OH-DPAT - dissolved in saline with care to exclude oxygen and kept frozen in aliquots.

diazepam - commercial product 'Valium' diluted in saline immediately prior to use.

βCCE - dissolved in saline by adding minimal 1N HCl.

ketanserin - dissolved in saline by adding minimal 0.1N HCl.

ritanserin - suspended in saline by addition of two drops of TWEEN-80.

cyproheptadine- dissolved in saline by adding 0.1N HCl.

10. Reagents used in Biochemical Assays.

REAGENTS	SOURCE
n-butanol (analar)	BDH
cysteine hydrochloride (anhydrous)	Sigma Ltd.
Decon-90	Fisons
ethanol (absolute)	BDH
n-heptane (analar)	BDH
hydrochloric acid (analar)	BDH
5-hydroxytryptamine sulphate creatinine complex	Sigma Ltd.
nitric acid (analar)	BDH
o-phthalaldehyde (OPT)	Sigma Ltd.

(a) Acidified butanol.n-butanol containing 0.85 ml concentrated hydrochloric acid per litre

b) Cysteine hydrochloride 2% and 0.2% in 1M hydrochloric acid.Kept frozen or used freshly prepared.

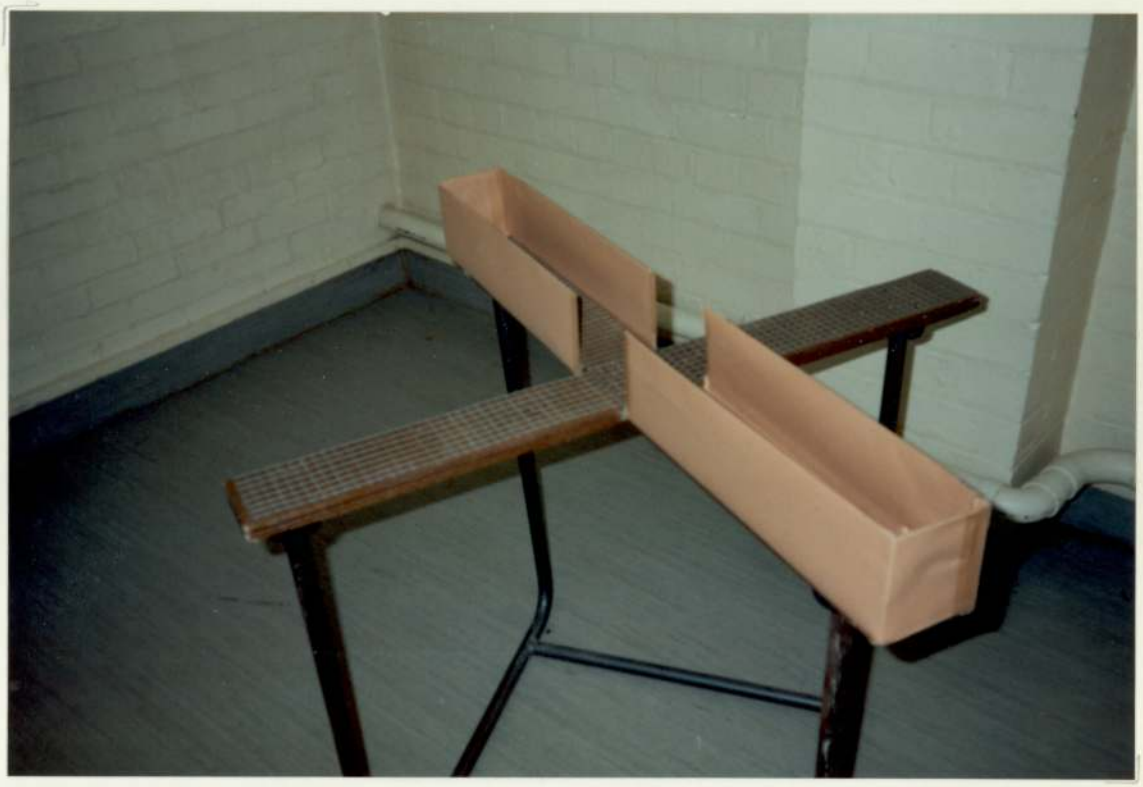
c) 5-hydroxytryptamine sulphate creatinine complex in 0.2% cysteine (in 1M HCl) 1mg/ml (base equivalent). These solutions were diluted with 0.2% cysteine/HCl to give reagent standards and extracted standards. Reagent blanks were prepared using 0.2% cysteine/HCl. These solutions were kept frozen in 1 ml aliquots and thawed once only.

(d) o-phthalaldehyde reagent (OPT)

o-phthalaldehyde crystals dissolved in concentrated hydrochloric acid - 4µg/ml. (10mg/250ml).

Plate 1.

THE ELEVATED X-MAZE



CHAPTER 1

THE EFFECT OF STANDARD ANXIOLYTIC AND ANXIOGENIC COMPOUNDS ON THE ELEVATED X-MAZE.

CHAPTER 1.

Introduction.

Although the actions of standard anxiolytic and anxiogenic agents have already been examined in this model, it is necessary to briefly re-examine them here in order to check their continued effectiveness under the present operating conditions. By their very nature, studies in behavioural pharmacology are prone to large inter-study variations necessitating the preliminary work described in this chapter.

Diazepam was chosen as the most frequently used representative of the benzodiazepines and the inverse benzodiazepine agonist βCCE was chosen as a representative anxiogenic agent. Finally as an alternative to a benzodiazepine-type compound, the α_2-adrenoceptor antagonist idazoxan was used as an anxiogenic agent since this class of compounds has been demonstrated to have anxiogenic-like activity (*see Lader and Bruce, 1986*).

Results.

1.1. Variability of control ratios.

Open/total entry ratios for control animals showed very little variation over a large number of animals (N=24). Approximately 2/3 of entries were made into enclosed arms with a resultant entry ratio of **0.302(±0.011)** [open/total±sem]. The open entry value was **4.13(±0.32)**, being significantly lower than enclosed arm entries: **9.21(±0.45)**.

1.2. The effect of diazepam.

Diazepam, (.50, 1.0 and 2.5 mg/kg) given i.p. 30 minutes prior to placing in the maze, caused a dose-dependent increase in the entry ratio to a maximum of 148% of control levels. At a higher dose (5.0 mg/kg) the ratio returned to approximately 120% of control levels. At all doses the open/total ratio produced was significantly elevated from control values (see Fig. 1.1). Throughout the dose range tested there were no signs of a significant change in the total number of entries (see Table 1.1). Observations made in the home cages prior to exposure to the maze indicated slight sedative effects at 5.0mg/kg.

1.3. The effect of ethyl-β-carboline-3-carboxylate (βCCE).

βCCE, (1.0-5.0 mg/kg) given i.p. 30 minutes prior to placing in the maze, produced a fall in the entry ratio to approximately 65-70% of control ratios in each case (see Fig. 1.2). The fall in ratio was significant for all doses. Total number of entries did not vary significantly from control values for each dose tested (see Table 1.1).

1.4. The effect of idazoxan.

Idazoxan (0.1 - 1.0 mg/kg) given i.p. 30 minutes prior to placing in the maze produced a dose-dependent fall in entry ratio to 55% of the control ratio which was significant at each dose used (Fig.1.3.). Total number of arm entries decreased with dose as did the entry ratio, but did not reach significance, the maximum fall being to 75% of control values (see Table 1.1).

Discussion.

As previously described by Mithani (1984), in the present model rats continually showed aversion to the open arm of the maze since only one third of entries, in untreated rats, were made into the open arms. The resultant entry ratio of 0.3 allows both increases and decreases to be seen enabling anxiogenic and anxiolytic activity to be studied.

As expected, diazepam produced an increase in the ratio of open to total arm entries over the whole of the dose-range tested. As discussed in the General Introduction an increase in the proportion of open arm exploration with no overall change in total arm entries is indicative of an anxiolytic compound. Diazepam and other benzodiazepine anxiolytics have already been demonstrated to behave in this way in this model of anxiety (*Handley and Mithani, 1984a; Pellow et al., 1985; Pellow, 1986*). It is important to note that the increase in ratio is obtained with no resultant significant change in the overall total activity. Only at the highest dose used (5.0 mg/kg) is there any indication of a fall in activity and this corresponds with the first signs of the sedative activity of diazepam which was observed in the home cage prior to exposure to the maze.

Ethyl-β-carboline-3-carboxylate (βCCE) is an inverse agonist at benzodiazepine receptors. Such compounds have been shown to be anxiogenic in man and animals (*Dorow et al., 1983; File and Pellow, 1984*) and have been demonstrated to show anxiogenic-like actions in the X-maze (*Pellow et al., 1987*). Under the present conditions βCCE

seems to behave in the same anxiogenic-like manner by reducing the proportion of open arms entered with a resultant fall in the ratio. Again over the dose-range tested βCCE produced no change in the total number of entries indicating no non-specific effects on motor behaviour or sedation.

Finally, the α_2-adrenoceptor antagonist idazoxan which has been shown to be anxiogenic in the X-maze (*Handley and Mithani, 1984a*) also showed an anxiogenic-like profile under present conditions producing a fall in the ratio with no resultant change in the total number of entries. This indicates that this model may be able to detect anxiolytic and anxiogenic effects in a range of compounds.

The initial criteria for a model of anxiety, i.e. that known anxiolytics and anxiogenics can be detected, have therefore been confirmed in the elevated X-maze as used under present conditions and with the current operator. This is an important point to establish since behavioural experiments by their very nature are prone to great inter-operator and inter-laboratory variation so although the model has already been validated with the above compounds it was necessary to re-examine them here.

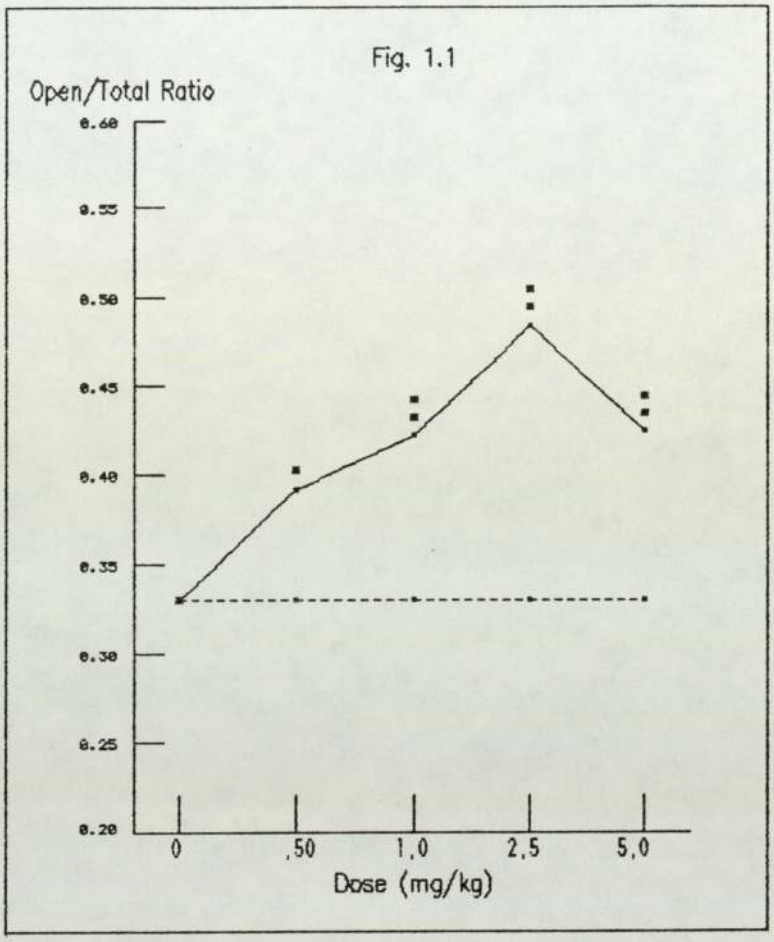


Fig.1.1. The effect of diazepam (0.5, 1.0, 2.5 & 5.0 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents the mean of concurrently run control values. Statistical comparisons were made on raw data (i.e. versus own control) using a t-test. * = $p<.05$ ** = $p<.01$.

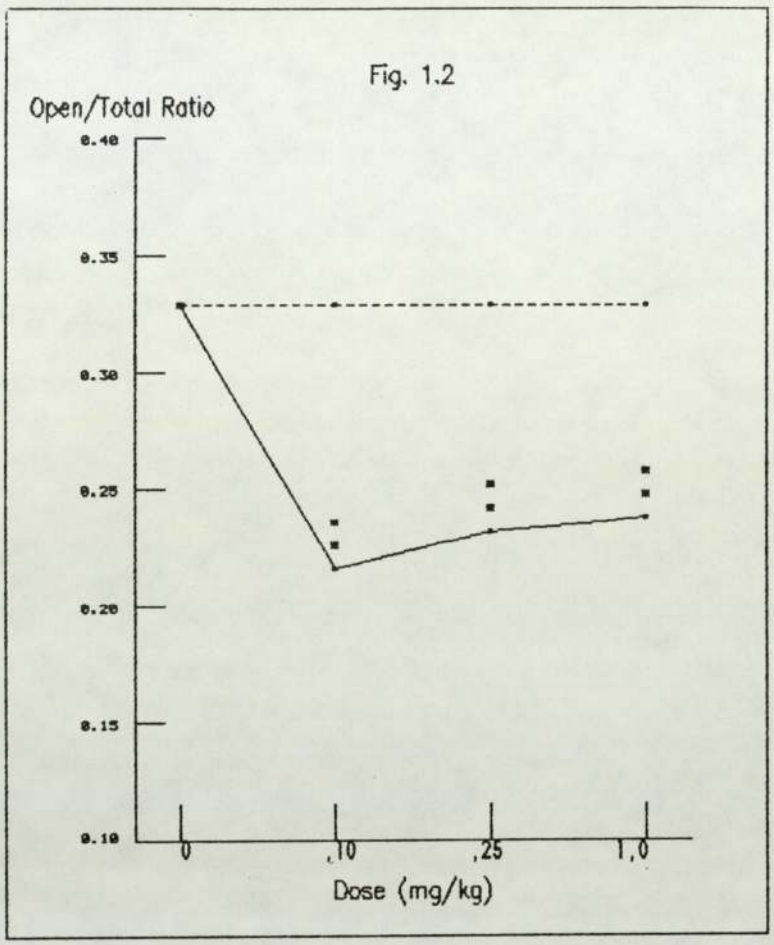


Fig.1.2. The effect of βCCE (1.0, 2.0 & 5.0 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents the mean of concurrently run control values. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

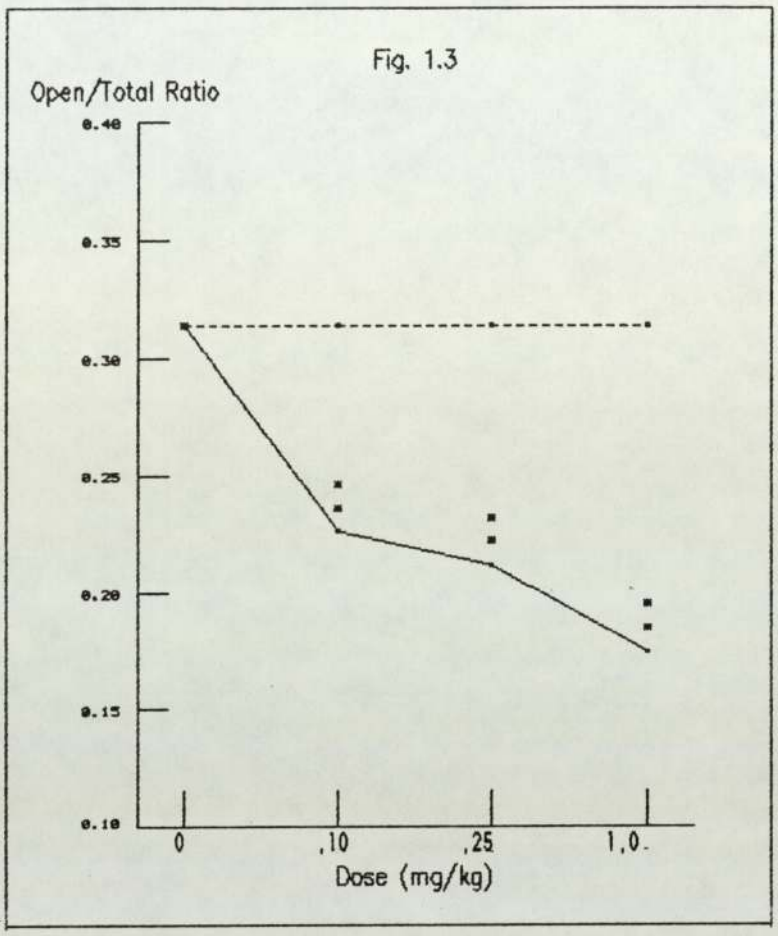


Fig.1.3. The effects of idazoxan (0.1, 0.25 & 1.0 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents the mean of concurrently run control values. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 1.1 Mean number of entries in 10 minutes
(Vehicle control values in brackets)

Drug/Dose mg/kg	Open	Total	Open/Total
Diazepam			
0.5	4.2±0.43	10.5±0.80	0.39±0.01*
	(4.2±0.52)	(11.0±1.70)	(0.33±0.01)
1.0	5.4±0.36	12.8±0.80	0.42±0.009**
	(4.3±0.55)	(12.5±0.80)	(0.33±0.02)
2.5	6.0±0.50	10.4±2.10	0.48±0.02**
	(4.8±0.76)	(14.2±1.80)	(0.33±0.03)
5.0	3.5±0.39	8.1±0.70	0.42±0.02**
	(4.2±0.52)	(11.0±1.70)	(0.33±0.01)
βCCE			
1.0	3.0±0.53*	13.2±0.95	0.22±0.03**
	(5.3±0.56)	(15.8±1.60)	(0.34±0.009)
2.0	3.3±0.30*	14.2±0.83	0.23±0.01**
	(5.3±0.56)	(15.8±1.60)	(0.34±0.009)
5.0	3.8±0.44	15.8±1.40	0.24±0.01**
	(4.8±0.33)	(14.8±0.82)	(0.32±0.004)
Idazoxan			
0.1	3.0±0.33	13.2±1.00	0.23±0.01**
	(4.2±0.15)	(13.6±0.73)	(0.31±0.02)
0.25	2.4±0.42**	10.4±1.50	0.21±0.02**
	(4.8±0.33)	(14.8±0.82)	(0.32±0.004)
1.0	2.0±0.33*	10.6±1.40	0.18±0.02**
	(4.2±0.15)	(13.6±0.73)	(0.31±0.02)

Table 1.1 The effect of diazepam, βCCE and idazoxan on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

CHAPTER 2

AGENTS PRODUCING AN ANXIOGENIC-LIKE PROFILE ON THE ELEVATED X-MAZE MODEL OF ANXIETY.

Introduction.

As outlined in the introduction there is considerable evidence suggesting a role for 5-HT in anxiety but until recently there has been a lack of selective ligands for the subtypes of central 5-HT receptors. This situation was rectified somewhat with the introduction of the simplified ergot congener 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). This compound produces biochemical and behavioural alterations consistent with central serotoninomimetic activity (*Hjorth et al., 1982*). Furthermore it shows marked selectivity for the 5-HT$_1$ receptor on the basis of ligand binding studies (*Middlemiss and Fozard, 1983*), and is regarded as a selective agonist for the 5-HT$_{1_a}$ subtype of the receptor (relative pIC_{50} values for the 5-HT1a,1b & 2 receptors are 8.5, 5.4 & <5.0 - *Tricklebank, 1985*). So far this compound has proved to be of enormous value as a pharmacological tool and has an ideal profile for use in the present investigation.

5-methoxy-3(tetrahydropyridin-4-yl)1H-indole (RU24969) is also a 5-HT agonist which shows selectivity for the 5-HT$_{1_b}$ subtype (*Sills et al., 1984*) and it too has proved to be of great value in the study of central 5-HT receptor function. (pIC_{50} values at 5-HT1a, 1b & 2 receptors are 7.9, 8.2 & 5.3 - *Tricklebank, 1985*).

5-Methoxy-N,N-dimethyltryptamine (5-MeODMT) and quipazine are relatively non-selective 5-HT agonists which do not appear to distinguish between the various receptor subtypes (pIC_{50} values at

5-HT1a, 1b & 2 receptors are 7.6, 7.0 & 5.9 and 5.8, 6.6 & 6.1 respectively - *Tricklebank, 1985*).

These compounds are therefore extremely useful for pharmacological investigation of the involvement of 5-HT systems in anxiety and behaviour and have been investigated here to determine their effect on the elevated X-maze model of anxiety.

Since these agonists exhibit various components of the '5-HT syndrome' (see *Tricklebank, 1985*), any such behavioural effects were also observed here.

Results.

2.1. The effect of the 5-HT_{1A} ligand 8-hydroxy-2-(di-n-propylamino) tetralin.

Fig. 2.1 shows how 8-OH-DPAT produces a large fall in the entry ratio over a wide dose range (0.015 - 1.0 mg/kg). This anxiogenic-like effect appears to be dose dependent up to 0.12mg/kg at which point the dose-response curve levels out indicating a maximum effect. This maximum corresponded to approximately 10% of the control ratio. The fall in the open/total ratio was significant at all doses tested. Very little change was seen in total number of entries with the exception of the two highest doses (0.5 & 1.0 mg/kg) when total entries were significantly reduced (see Table 2.1). 8-OH-DPAT was administered i.p. 10 minutes prior to the maze since preliminary experiments showed this to be the optimum pre-treatment time. 8-OH-DPAT (0.02mg/kg) was given 10, 20 and 30 minutes prior to the maze exposure. The anxiogenic-like effect was most pronounced after 10 minutes and was not present to any significant extent after 30 minutes. Signs of the '5-HT syndrome' appeared at doses of 0.12 mg/kg and above. The first and most prominent component was the flat body posture which was observed at all doses of 0.12 mg/kg and above. Other components of the 'syndrome', namely reciprocal fore-paw treading and hindlimb abduction did not appear until a dose of 1.0 mg/kg was attained and even then these effects were only slight and sporadic. Other behavioural changes seen could be described as 'fear-like' behaviours. At doses corresponding to the appearance of the 'syndrome' the animals showed increased defeacation, had freezing episodes when on

the maze, were hyperreactive to sound and movement and squealed when handled.

2.2. The effect of the 5-HT ligand 5-methoxy-3(tetrahydropyridin-4-yl)1H-indole (RU24969).

RU24969 administered i.p. 30 minutes prior to exposure to the maze produced a fall in the entry ratio characteristic of an anxiogenic-like profile over a dose range of 0.1 - 3.0 mg/kg (Fig. 2.2). The resultant dose-response curve was steep with a maximum effect being achieved at a dose of 1.0 mg/kg. This maximum corresponded to approximately 17% of the control ratio. The fall in ratio was significant at doses of 0.5 mg/kg and above. Total number of entries remained unchanged from controls until the two highest doses (1.0 & 3.0 mg/kg) were reached, at which point the number of entries increased. However, this increase was not significant when compared with controls. (see Table 2.1). Few other behavioural changes were observed with RU24969, the most notable being the distinct hyperactivity causing the increased entries mentioned at the highest dose used (3.0 mg/kg). The 'fear-like' behaviour observed with 8-OH-DPAT was present only at the highest dose and to a much less extent.

2.3.The effect of the 5-HT agonists 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and quipazine.

5-MeODMT given i.p. 15 minutes prior to maze exposure also produced a fall in the entry ratio characteristic of an anxiogenic-like response over a dose-range of 0.25 - 2.5 mg/kg (Fig.2.3). The open/total ratio fell rapidly to approximately 10% of the control

ratio being significant at all but the lowest dose (0.25 mg/kg). The only behavioural change observed was the onset of flat body posture at the highest dose (2.5 mg/kg). Total entries varied slightly around control values but at no dose was this significantly different from control values for total entries (See Table 2.2).

Quipazine given i.p. (0.5 - 2.0 mg/kg) 30 minutes prior to maze exposure also significantly decreased the proportion of open arm entries dose-dependently (Fig.2.4). Total entries again did not vary significantly from control values (Table 2.2). Observations in the home cage prior to maze exposure showed some hyperreactivity and at the highest dose (2.0 mg/kg) some indications of the 5-HT 'syndrome' were visible.

2.4.The effect of repeated dosage of 5MeODMT and RU24969.

The anxiogenic-like fall in entry ratio with the two agonists remained almost constant over 6 weekly exposures of 0.5 mg/kg with the 5MeODMT response increasing over the final two exposures. In corresponding vehicle control treated rats the entry ratio remained constant over the six exposures. (Fig.2.5, Table 2.3).

Discussion

Since 8-OH-DPAT was first described (*Arvidsson, et al., 1981; Hjorth et al., 1982*) it has been the focus of much attention in this field and has provided us with a great deal of information in terms of determining the functional relevance of the various subtypes of the 5-HT receptor. This agent is a selective agonist for the 5-HT_{1a} subtype, in terms of binding studies (*Middlemiss and Fozard, 1983; Tricklebank, 1985*) and induces a complex behavioural syndrome consisting of head-weaving, flat body posture, hyperlocomotion and reciprocal forepaw treading (*Arvidsson et al., 1981; Hjorth et al., 1982*). Such behaviours seem identical with the so-called 5-HT 'syndrome' which were thought to be activated by the 5-HT_{2} receptor subtype (*Green et al., 1983*) but which now, at least in part, seem to be mediated by the 5-HT_{1a} subtype (*Tricklebank et al., 1985*). 8-OH-DPAT therefore was selected as an important ligand in the present studies especially since it has already been connected with anxiety. Engel et al., (1984) reported that 8-OH-DPAT increased the number of shocks accepted in the punished drinking model of anxiety in rats, and thus 8-OH-DPAT acquired the label of 'putative' anxiolytic. This clearly differs from the results obtained here in the X-maze where 8-OH-DPAT was consistently anxiogenic. However, it is interesting to note that the anti-conflict effect seen by Engel was altered to an anxiogenic-like increase in suppression after 5-HT depletion with p-chlorophenylalanine (*Engel et al., 1984*). The release of punishment-suppressed responding was interpreted as being due to the effect of 8-OH-DPAT inhibiting 5-HT-neuronal firing via its activation of

5-HT_{1A} receptors on cell bodies (*Hutson et al., 1986*). As for the anxiogenic-like effect after pCPA this was suggested to be as a result of the agonist effects of 8-OH-DPAT on post-synaptic receptors made supersensitive by 5-HT depletion (*Engel et al., 1984*). Clearly our studies need to investigate the effects of pCPA on the 8-OH-DPAT response in the maze and this will be covered in another chapter. It must be pointed out however, that since the punished drinking model obviously involves measurement of the withholding of a consummatory response then it is open to the suggestion that any effects seen could possibly be attributed to changes in the capacity of the animals to withhold responding (*Soubrié, 1986*). However, in a conflict proceedure 8-OH-DPAT has also been shown to be anxiolytic given i.p. and anxiogenic given into the amygdala (*Hodges et al., 1987*).

The behaviours seen after injection of 8-OH-DPAT are indicative of typical fear-like behaviours in animals and as such are consistent with the anxiogenic-like profile of the compound. These behaviours were so marked at the high doses that the freezing behaviour observed was probably the causative factor in the significant fall in total entries. The animals appeared so fearful that their exploratory behaviour was interrupted by long periods of complete immobility. This did not appear to be caused by sedation since the animals attained a classic 'freezing' posture when even such behaviours as grooming and sniffing, which can still be present in sedation, were absent.

RU24969 has been proposed as a selective agonist at 5-HT_1 receptors (*Gardner and Guy, 1983*) which reduces 5-HT turnover in the CNS in vivo (*Euvrard and Boissier, 1980*). Studies indicate that it has some selectivity for the 5-HT_{1B} subtype (*Sills et al., 1984*) although an action on the other 5-HT_1 subtypes cannot be excluded. These features present RU24969 as a useful pharmacological tool for such investigations as the present, especially when used in comparison with other ligands with varying selectivities. It too induces various behavioural components, the most prominent of which is a distinct hyperlocomotion which may represent activation of a 5-HT_1 receptor (*Green et al., 1984*) which may or may not be identical with the mechanism by which 8-OH-DPAT induces a hyperlocomotor effect in conjunction with the other effects seen. Overall, however, RU24969 and 8-OH-DPAT produce different functional responses in mice, the former inducing locomotor activity (*Gardner and Guy, 1983*) and the latter inducing hypothermia (*Goodwin et al., 1985*), a factor which may be important in the light of their actions on experimentally-induced anxiety. In the present study, RU24969 produced an anxiogenic-like fall in the entry ratio of similar magnitude to 8-OH-DPAT. However, the dose required to produce this effect was some 10-fold greater than with 8-OH-DPAT. Since RU24969 has only a slightly higher binding affinity (pIC50) for 5-HT_{1B} over 5-HT_{1A} sites (*Tricklebank, 1985*) this would suggest, assuming no major differences in brain penetration, that the effect seen in the maze is at a dose which will bind to 5-HT_{1A} sites which therefore could be responsible for the 8-OH-DPAT-like anxiogenic effect. The hyperlocomotor effect of RU24969 was present at the doses tested but not to such an extent that it

invalidated the arm entry data, since the increase in total entries did not reach significance. Previous studies with RU24969 in anxiety using punished responding indicated little activity (*Shephard et al., 1982*) or a depression of punished licking (*Gardner, 1985*), although the latter response may have been subject to disruption by the hyperactivity produced. This is clearly an important advantage with the present model since such factors can be discounted. Thus using a similar model to the X-maze used here Pellow and co-workers have demonstrated similar anxiogenic-like activity with RU24969 in the same dose range (*see Pellow, 1986*).

5-Methoxy-N,N-dimethyltryptamine (5MeODMT) is regarded as a classical agonist of 5-HT receptors. Its binding profile shows minimal selectivity between the 5-HT, subtypes whilst it shows somewhat greater differentiation between 5-HT, and 5-HT_2 sites (*Tricklebank, 1985*), being more selective for 5-HT, sites. It too induces a behavioural syndrome identical to that seen with 8-OH-DPAT (*Grahame-Smith, 1971b*). Like 8-OH-DPAT and RU24969, 5MeODMT also produced an anxiogenic-like response of comparable magnitude without significantly altering overall activity. The dose to produce such effects was again higher than with 8-OH-DPAT but slightly less than with RU24969. Since 5MeODMT shows no appreciable selectivity for 5-HT, subtypes then it is not possible to attribute this effect to a particular subtype at present. In a conflict procedure, 5MeODMT showed no effect on punished responding but tended to reduce unpunished responding, but was however, able to reverse the anti-conflict effects of chronic pCPA (*Shephard et al., 1982*).

The anxiogenic-like activity with quipazine was less pronounced and this, along with the higher dose needed, is not incompatible with the contention that one particular subtype of the 5-HT receptor may be responsible for the anxiogenic effect since quipazine is the least selective of the agonists employed here (see *Tricklebank, 1985*). Quipazine has previously been demonstrated to have effects on anxiety models, being similarly anxiogenic in the X-maze (*Mithani, 1984*) and the plus-maze (*Pellow, 1986*). These two maze models are essentially identical with one or two exceptions; the walls of the enclosed arms of the plus-maze being significantly taller and the elevation from the floor being less than in the X-maze. In conflict models, however, quipazine depressed both punished and unpunished responding (*Commisaris and Rech, 1982*).

When taken together, these results obviously suggest a role for the activation of 5-HT receptors in the production of anxiety in this model since four different agonists produce similar results, only varying in the dose required to produce such effects. The relative potencies vary in such a manner to suggest that probably the 5-HT_1 receptor and possibly the 5-HT_{1_B} subtype is crucially involved. Whether pre- or post-synaptic receptor activation is involved cannot be postulated here since further experiments are required. This will therefore be discussed in the relevant section later in this study.

Finally, the repeated dosage study with RU24969 and 5MeODMT was undertaken to determine the feasibility of re-using experimental animals in the X-maze. Each rat received the same treatment on six

consecutive occasions a week apart to determine any habituation or tolerance to the model since the discussion of the concepts involved in the model in the General Introduction suggest that novelty is a crucial factor. Although 5MeODMT showed a potentiated effect after four exposures, RU24969 and more importantly vehicle controls did not vary significantly over the whole experiment. Since it was not expected that animals would receive the same treatment on multiple exposures in future experiments the vehicle control results suggested that it was possible to re-use rats in this model. Care must be taken, however, to allow adequate 'washout' times after each experiment and therefore rats were never re-exposed to the maze until at least one week after single administration of a drug. Careful notes of each animal's 'drug history' were therefore needed and where experiments were repeated a different and preferably näive group of animals were used.

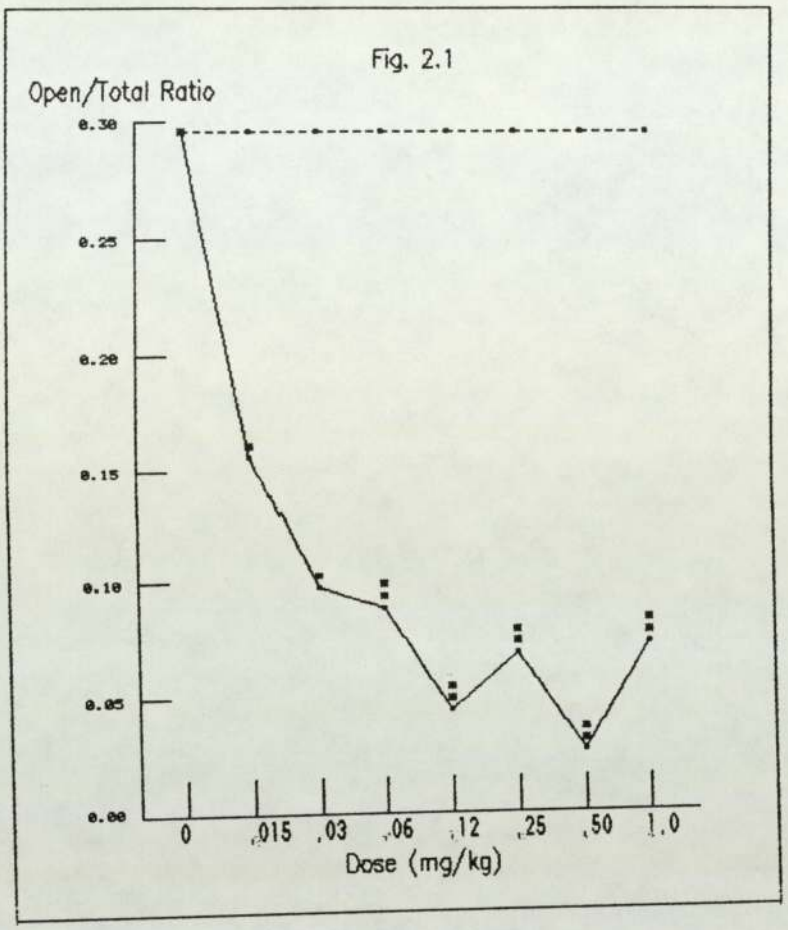


Fig.2.1 The effect of **8-OH-DPAT** (.015, .03, .06, .12, .25, .50 & 1.0 mg/kg) given i.p. (10 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The baseline represents concurrently run control values. Statistical comparisons were made using a t-test. * = $p<.5$ ** = $p<.01$.

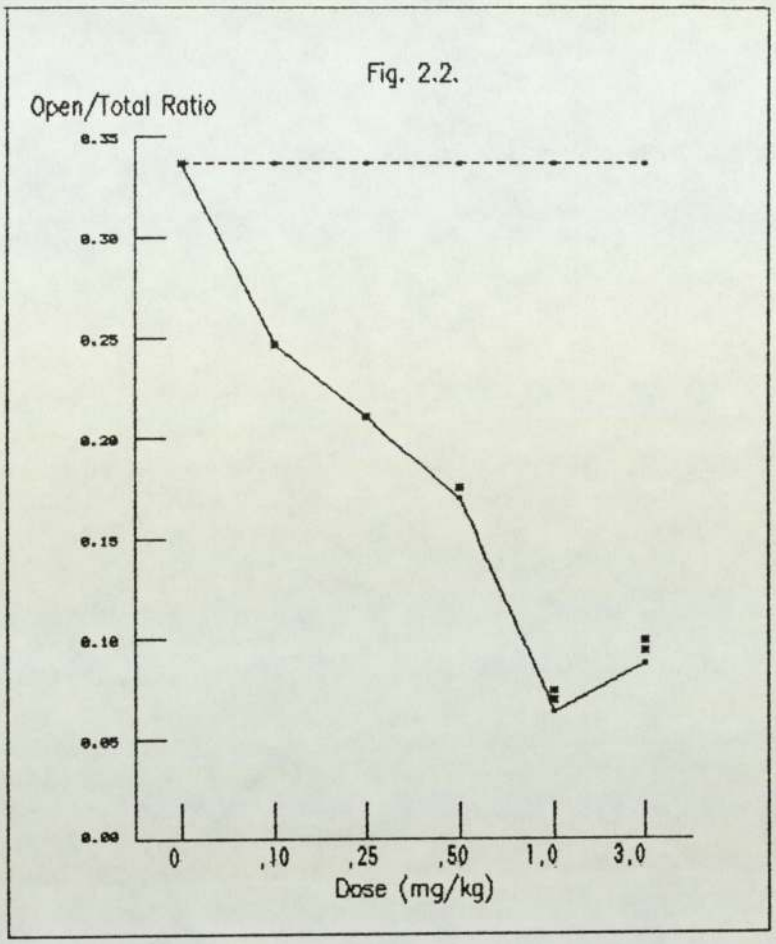


Fig.2.2. The effect of **RU24969** (.10, .25, .50, 1.0 & 3.0 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents concurrently run control values. Statistical comparisons were made using a t-test. * = $p<.05$ ** = $p<.01$.

Table 2.1 Mean number of entries in 10 minutes.
(Vehicle control values in brackets)

Drug/Dose mg/kg	Open	Total	Open/Total
8-OH-DPAT			
0.015	1.4±.35	8.6±.72	0.16±.03*
	(2.0±.28)	(6.8±.60)	(0.29±.02)
0.03	1.0±.50	8.2±1.8	0.097±.03**
	(2.0±.28)	(6.8±.60)	(.29±.02)
0.06	1.0±.29**	10.6±.80	0.088±.02**
	(3.3±.56)	(10.2±1.6)	(0.33±.03)
0.12	0.4±.22**	8.0±1.3	0.044±.02**
	(3.3±.56)	(10.2±1.6)	(0.33±.03)
0.25	0.5±.20**	5.3±.87	0.068±.03**
	(2.2±.16)	(7.6±.44)	(0.29±.01)
0.50	0.2±.18**	4.0±1.0*	0.026±.02**
	(2.2±.16)	(7.6±.44)	(0.29±.01)
1.0	0.4±.45**	4.0±.63**	0.072±.04**
	(3.0±.49)	(11.2±.87)	(0.27±.04)
RU24969			
0.1	4.0±1.3	13.6±2.3	0.25±.06
	(3.4±1.5)	(10.2±3.1)	(.26±.06)
0.25	3.2±.77	14.2±1.9	0.21±.03
	(4.0±.00)	(12.0±.28)	(0.33±.007)
0.5	2.7±.83*	14.8±1.8	0.17±.04*
	(5.2±.44)	(13.8±1.7)	(0.39±.04)
1.0	1.3±.30**	18.3±2.5	0.063±.01**
	(5.3±.61)	(14.5±.99)	(0.36±.02)
3.0	2.0±.75**	22.5±1.3	0.087±.03**
	(6.3±.61)	(18.6±1.6)	(0.34±.02)

Table 2.1. The effect of 8-OH-DPAT and RU24969 on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

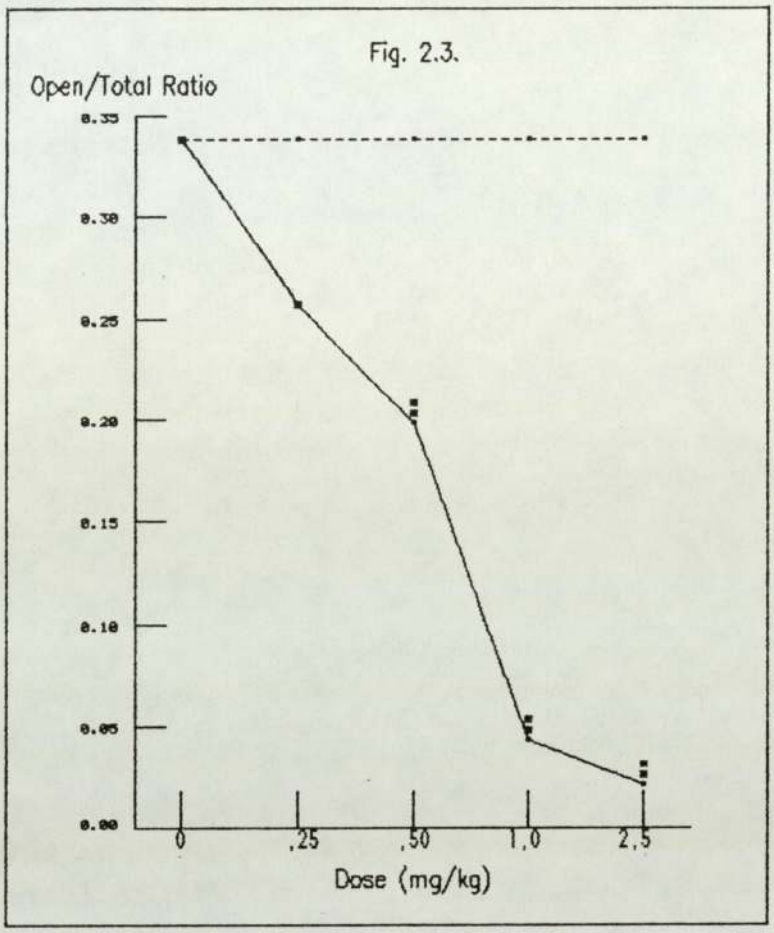


Fig.2.3. The effect of **5MeODMT** (.25, .50, 1.0 & 2.5 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents concurrently run control values. Statistical comparisons were made using a t-test. * = $p<.05$ ** = $p<.01$.

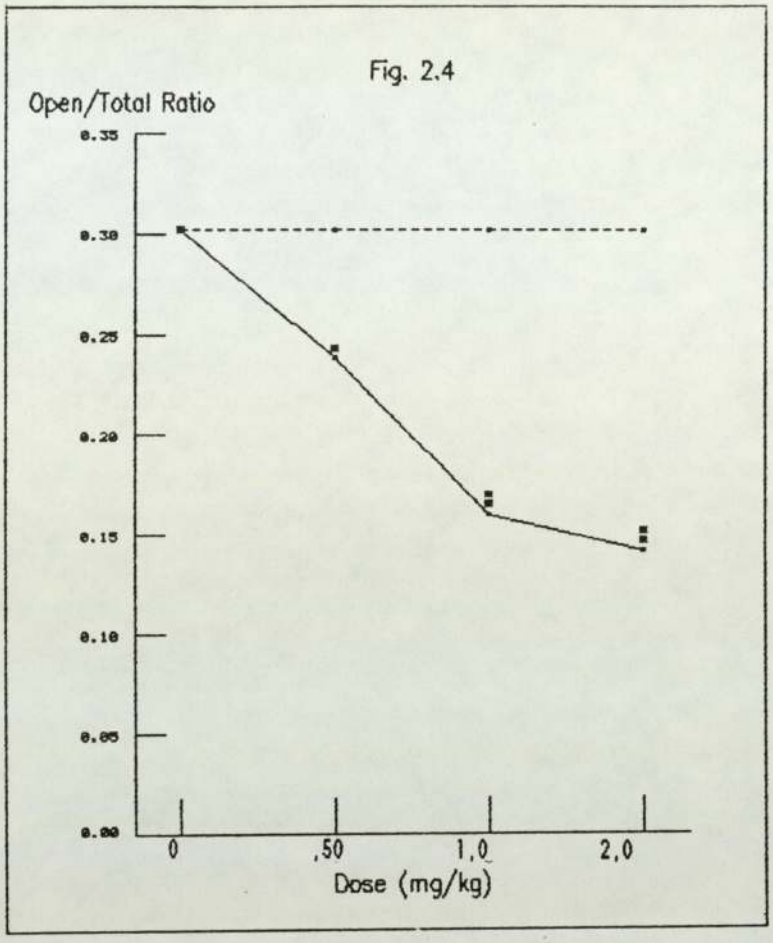


Fig.2.4. The effect of **quipazine** (.50, 1.0 & 2.0 mg/kg) on the open/total entry ratio of the elevated X-maze. The baseline represents concurrently run control values. Statistical comparisons were made using a t-test. * = $p<.05$ ** = $p<.01$.

Table 2.2. **Mean number of entries in 10 minutes.**
(Vehicle control values in brackets)

Drug/Dose mg/kg	Open	Total	Open/Total
5MeODMT			
0.25	2.3±.65*	9.3±1.7	0.26±.09
	(5.2±.44)	(13.8±1.7)	(0.39±.04)
0.5	4.0±.88	18.5±2.5	0.19±.03**
	(5.3±.61)	(14.5±.99)	(0.36±.02)
1.0	0.66±.45**	11.5±1.9	0.043±.03**
	(6.3±.61)	(18.6±1.6)	(0.34±.02)
2.5	0.16±.15	7.2±1.6	0.021±.02**
	(3.4±1.5)	(10.2±3.1)	(0.26±.06)
Quipazine			
0.5	2.2±.18*	9.2±.52	0.24±.01*
	(4.4±.73)	(14.2±1.8)	(0.30±.03)
1.0	2.3±.38	14.0±1.4	0.16±.02**
	(3.5±.39)	(13.8±1.1)	(0.25±.01)
2.0	1.8±.18*	13.2±1.8	0.14±.02**
	(4.4±.18)	(14.2±1.8)	(0.30±.03)

Table 2.2. The effect of 5MeODMT and quipazine on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

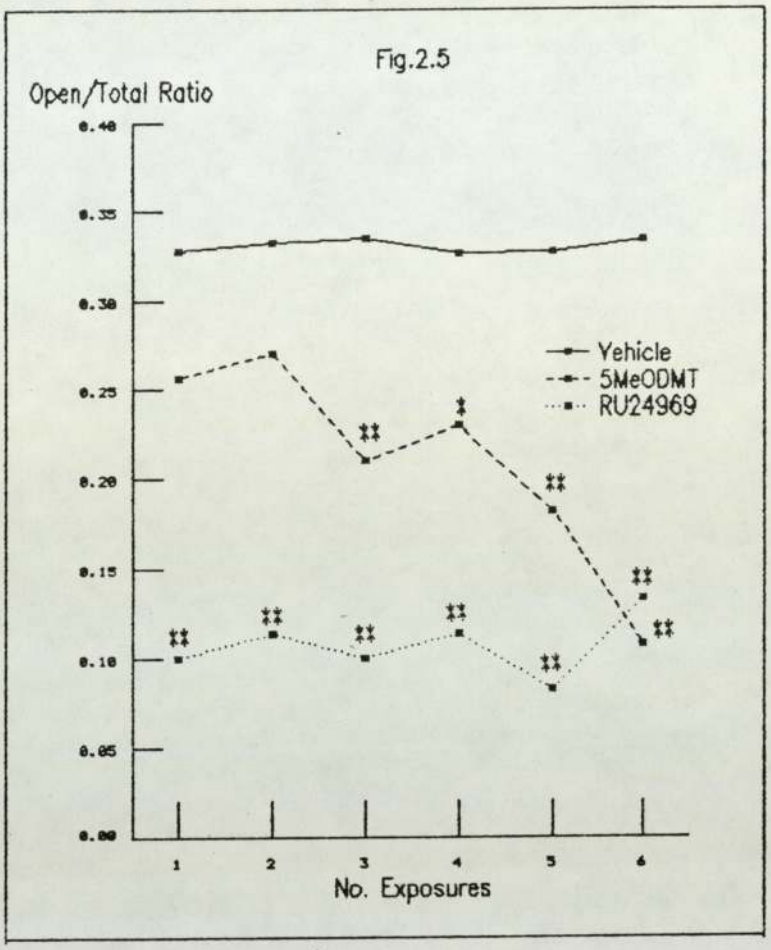


Fig.2.5. The effect of 6 consecutive weekly exposures to the X-maze on the open/total entry ratio to vehicle controls, RU24969 (.50 mg/kg) and 5MeODMT (.50 mg/kg). Statistical comparisons were made using a t-test. * = $p<.05$ ** = $p<.01$.

Table 2.3. **Mean number of entries in 10 minutes.**

Treatment & Exposures.	Open	Total	Open/Total
Vehicle			
1st	4.8±.91	14.2±2.3	0.33±.02
2nd	4.0±.28	12.2±.82	0.33±.02
3rd	3.8±.89	10.8±1.7	0.34±.04
4th	4.0±1.1	13.2±2.5	0.33±.02
5th	4.2±.52	12.6±1.0	0.33±.02
6th	4.8±1.5	14.0±4.1	0.33±.02
5MeODMT (.5mg/kg)			
1st	3.8±1.2	13.4±3.2	0.26±.03
2nd	3.3±.54	11.8±.82	0.27±.03
3rd	2.5±.56	11.5±1.7	0.21±.03
4th	1.6±.36	6.8±1.4	0.23±.01
5th	1.8±.44	9.0±1.2	0.18±.04
6th	0.8±.18	6.8±.76	0.11±.03
RU24969 (.5mg/kg)			
1st	2.0±.35	20.8±1.2	0.10±.02
2nd	1.4±.22	12.0±1.2	0.11±.008
3rd	1.5±.43	12.5±3.0	0.10±.03
4th	1.8±.18	16.4±1.1	0.11±.009
5th	1.0±.28	11.6±1.4	0.082±.02
6th	1.8±.77	11.6±1.2	0.13±.05

Table 2.3. The effect of 6 weekly exposures of vehicle 5MeODMT and RU24969 on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

CHAPTER 3

PUTATIVE ANXIOLYTIC AGENTS IN THE ELEVATED X-MAZE MODEL OF ANXIETY.

Introduction

As mentioned in the introduction to this thesis, antagonists at 5-HT receptors appear to have a role in anxiety, although the results so far are far from conclusive. Most of the studies thus far have used the various 'classical' 5-HT receptor antagonists which show little selectivity for 5-HT receptor subtypes, and often have measurable affinity for other receptor systems. Two of these compounds, methysergide and cyproheptadine, were examined here. However, more recent work has resulted in the development of several antagonists that act at subtypes of the 5-HT receptor, principally the 5-HT_2 receptor. Ketanserin was the prototype of a series of such compounds (*Leysen et al., 1981*) followed by the more potent and selective analogue ritanserin. Ritanserin is claimed to be a 5-HT_2-receptor antagonist with no partial agonist activity (*Niemegeers et al., 1984; Colpaert et al., 1985*). A series of analogues followed including altanserin, setoperone and R56413 (seganserin) (see *Janssen, 1985* for review). All are claimed to antagonise events known to be mediated by the 5-HT_2 receptor e.g. serotonin-induced vasoconstriction, bronchoconstriction and platelet aggregation. With the exception of ketanserin, which has some peripheral actions, the compounds are potent antagonists of LSD and other centrally acting serotonomimetics (*Janssen, 1985*). Taking these factors into account and considering the results gained from the use of less selective antagonists in animal models of anxiety, these selective antagonists were chosen for use in our elevated X-maze model with the hope of explaining some of the earlier controversy with 5-HT antagonists. Ketanserin was chosen as the prototype compound together with the highly potent and selective

ritanserin. Also a newer addition to the field R56413, or seganserin, was examined.

Recently there has been much work carried out on two *new* putative anxiolytics ipsapirone (TVXQ7821) and buspirone. Ipsapirone was first described by Schuurman and co-workers (1984) as being active in several animal models of anxiety and was subsequently found to bind to 5-HT receptors (*Traber et al., 1984; Glaser and Traber, 1985*). Buspirone was demonstrated to have anxiolytic properties in man as long ago as 1979 (*Goldberg, 1979; Goldberg and Finnerty, 1979*) but its mechanism of action remained unclear until it too was demonstrated to bind to 5-HT receptors (*Glaser and Traber, 1983; Skolnick et al., 1984*). Since both ipsapirone and buspirone are non-benzodiazepine-like, putative anxiolytics, and have links with the serotonergic system they are proving to be an essential part of any investigation such as the present.

Pindolol and alprenolol, although principally known as β-adrenoceptor antagonists have considerable actions on 5-HT receptors (*Middlemiss et al., 1977; Nahorski and Wilcocks, 1983*) and have gained much use as antagonists of 5-HT$_1$ receptors in the absence of any selective antagonists for this receptor subtype. Studies have shown that of the β-antagonists which bind to 5-HT receptors, greater stereoselectivity for 5-HT$_1$ receptors is achieved with the racemic form and particularly the (-)-isomer (*Nahorski and Wilcocks, 1983*). Whilst studies have to be carefully controlled in view of their adrenergic activity, these compounds obviously have a role to play in such

studies as the present, especially in the light of the results in chapter 1 of this thesis.

Additionally as discussed in the Introduction, the 5-HT depletor p-chlorophenylalanine (pCPA) produces an ant-conflict action in operant conditioning models of anxiety and it was deemed important to be able to demonstrate such an anxiolytic action in the X-maze.

Finally, recent demonstration of selective 5-HT_3 antagonists and possible anxiolytic activity thereof (*Jones et al., 1987*) has proved it essential to include these agents in this study. However, since these developments are very recent, only a preliminary examination of the 5-HT_3 antagonists currently available has been possible.

Results

3.1. The effects of the 5-HT_2 receptor antagonists ketanserin, ritanserin and seganserin.

Fig. 3.1 shows how ketanserin injected i.p. 30 minutes prior to the maze exposure produced a small, but significant, anxiolytic-like rise in entry ratio at doses of 0.1 and 0.5 mg/kg; an effect which was no longer present at a dose of 1.0 mg/kg. Over the same dose range total number of arm entries showed a slight downward trend which was not significant (Table 3.1). No sedation or behavioural changes were observed at any time during the studies with ketanserin.

Fig.3.2 shows how ritanserin given i.p. 30 minutes prior to the maze produced an anxiolytic-like increase in the open/total ratio in a dose dependent manner over a dose range of 0.025 - 5.0 mg/kg, being significant at all doses. The maximum increase in ratio corresponded to approximately 160% of the control ratio. Over the same dose range the total number of entries did not vary from controls with the exception of the highest dose (5.0 mg/kg) which produced an increase to approximately 170% of control values (Table 3.1). Throughout the studies with ritanserin no sedation or behavioural changes were observed.

Fig.3.3 shows the effect of seganserin (R56413) on the open/total entry ratio given i.p. 30 minutes prior to the maze. A dose of 0.1 mg/kg produced a non-significant fall in entry ratio to approximately 85% of control values. At a dose of 0.5 mg/kg the ratio increased

significantly to 133% of control ratio. Finally at a dose of 1.0 mg/kg there was no change in the ratio compared with controls. Table 3.1 shows that total number of entries remained similar to control values for each dose used. Table 3.1 contains the full data for the 5-HT_2 antagonists with their corresponding control values.

3.2. The effect of the 5-HT antagonists methysergide and cyproheptadine.

Methysergide produced a significant anxiolytic-like rise in the entry ratio at 2.5 mg/kg but was without effect at the other doses examined (1.0, 5.0 & 10.0 mg/kg) - see Fig.3.4. However, at the anxiolytic dose (2.5 mg/kg) total number of entries was also siginficantly increased, whilst at the other doses no change in total entries was observed (Table 3.2).

Cyproheptadine 1.0 - 10.0 mg/kg failed to produce any significant change in either the open/total ratio (Fig.3.5) or the total number of arm entries (Table 3.2).

3.3 The effect of the 5-HT ligands ipsapirone (TVXQ7821) and buspirone.

Ipsapirone given i.p. 30 minutes prior to the maze produced an anxiolytic-like increase in the open/total ratio which was significant at all doses (Fig.3.6). From 0.25 - 2.5 mg/kg the ratio increased dose-dependently to approximately 135% of control values. At the highest dose of 5.0 mg/kg the increase in the ratio approximated to 125% of control values. Table 3.3 shows how total number of entries

increased steadily over the dose range and became significantly elevated from controls at the highest dose (5.0mg/kg), being approximately 145% of control totals. The only behavioural change observed with ipsapirone was hyperactivity at the highest doses used which was responsible for the significant increase in total entries.

Buspirone 0.025 - 0.10 mg/kg produced open/total ratios and total entries similar to control values and at higher doses 0.25 - 5.0 mg/kg produced a general depression of both open/total entries and total entries (Fig.3.7 and Table 3.3). At the highest dose (5.0 mg/kg) this resulted in an open/total ratio of zero and total entries were depressed to 35% of control values. The only behavioural change observed was that of increasingly severe sedation with doses above 0.25 mg/kg.

3.4. The effect of the β-adrenergic and 5-HT$_1$ receptor antagonists pindolol and alprenolol.

(±)pindolol given i.p. 30 minutes prior to the maze produced a complex dose response curve involving an anxiolytic-like increase in entry ratio at doses below 0.5 mg/kg and an anxiogenic-like fall in the ratio at 1.0 mg/kg, returning to control-like ratios at 2.0 mg/kg (fig.3.8). From 0.05 - 0.25 mg/kg the anxiolytic-like response increased dose dependently to approximately 130% of control values. The anxiogenic-like response at 1.0 mg/kg produced ratios approximating to 60% of control values. Several repeats of this dose-response curve produced an identical profile, but the doses at which

each effect occurred varied slightly. Total number of entries as shown in Table 3.4 did not vary significantly from controls throughout the whole dose range and no sedation or behavioural changes were observed.

(-)alprenolol produced a small but significant increase in the open/total ratio at 0.1 mg/kg but had no effect at the other doses examined (0.25 - 1.0 mg/kg) (Fig.3.9). Total entries remained unchanged throughout the dose-range tested (Table 3.4) and no behavioural changes were observed.

3.5. The effect of the 5-HT depletor p-chlorophenylalanine.

pCPA given for 3 days prior to maze exposure at 300mg/kg and an injection volume of 3ml/kg, produced a small but significant increase in entry ratio to approximately 125% of similarly treated vehicle controls. Total entries were no different from the corresponding controls. See Table 3.6.

3.6. The effects of the 5-HT_3 antagonists GR38032F, MDL72222, ICS205-930 and BRL24924.

GR38032F (0.001 - 0.10 mg/kg) failed to produce any effect on the open/total ratio (Fig.3.10) and decreased the total number of entries at 0.001 mg/kg to 75% of control levels (see Table 3.5).

MDL72222 (0.01 - 1.0 mg/kg), ICS205-930 (0.001 - 0.1 mg/kg) and BRL24924 (0.1 - 1.0 mg/kg) all failed to produce any change in the open/total ratio (Fig.3.10) or the total number of arm entries (see Table 3.5 & 3.6).

Discussion.

The results with ritanserin show a convincing anxiolytic-like effect over a wide dose range since the entry ratio is increased with no effect on totals, except at high dose. This effect is in agreement with other studies with ritanserin on anxiety. In a light/dark box type model ritanserin showed an anxiolytic profile remarkably similar to that seen here (*Colpaert et al., 1985*). However, the same workers found little activity with the compound in a licking conflict model and another study (*Gardner, 1986*) indicated no anxiolytic activity in either a food-motivated conflict paradigm or in social interaction. The results with ketanserin and especially seganserin were, however, less convincing. Ketanserin did show an anxiolytic effect, although less marked than ritanserin. In a previous study using the X-maze (*Mithani, 1984*) ketanserin was only anxiolytic at one single low dose. Seganserin only raised the open/total ratio at one of the tested doses, and decreased it at one other dose. This also agrees with the results gained in the light/dark box model (*Colpaert et al., 1985*). A possible explanation of this is the fact that seganserin appears to behave as only a partial antagonist in the LSD-discrimination test (*Colpaert et al., 1985*), and as such resembles methysergide, cyproheptadine and several other 5-HT antagonists (*Colpaert et al., 1982*). Of these antagonists used here only ritanserin has been examined clinically and has been suggested to have potential anxiolytic properties which may be qualitatively different from those of the benzodiazepines (*Arriaga et al., 1984; Ceulemans et al., 1984*).

Ketanserin, whilst having a high affinity for 5-HT_2 receptors is now known to possess α_1-adrenergic blocking activity (*Fozard, 1983*), a property which is probably responsible for its hypotensive effect (*Kalkman et al., 1983*). Thus the possibility that the anxiolytic-like effect of ketanserin may be at least partly due to its α_1-adrenoceptor blocking activity cannot be excluded. Bearing this in mind, close scrutiny of the binding profile of ritanserin reveals that it too binds to α_1-adrenoceptors. Although ritanserin is clearly more potent at 5-HT_2 receptors than ketanserin (pIC_{50} 9.5 vs 8.8), the respective values at α_1-receptors are 7.0 and 7.5 (see *Tricklebank, 1985* for binding data) indicating far from negligible binding activity. When this is considered with the fact that the α_1-adrenoceptor antagonist prazosin shows some anxiolytic-like activity in the X-maze (*Handley and Mithani, 1984a*) it seems likely that the adrenergic receptors may have some involvement in the the production of the anxiolytic-like effects with these antagonists in this model. Furthermore, Colpaert et al., (1985) reported that the vast differences seen between ritanserin and seganserin in the anxiety tests were no longer apparent when examined against 5-HT-induced hypothermia and 5-HT-induced head-twitch. This suggests that the putative anxiolytic effects of ritanserin cannot be simply ascribed to a pharmacologically defined action at 5-HT receptors. Further work is needed to clarify the role of 5-HT_2 receptors in anxiety, especially when considering the implications for 5-HT_1 receptors suggested in chapter 1.

Results with the two antagonists methysergide and cyproheptadine were consistent with several other studies indicating either no effects or small non-repeatable effects. Methysergide has previously been shown to increase punished responding (*Cook and Sepinwall, 1975b*) and to be inactive in the same procedure (*Kilts et al.,1982*). Cyproheptadine has a similar profile of inconsistent effects. The results here add the X-maze to the list of anxiety models in which these compounds show little activity. The fact that cyproheptadine binds to the GABA/chloride ionophore (*Squires et al., 1983*) is one possible reason why it shows some activity in the anti-conflict models, all of which seem to respond well to benzodiazepine-like compounds.

Ipsapirone displayed an anxiolytic profile in the elevated X-maze over a dose range and thus agrees with other studies with this compound using various other models (*Glaser et al., 1984; Traber et al., 1984; Schuurman et al., 1984*) including footshock induced aggression in mice, passive avoidance, punished drinking and social interaction. Further studies have shown that this ligand binds to 5-HT_1 receptors (*Dompert et al., 1985*) and furthermore to the 5-HT_{1A} subtype (*Peroutka, 1985; Spencer and Traber, 1987*). There is some controversy as to whether ipsapirone (and buspirone) are actually agonists or antagonists at 5-HT receptors. Both ligands behave in a similar manner to 8-OH-DPAT in that they result in a stimulation of corticosterone secretion and decrease body temperature (*Koenig et al., 1986*). Both ligands will substitute for 8-OH-DPAT in drug discrimination studies (*Cunningham et al., 1985; 1987*) and both will

increase food intake, like 8-OH-DPAT (*Dourish et al., 1986c*). Thus there is ample evidence that these agents act as agonists at 5-HT receptors. However ipsapirone has been reported to dose-dependently antagonise 8-OH-DPAT-induced hypothermia in rats and mice (*Goodwin et al., 1986*) and to accelerate mouse brain 5-HT synthesis (*ibid*). The former action indicates a presynaptic 5-HT_{1_A} antagonist action of ipsapirone as does the latter since 8-OH-DPAT decreases the rate of brain 5-HT synthesis in mice (*Goodwin and Green, 1985*) and in rats (*Hjorth et al., 1982*). However, the latter also reported evidence of induction of some 5-HT 'syndrome' effects with ipsapirone suggesting 5-HT agonistic actions. Also Goodwin et al., (1986) reported that ipsapirone had no effect on the 8-OH-DPAT-induced 'syndrome' (possibly a post-synaptically-mediated effect). Overall the effects on drug discrimination, corticosterone levels, hypothermia (possibly) and previous anxiety studies, suggest that buspirone and ipsapirone, along with 8-OH-DPAT are agonists at 5-HT_{1_A} receptors (probably somato-dendritic autoreceptors). The work on 5-HT synthesis rate, Goodwin and co-workers hypothermia results, and the 'syndrome' effects suggest that ipsapirone is a presynaptic 5-HT_{1_A} antagonist. Thus far the work in this thesis indicates a possible antagonistic action of ipsapirone although as yet one cannot speculate as to the neuronal location of such an action. The suggestion that ipsapirone and buspirone have partial agonist activity (*Clague and Spedding, 1987*) may of course be a possible explanation of the anomalies seen.

The results with buspirone unfortunately do not allow a great deal to be concluded since it only produced a general depression of

exploratory activity in the X-maze. The lack of effect on the X-maze is consistent with other studies indicating a lack of efficacy for buspirone in animal models (*Goldberg et al., 1983; File, 1985b; Gardner, 1986*), although these studies do not neccessarily implicate a general depression of activity as the causative factor. Buspirone does, however, indicate anxiolytic activity inconsistently in punished responding models and appears to be anxiolytic in the social interaction model (*Gardner and Guy, 1985*).

Pindolol, although producing a complex but repeatable curve, indicates overall anxiolytic activity which is consistent with the role of ipsapirone as an antagonist with anxiolytic effects and 8-OH-DPAT as an agonist with anxiogenic effects. Alprenolol, however, did not produce such convincing results, being anxiolytic at only one dose. This exemplifiess the need for more 5-HT$_1$ antagonists to confirm such results. The biphasic effect with pindolol could possibly be due to the fact that the racemic form was used or to the fact that it has been suggested to be a mixed agonist/antagonist at central 5-HT rceptors (*Hjorth and Carlsson, 1986*). Further controlled studies are necessary to discount any β-adrenergic involvement in the actions of pindolol in this test, a matter which will be addressed later in this thesis.

Finally the evolution of the 5-HT$_3$ antagonists recently has aroused a great deal of interest (see *Bradley et al., 1986; and Richardson and Engel, 1986* for review). GR38032F is a novel 5-HT$_3$ selective antagonist (*Brittain et al., 1987*) which amongst other possible

actions has been shown to have some anxiolytic activity in the mouse light/dark box and Social Interaction test (*Costall et al., 1987; Jones et al., 1987*). Using doses which were reported active in social interaction, the X-maze indicated no anxiolytic activity for this compound. Similarly the other available 5-HT_3 antagonists MDL72222 (*Fozard, 1984*), ICS205-930 (*Richardson et al., 1985*), which have also recently been reported anxiolytic (*Tyers et al., 1987*) and BRL24924 (a less potent analogue of the selective 5-HT_3 receptor antagonist BRL43694, *Fake et al., 1987*) indicated no anxiolytic activity in the X-maze. However these results are preliminary and further examination of these compounds is necessary in the future over a wider dose range and in different models to confirm whether or not 5-HT_3 receptors have a role to play in anxiety.

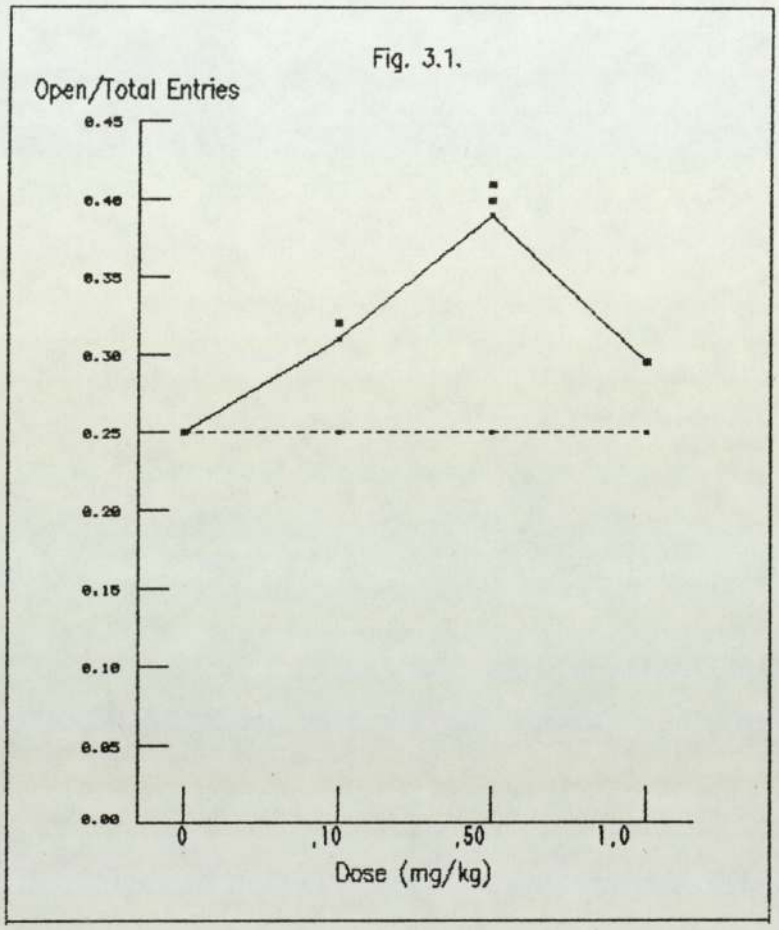


Fig.3.1. The effect of **ketanserin** (.10, .50 & 1.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

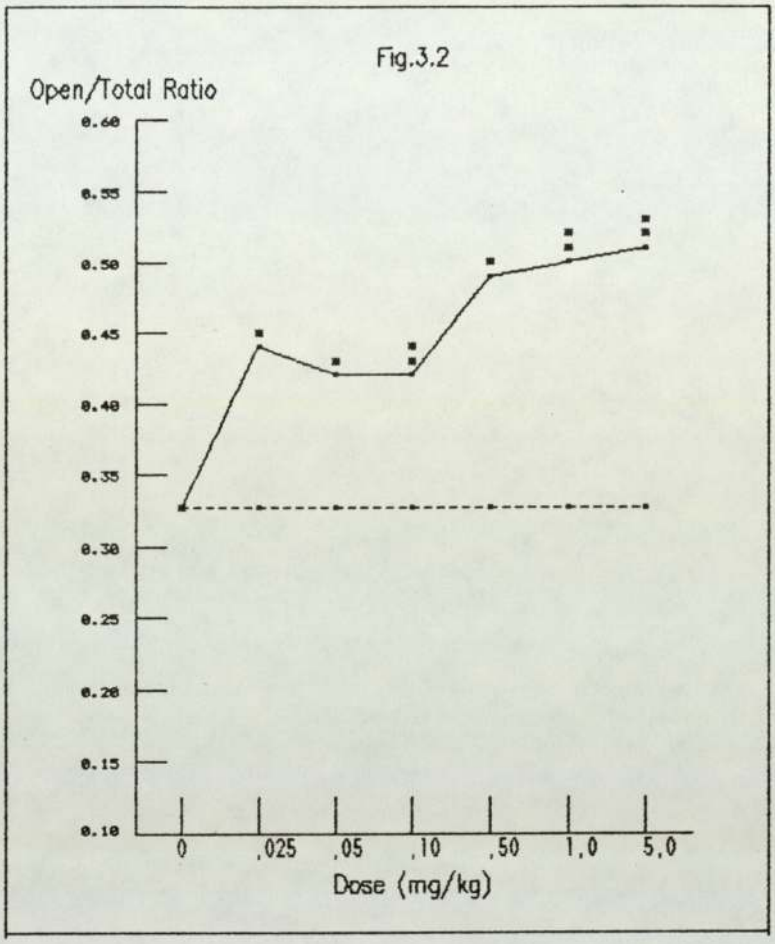


Fig.3.2. The effect of **ritanserin** (.025, .05, .10, .50, 1.0 & 5.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 3.1

Mean number of entries in 10 minutes (±sem).
(Vehicle control values in brackets).

Drug/Dose mg/kg	Open	Total	Open/Total
Ketanserin			
0.1	5.0±.78	15.6±1.7	0.31±.01*
	(3.5±.39)	(13.8±1.1)	(0.25±.01)
0.5	3.4±.36	8.8±1.0	0.39±.02**
	(2.0±.56)	(7.4±1.5)	(0.25±.03)
1.0	1.8±.41	5.8±1.0	0.29±.04
	(2.0±.56)	(7.4±1.5)	(0.25±.03)
Ritanserin			
0.025	6.4±.67	14.4±1.4	0.44±.01*
	(5.4±.96)	(15.0±2.4)	(0.35±.02)
0.05	5.2±.64	12.2±1.3	0.42±.03*
	(3.7±1.1)	(11.2±2.7)	(0.29±.03)
0.1	6.8±1.4*	15.8±2.8	0.42±.02**
	(3.7±1.1)	(11.2±2.7)	(0.29±.03)
0.5	8.3±1.2	16.5±1.8	0.49±.02*
	(5.2±1.2)	(15.4±2.2)	(0.31±.05)
1.0	5.8±.76	11.7±1.5	0.50±.02**
	(5.2±1.2)	(15.4±2.2)	(0.31±.05)
5.0	10.5±1.1**	20.7±2.2*	0.51±.02**
	(4.3±.51)	(12.2±1.4)	(0.36±.02)

Table 3.1. The effect of ketanserin and ritanserin on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

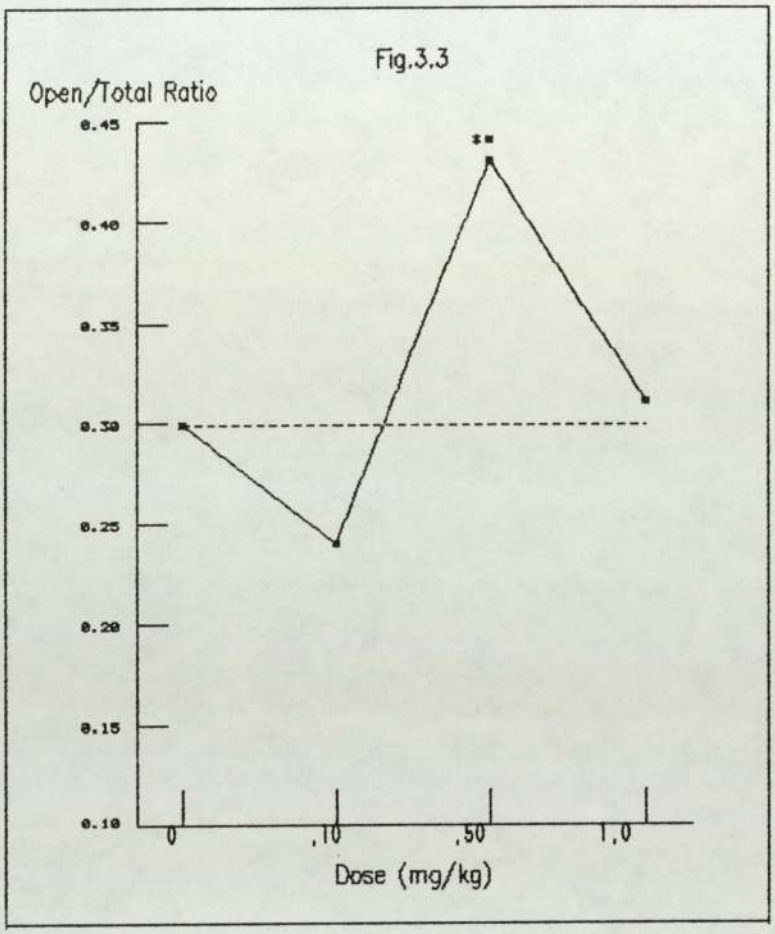


Fig.3.3. The effect of **seganserin** (R56413) (.10, .50 & 1.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

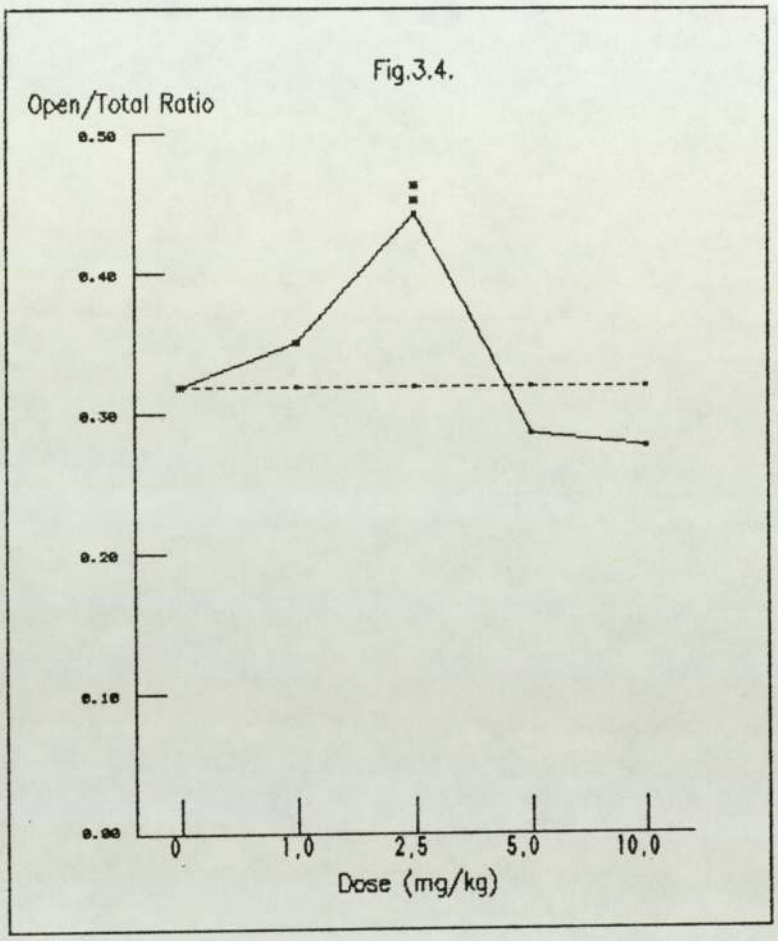


Fig.3.4. The effect of **methysergide** (1.0, 2.5, 5.0 & 10.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

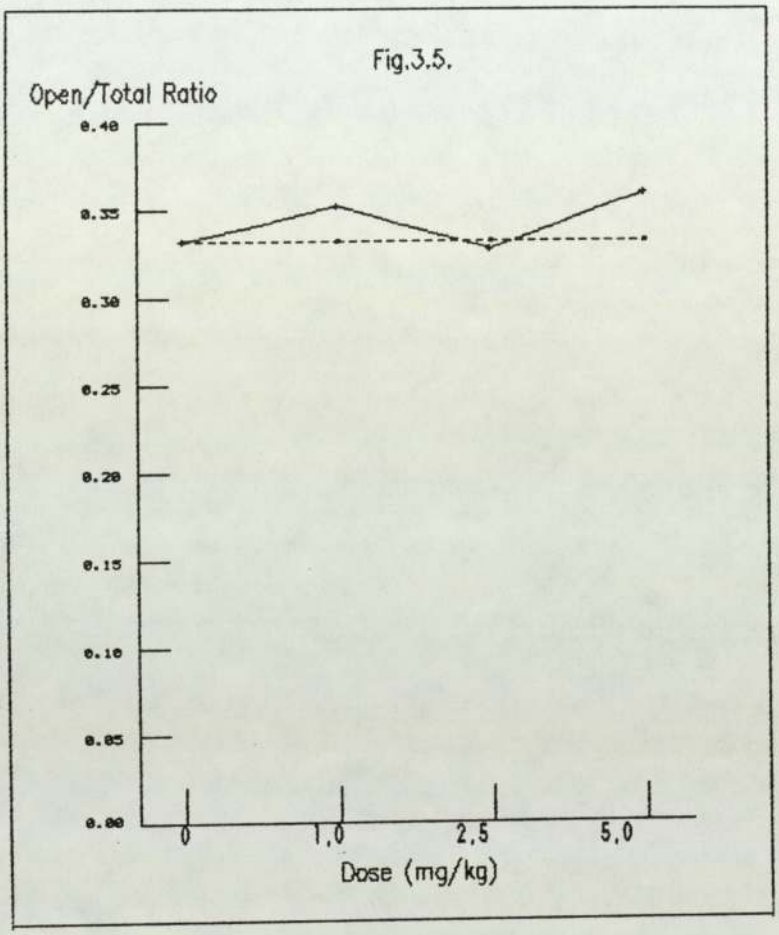


Fig.3.5. The effect of **cyproheptadine** (1.0, 2.5 & 5.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test.

Table 3.2

Mean number of entries in 10 minutes (±sem)

(Vehicle controls in brackets)

Drug/Dose mg/kg	Open	Total	Open/total
Seganserin			
0.1	2.7±.45	10.8±1.1	0.24±.03
	(3.2±.83)	(9.5±1.6)	(0.28±.03)
0.5	3.2±.44	7.0±1.1	0.43±.03*
	(3.6±.36)	(9.8±1.1)	(0.33±.02)
1.0	2.8±.96	8.0±1.5	0.31±.05
	(3.6±.36)	(9.8±1.1)	(0.33±.02)
Methysergide			
1.0	5.6±.78	15.6±1.5	0.35±.02
	(4.4±.46)	(14.0±1.4)	(0.314±.01)
2.5	8.0±.28**	18.0±.49**	0.442±.007**
	(4.2±.33)	(13.0±1.0)	(0.33±.009)
5.0	3.4±.73	11.2±1.1	0.28±.04
	(4.2±.33)	(13.0±1.0)	(0.33±.009)
10.0	3.8±.82	13.6±1.4	0.28±.04
	(4.2±.33)	(13.0±1.0)	(0.33±.009)
Cyproheptadine			
1.0	4.2±.18	11.8±.33	0.35±.009
	(3.6±.46)	(10.8±1.0)	(0.33±.005)
2.5	4.4±.45	13.4±1.3	0.33±.02
	(3.6±.46)	(10.8±1.0)	(0.33±.005)
5.0	3.4±.22	9.6±.80	0.36±.02
	(3.6±.46)	(10.8±1.0)	(0.33±.005)

Table 3.2. The effect of seganserin, methysergide and cyproheptadine on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

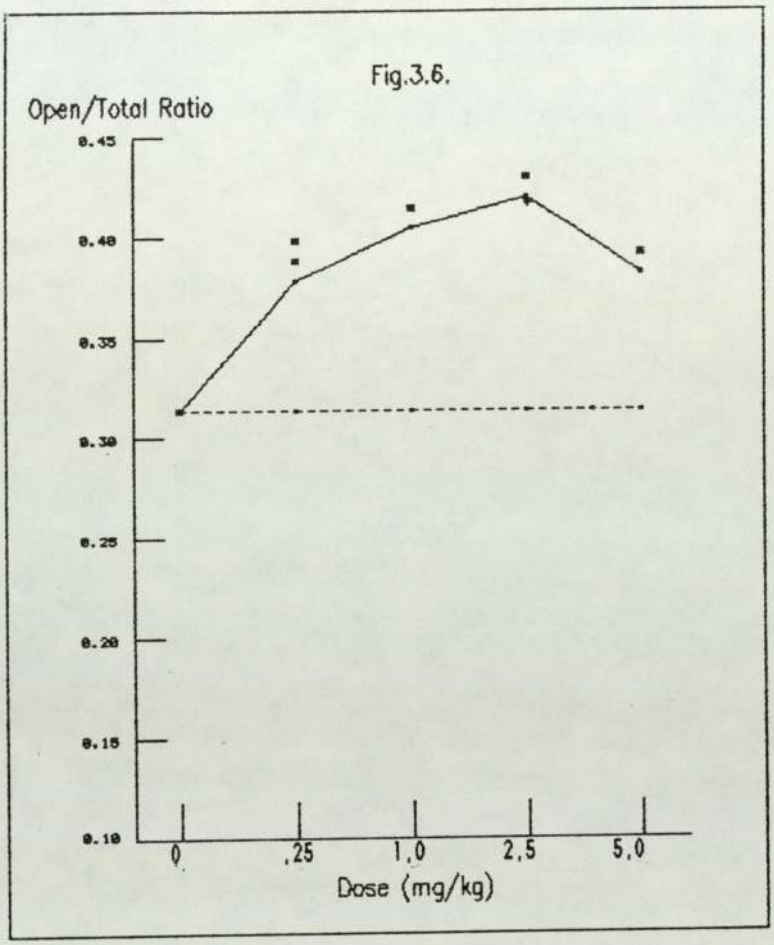


Fig.3.6. The effect of **ipsapirone** (TVXQ7821) (.25, 1.0, 2.5 & 5.0 mg/kg) given i.p.(30 mins. pretreatment) on the open/total entry ratio in the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

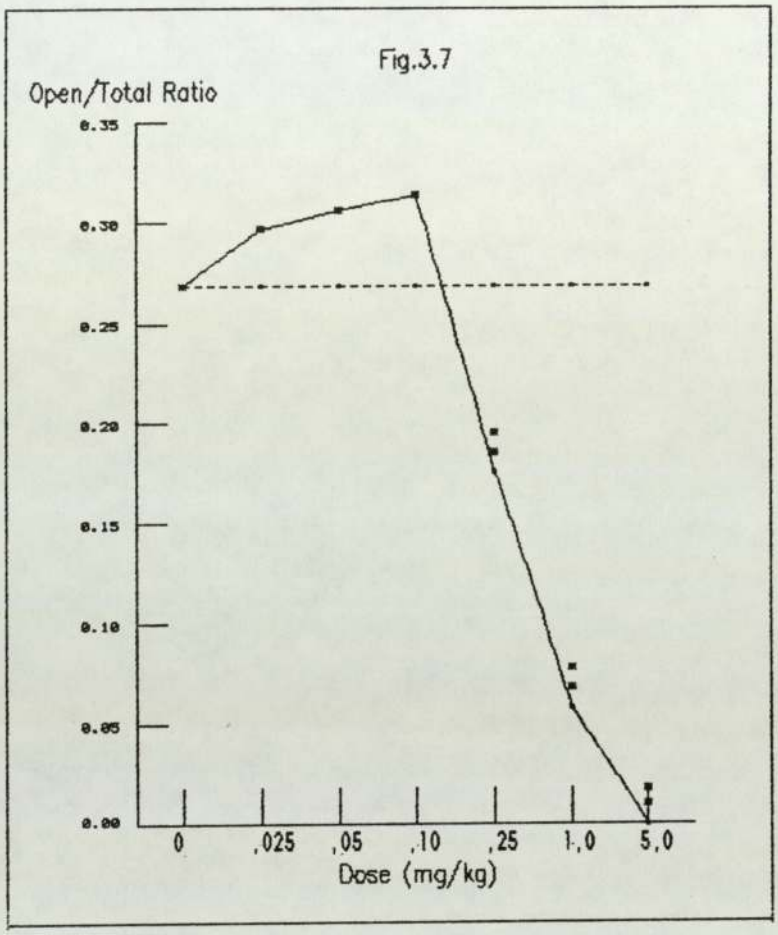


Fig.3.7. The effect of **buspirone** (.025, .05, .10, .25, 1.0 & 5.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 3.3

Mean number of entries in 10 minutes ±sem.

(Vehicle control values in brackets.)

Drug/Dose mg/kg	Open	Total	Open/Total
Ipsapirone			
0.25	7.0±.58*	18.5±1.6	0.38±.01**
	(5.0±.33)	(17.2±.92)	(0.29±.004)
1.0	7.8±.64**	19.8±1.8	0.41±.03*
	(5.0±.33)	(17.2±.92)	(.29±.004)
2.5	10.3±1.3*	24.3±2.7	0.42±.02*
	(6.0±.62)	(17.8±.86)	(0.34±.03)
5.0	9.7±1.1*	25.2±1.8	0.38±.03**
	(6.0±.62)	(17.8±.86)	(0.34±.03)
Buspirone			
0.025	3.2±.50	9.6±.87	0.30±.03
	(2.3±.19	(8.8±.64)	(0.27±.01)
0.05	3.2±.50	10.0±1.2	0.31±.02
	(1.7±.19)	(6.6±.70)	(0.24±.02)
0.1	3.8±.33	11.4±.83	0.31±.01
	(2.3±.19	(8.8±.64)	(0.27±.01)
0.25	1.3±.22	7.3±.97	0.18±.01
	(1.7±.19)	(6.6±.70)	(0.24±.02)
1.0	0.6±.36*	7.2±1.3**	0.06±.03**
	(5.4±.46)	(18.0±1.6)	(0.30±.008)
5.0	0.0±.00**	3.8±.33**	0.00±.00**
	(5.4±.46)	(18.0±1.6)	(0.30±.008)

Table 3.3. The effect of ipsapirone and buspirone on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

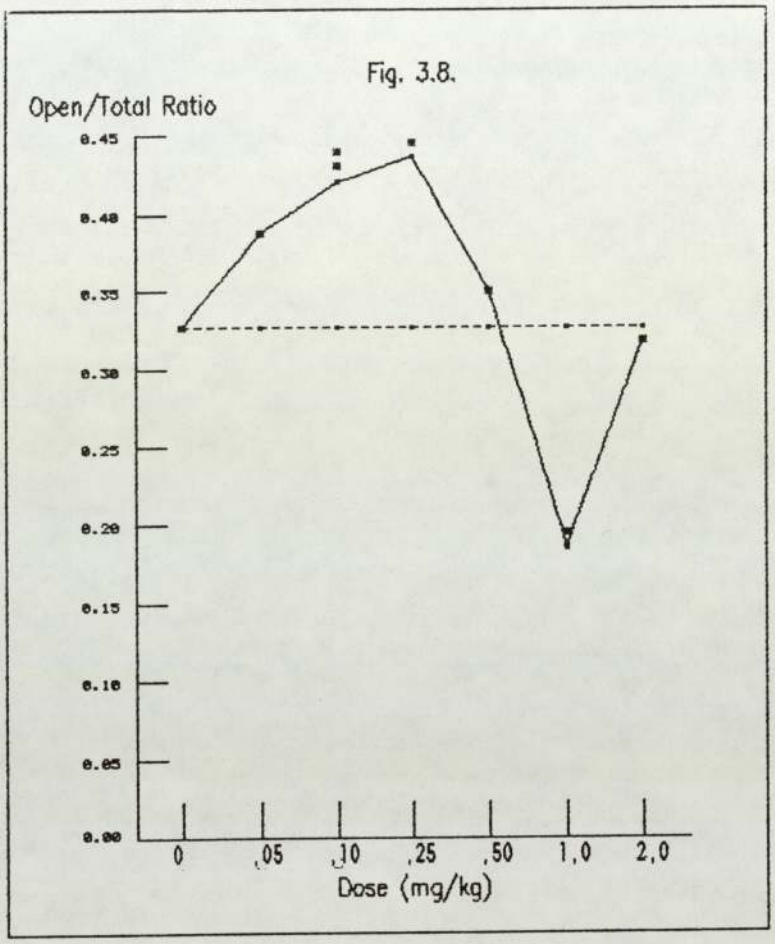


Fig.3.8. The effect of **(±)pindolol** (.05, .10, .25, .50, 1.0 & 2.0 mg/kg) given i.p.(30 mins. pretreatment) on the open/total entry ratio in the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

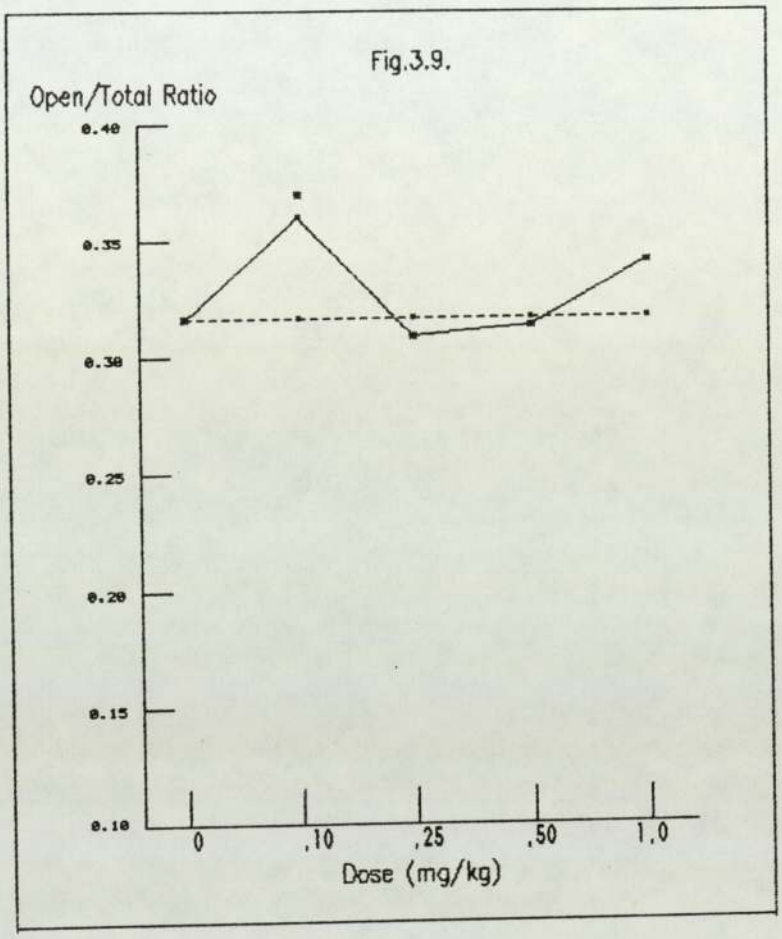


Fig.3.9. The effect of **(-)alprenolol** (.10, .25, .50 & 1.0 mg/kg) given i.p. (30 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$

Table 3.4.

Mean number of entries in 10 minutes (±sem)

(Vehicle control values in brackets)

Drug/Dose	Open	Total	Open/total
(±)pindolol			
0.05	4.0±.57	10.2±1.0	0.39±.03
	(4.2±.66)	(12.0±1.5)	(0.35±.02)
0.1	6.4±.54	15.2±1.1	0.42±.01**
	(4.0±.26)	(12.5±.50)	(0.32±.009)
0.25	5.6±.73	12.8±1.4	0.44±.02*
	(3.8±.82)	(10.8±2.1)	(0.34±.02)
0.5	5.5±.81	15.0±1.2	0.35±.03
	(4.7±.90)	(14.2±1.9)	(0.31±.03)
1.0	3.3±1.1	15.8±2.3	0.19±.03*
	(4.7±.90)	(14.2±1.9)	(0.31±.03)
2.0	4.3±.87	13.0±2.4	0.32±.02
	(4.0±.26)	(12.5±.50)	(0.32±.009)
(-)alprenolol			
0.1	4.3±.65	11.8±1.6	0.36±.01*
	(4.0±.50)	(13.5±2.2)	(0.31±.01)
0.25	4.3±.41	14.0±1.3	0.31±.03
	(4.0±.41)	(12.3±1.2)	(0.33±.02)
0.5	4.3±.45	13.6±1.0	0.31±.01
	(4.0±.50)	(13.5±2.2)	(0.31±.01)
1.0	5.0±.40	14.8±1.3	0.34±.02
	(4.0±.41)	(12.3±1.2)	(0.33±.02)

Table 3.4. The effect of pindolol and alprenolol on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

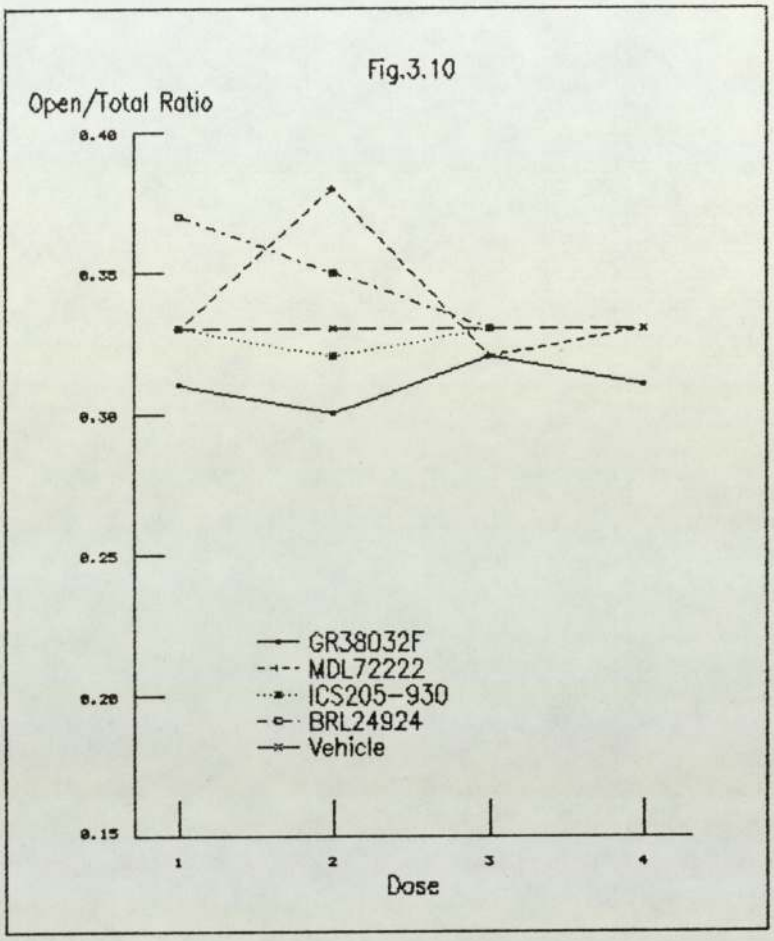


Fig.3.10. The effect of **GR38032F** (.001, .01 & .10 mg/kg), **MDL72222** (.01, .10 & 1.0 mg/kg), **ICS205-930** (.01 & .10 mg/kg) and **BRL24924** (.10 & 1.0 mg/kg) given i.p. (20 mins. pretreatment) on the open/total entry ratio of the elevated X-maze. The dotted line represents the mean of concurrently run controls. Statistical comparisons were made on raw data using a t-test.

Table 3.5.

Mean number of entries in 10 minutes (±sem).

(Vehicle control values in brackets).

Drug/Dose mg/kg	Open	Total	Open/total
GR38032F			
0.001	3.0±.28	10.0±.89*	0.30±.009
	(4.2±.18)	(13.4±.45)	(0.31±.006)
0.01	3.6±.45	11.2±.90	0.32±.01
	(4.2±.18)	(13.4±.45)	(0.31±.006)
0.1	3.2±.60	9.7±1.3	0.31±.04
	(3.7±.19)	(11.8±1.0)	(0.32±.01)
MDL72222			
0.01	7.2±.96	18.6±1.7	0.38±.02
	(5.2±.44)	(15.5±1.2)	(0.33±.006)
0.1	3.3±.51	10.8±2.1	0.32±.02
	(3.2±.28	(10.8±.85)	(0.32±.009)
1.0	5.0±.78	14.8±1.4	0.33±.03
	(5.2±.28)	(15.5±1.2)	(0.33±.006)

Table 3.5. The effect of GR38032F and MDL72222 on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test.

Table 3.6.

Mean number of entries in 10 minutes (±sem).

(Vehicle control values in brackets).

Drug/Dose mg/kg	Open	Total	Open/total
ICS205-930			
0.01	4.0±.56	12.3±1.5	0.32±.03
	(4.4±.36)	(13.6±.51)	(0.33±.03)
0.1	5.0±.62	14.7±1.5	0.33±.02
	(4.4±.36)	(13.6±.51)	(0.33±.03)
BRL24924			
0.1	7.8±.69	22.3±1.6	0.35±.02
	(8.0±.85)	(21.3±2.2)	(0.37±.02)
1.0	5.8±1.0	17.2±3.6	0.33±.02
	(8.0±.85)	(21.3±2.2)	(0.37±.02)
p-chlorophenylalanine			
	5.3±.40	**13.8±.97**	**0.38±.02***
	(5.1±.50)	(16.2±1.3)	(0.31±.02)

Table 3.6. The effect of ICS205-930, BRL24924 and pCPA on exploration in the elevated X-maze. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

CHAPTER 4

DRUG INTERACTIONS IN THE ELEVATED X-MAZE.

Introduction

In order to learn more about the nature of the responses already seen in chapters 1 and 2 it was necessary to undertake antagonism studies between anxiogenic-like compounds and those showing an anxiolytic profile. The anxiogenic response to 8-OH-DPAT, presumably 5-HT_{1A} receptor mediated, was tested after pretreatment with a number of ligands with differing receptor selectivity. Ritanserin was tested against 8-OH-DPAT in order to examine the role of 5-HT_{2} receptors in the anxiolytic response. Pindolol and alprenolol were used as 5-HT, antagonists in order to further examine the role of 5-HT_{1A} receptors in anxiety. Because of the β-adrenergic activity of the latter two compounds, it was necessary to examine those β-adrenoceptor antagonists with no activity at 5-HT receptors to clarify the receptor groups involved.

The effect of diazepam on the 8-OH-DPAT response was tested for comparison purposes.

Finally the 8-OH-DPAT response was examined after depletion of central 5-HT levels with 3 days of treatment with p-chlorophenylalanine. This would help to indicate whether the anxiogenic response to 8-OH-DPAT was pre- or post-synaptically mediated.

Results

4.1. The effect of ritanserin on the response to 8-OH-DPAT and 5MeODMT.

Ritanserin (0.1 mg/kg) produced an anxiolytic-like response and 8-OH-DPAT (0.05 mg/kg) produced an anxiogenic response. 8-OH-DPAT administered in the presence of ritanserin produced an anxiogenic response of equal magnitude to that produced by 8-OH-DPAT alone (Fig.4.1). Total entries did not vary from control levels with either treatment or combination (see Table 4.1).

5MeODMT (0.25 mg/kg) produced an anxiogenic-like response which again remained as potent after pretreatment with an anxiolytic dose of ritanserin (0.1 mg/kg) (Fig.4.1). Total entries did not vary significantly from control values for either treatment or combination (see Table 4.1).

4.2. The effect of diazepam on the response to 8-OH-DPAT.

Diazepam at a dose of 2.5 mg/kg was significantly anxiolytic whilst 8-OH-DPAT (0.03 mg/kg) produced an anxiogenic-like response. When given in the presence of diazepam, 8-OH-DPAT produced complete abolition of open arm entries (Fig.4.2) and a drastic reduction in total number of entries (Table 4.2). The combination treatment resulted in a severe muscle hypotonia with the consequent depressant effect on maze exploration.

Diazepam at a lower but still significantly anxiolytic dose (1.0 mg/kg) failed to antagonise the anxiogenic response to 8-OH-DPAT (Fig.4.2) and produced no change in total entries (see Table 4.2).

4.3. The effect of (±)pindolol and (−)alprenolol on the response to 8-OH-DPAT.

Pindolol at an anxiolytic dose (0.10 mg/kg) significantly antagonised the anxiogenic response to 8-OH-DPAT (0.03 mg/kg) - Fig.4.3. The open/total ratio for 8-OH-DPAT was 40% of control values and was returned to 85% of control levels in combination with pindolol. Total entries did not vary from control values with either treatment or combination (Table 4.3).

Alprenolol (0.1 mg/kg) did not produce any significant change in the open/total ratio but did significantly antagonise the fall in ratio produced by 8-OH-DPAT. (Fig.4.3.). The entry ratio after 8-OH-DPAT fell to 20% of control values, and was 45% of control values after pretreatment with alprenolol. Total entries did not vary from control levels with either treatment or combination (Table 4.3).

4.4. The effect of ipsapirone and buspirone on the response to 8-OH-DPAT.

Ipsapirone at an anxiolytic dose (1.0 mg/kg) significantly antagonised the fall in entry ratio produced by 8-OH-DPAT (Fig.4.4.). The entry ratio which fell to 60% of control values with 8-OH-DPAT remained at 110% of control levels after pretreatment with ipsapirone. Total number of entries were significantly increased in

the combination group but remained similar to control levels in single treatment groups (Table 4.4.)

Buspirone (0.05 mg.kg) failed to produce any effect on the entry ratio and also failed to antagonise the anxiogenic response to 8-OH-DPAT (Fig.4.4). In fact the combination depressed the ratio further (although not significantly so). Total number of entries did not vary from control levels with either treatment or combination (Table 4.4).

4.5. The effect of metoprolol and ICI 118,551 on the response to 8-OH-DPAT.

Metoprolol (3.0 mg/kg) failed to produce any effect on the entry ratio and also failed to significantly antagonise the response to 8-OH-DPAT (Fig.4.5). Total number of entries did not vary from control levels with either treatment or combination (Table 4.5).

ICI 118,551 (1.0 mg/kg) failed to produce any effect on the entry ratio and also failed to antagonise the response to 8-OH-DPAT (Fig.4.5). Total number of entries were significantly increased by the combination but remained similar to control levels with the single treatments (Table 4.5).

4.6. The effect of p-chlorophenylalanine (pCPA) pretreatment on the response to 8-OH-DPAT.

Three daily pretreatments with pCPA (300 mg/kg) produced an anxiolytic-like response in the X-maze 24 hrs. after the last pCPA injection. The response to 8-OH-DPAT was antagonised in pCPA treated rats; the ratio changing from 75% of controls with 8-OH-DPAT to 110% of controls after pretreatment with pCPA (Fig.4.6). Total entries did not vary from control levels with either treatment or combination (Table 4.6). Mean forebrain 5-HT levels in pCPA treated rats fell by 79% compared with vehicle controls.

Mean 5-HT level in controls = 315.2 ±10.2 ng/g N=6

Mean 5-HT level after pCPA = 67.3 ±2.7 ng/g N=6

Discussion

The fact that the 5-HT_2 receptor antagonist ritanserin did not affect the anxiogenic-like response to either 8-OH-DPAT or 5MeODMT suggests that 5-HT_2 receptors are not involved in the agonist response. The exact role of the 5-HT_2 antagonists is therefore difficult to assess in the present experiments. One possible explanation is that the anxiolytic effect seen with the antagonists may be due to their actions on α_1-adrenoceptors since possible anxiolytic effects of α_1-antagonists have been shown in this model (*Handley and Mithani, 1984a; Mithani, 1984*). However, a separate involvement of 5-HT_2 receptors in anxiety cannot be ruled out at this stage.

The antagonism of the 8-OH-DPAT response by the putative 5-HT_1 antagonists alprenolol and pindolol and the 5-HT_{1_m} ligand ipsapirone is consistent with an action of these drugs at 5-HT_1 receptors. Pindolol and ipsapirone both completely blocked the 8-OH-DPAT response, and whilst alprenolol was without effect on its own it did significantly antagonise the 8-OH-DPAT response. Since ipsapirone and 8-OH-DPAT bind essentially only to 5-HT_1 (5-HT_{1_m}) receptors (*Peroutka, 1985; Middlemiss and Fozard, 1983; Hamon et al., 1986*) then this is strong evidence that the anxiogenic and anxiolytic responses are as a result of agonist and antagonist action respectively at 5-HT_1 (5-HT_{1_m}) receptors in this model. Whilst the results with pindolol and alprenolol tend to agree with such a conclusion care must be taken to consider the implications of their actions at β-adrenoceptors. For this purpose agents were

sought which were centrally active at β-adrenoceptors but were without effect on 5-HT receptors.

Metoprolol and ICI 118,551 are selective antagonists for the β_1 and β_2-adrenoceptors respectively (*Ablad et al., 1983; Biliski et al., 1983*) and are devoid of actions at 5-HT receptors (*Costain and Green, 1978; Middlemiss, 1986a*). When given on their own no changes in open/total ratio or total entries were seen and neither were able to antagonise the anxiogenic-like effect of 8-OH-DPAT. This therefore suggests that the β-adrenoceptors may not be involved in the modulation of the 8-OH-DPAT response by pindolol and alprenolol and thus the results are still consistent with a role for 5-HT, or 5-HT_{1_m} receptors.

The fact that an anxiolytic-like effect (with pindolol or ipsapirone) antagonises an anxiogenic-like effect (with 8-OH-DPAT) may be explained by a 'physiological antagonism' since the effects are opposite and a combination could therefore be expected to produce an intermediate response. However, the results with diazepam, and indeed with ritanserin, suggest this is not the explanation since their anxiolytic-like effect failed to antagonise the anxiogenic-like effect of 8-OH-DPAT. The failure of diazepam to modify the 8-OH-DPAT effect is interesting in its own right, since it could indicate that the two drugs act by independent mechanisms. It has been shown that the apparent involvement of 5-HT in the anti-anxiety effects of benzodiazepines contains many inconsistencies (*Thiébot, 1986*). The benzodiazepines may render the animal less able to wait for reward,

and the involvement of 5-HT may actually be in this aspect of the benzodiazepine response (*ibid*). The higher dose of diazepam appeared to render the animal unable to co-ordinate movement when in combination with 8-OH-DPAT. This effect was best described as severe muscle hypotonia and may reasonably be assumed to be a potentiation of the muscle relaxant effect of diazepam. This effect is also interesting since benzodiazepines appear to potentiate certain other effects of 5-HT agonists (*Handley and Singh, 1985; Moser and Redfern, 1986*).

The anxiolytic effect of pretreatment with pCPA was expected and is well documented (see Introduction). However, the antagonism of the 8-OH-DPAT response by pCPA pretreatment is interesting since it suggests a pre-synaptic action of 8-OH-DPAT. Previously 8-OH-DPAT has been shown to be anxiolytic in punished drinking, an effect which is reversed in 5-HT depleted rats by pretreatment with pCPA (*Engel et al., 1984*). This anti-conflict effect may be due to the inhibition of neuronal firing consequent on activation of 5-HT_{1a} receptors on cell bodies (*Hutson et al., 1986*) or by a presynaptically generated increase in impulsivity (*Engel et al., 1984; Soubrié, 1986a*). The anxiogenic-like effect after 5-HT depletion has been attributed to agonist effects of 8-OH-DPAT on post-synaptic receptors made supersensitive by the 5-HT depletion (*Engel et al., 1984*). The results from the present study suggest, however, that 8-OH-DPAT is producing its anxiogenic effect via the presynaptic 5-HT_1 receptor. However, of the presynaptic 5-HT receptors identified, the 5-HT_{1a} subtype would appear to be located

on the cell body and mediates an inhibitory action on terminal 5-HT release by decreasing neuronal firing (see *Dourish et al., 1986*a). Such an action would be expected to produce an anxiolytic-like effect since previous studies suggest that manipulations known to reduce 5-HT neurotransmission produce anti-conflict (or anxiolyitc-like) effects. The terminal autoreceptor is believed to be of the 5-HT_{1B} subtype (*Engel et al., 1986; Bonnano et al., 1986; Maura et al., 1986*), although this view is not universally held with other evidence impicating the 5-HT_{1A} receptor subtype (*Gozlan et al., 1983; Hamon et al., 1984*). However, activation of this receptor would also be expected to produce an anxiolytic effect by virtue of the resultant decrease in 5-HT release. Verge and colleagues (1985) suggested that 8-OH-DPAT acts on autoreceptors on cell bodies in the raphé, but not on the presynaptic terminal receptor. Our proposed presynaptically generated anxiogenic effect of 8-OH-DPAT is clearly at odds with previous work. The possibility remains that the nature of the receptor depends on the brain area studied.

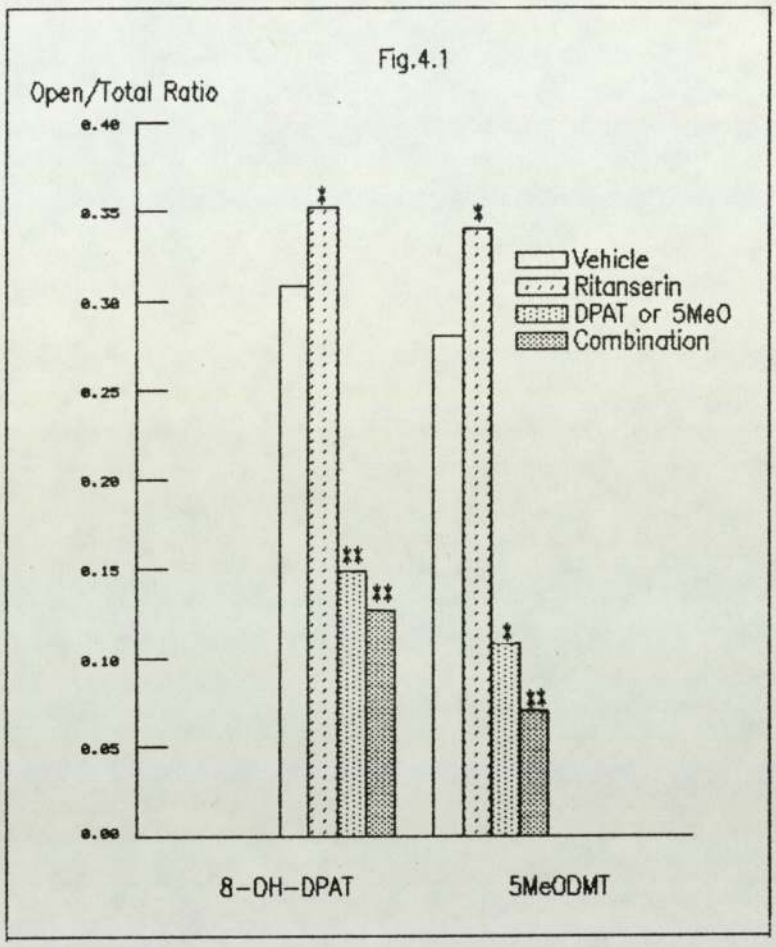


Fig.4.1. The effect of **ritanserin** (0.1 mg/kg) in combination with either **8-OH-DPAT** (0.03mg/kg) or **5MeODMT** (0.25mg/kg) on the open/total entry ratio of the elevated X-maze. Statistical comparisons relative to vehicle control were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 4.1.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	3.6±.46	11.6±1.3	0.30±.008
Ritanserin	4.4±.73	12.2±1.6	0.35±.02*
8-OH-DPAT	2.2±.77	14.4±2.0	0.15±.04**
Ritanserin + 8-OH-DPAT	2.0±.40*	15.4±1.5	0.13±.02**
Vehicle	5.3±1.1	18.3±2.6	0.28±.03
Ritanserin	4.8±.96	14.0±2.8	0.34±.02*
5MeODMT	1.3±.55*	10.0±1.5*	0.11±.04*
Ritanserin + 5MeODMT	0.75±.41**	10.8±.75*	0.07±.03**

Table 4.1. The effect of ritanserin on the anxiogenic response to 8-OH-DPAT and 5MeODMT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

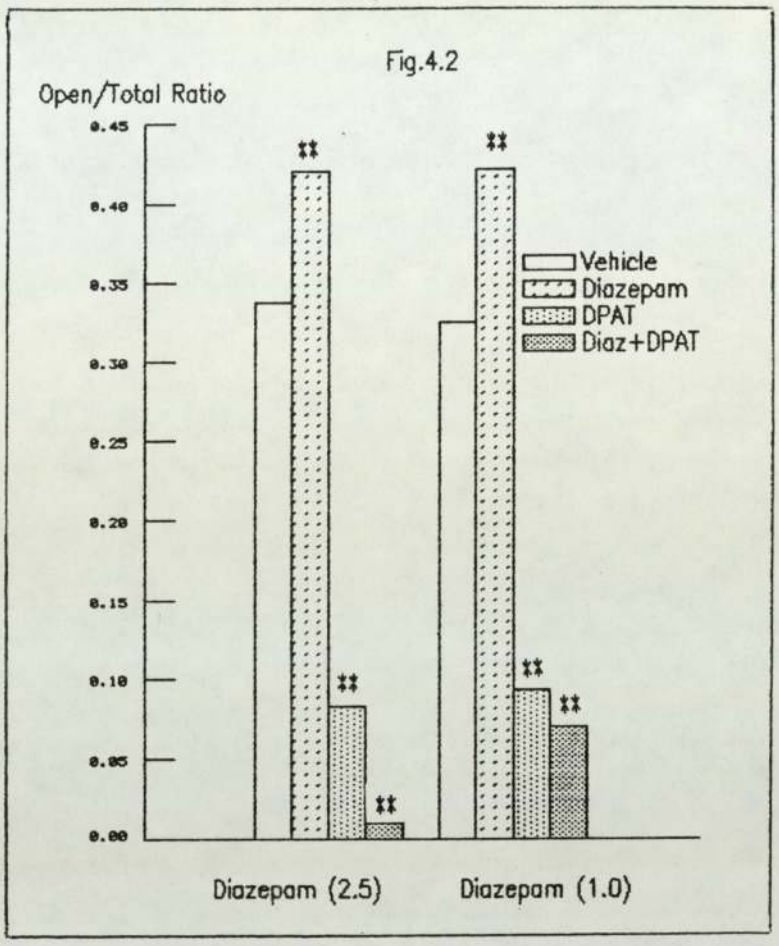


Fig.4.2. The effect of **diazepam** (1.0 & 2.5 mg/kg) on the response to 8-OH-DPAT (0.03mg/kg)(*DPAT*) on the open/total entry ratio of the elevated X-maze. Statistical comparisons relative to vehicle controls were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 4.2.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	4.3±.22	12.5±.75	0.34±.006
Diazepam 2.5mg/kg	4.0±.61	8.5±.60	0.42±.01**
8-OH-DPAT	1.0±.35**	10.5±1.4	0.083±.02**
Diazepam + 8-OH-DPAT	0.0±.00**	4.0±.61**	0.00±.00**
Vehicle	4.3±.54	12.5±.80	0.33±.02
Diazepam 1.0mg/kg	5.4±.40	12.8±.80	0.42±.009**
8-OH-DPAT	1.0±.00**	11.3±1.1	0.09±.007**
Diazepam + 8-OH-DPAT	0.6±.22**	8.0±1.3	0.07±.03**

Table 4.2. The effect of diazepam on the anxiogenic response to 8-OH-DPAT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

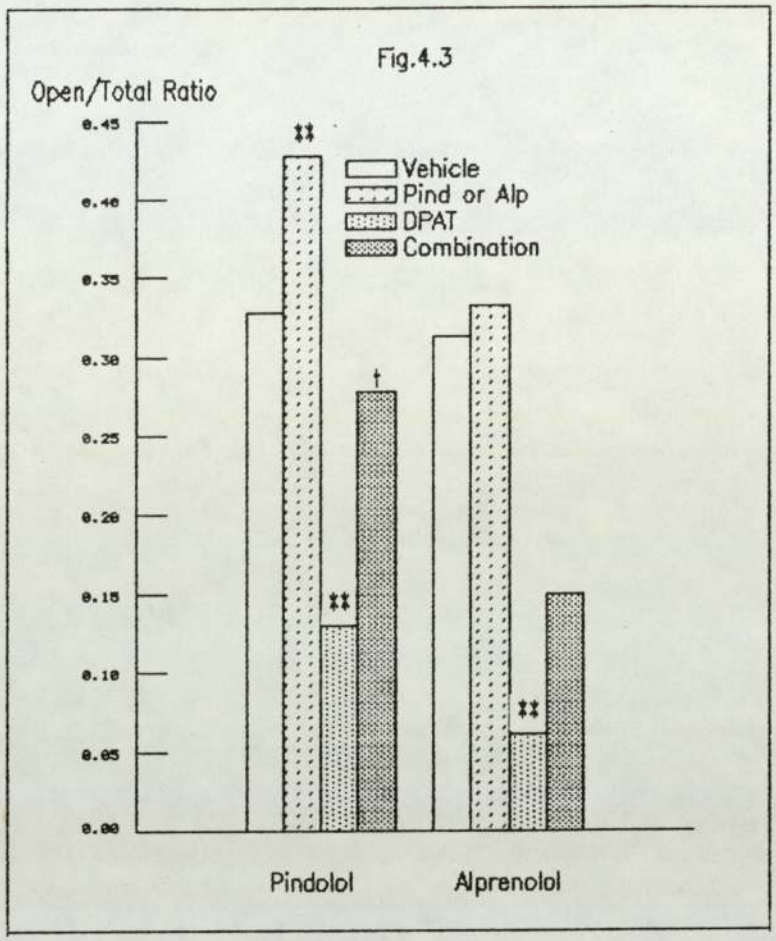


Fig.4.3. The effect of **pindolol** (0.1mg/kg) and **alprenolol** (0.1mg/kg) on the response to **8-OH-DPAT** (0.03 & 0.1 mg/kg respectively)(*DPAT*) on open/total entry ratio in the elevated X-maze. Statistical comparisons relative to vehicle controls were made on raw data using a Two-Way ANOVA followed by Tukey's test for unconfounded means. * = $p<.05$ ** = $p<.01$ relative to vehicle.

† = $p<.05$ relative to 8-OH-DPAT alone after Tukey's test.

Table 4.3.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	4.2±.41	12.6±1.1	0.33±.006
Pindolol	5.5±.40	13.0±1.0	0.43±.02**
8-OH-DPAT	1.7±.32**	11.8±.80	0.13±.02**
Pindolol + 8-OH-DPAT	4.1±.74	13.2±1.4	0.28±.03††
Vehicle	4.7±1.1	14.0±2.8	0.31±.03
Alprenolol	4.6±.36	13.8±.77	0.33±.01
8-OH-DPAT	1.0±.40**	13.0±2.5	0.06±.02**
Alprenolol + 8-OH-DPAT	2.0±.40	14.0±2.1	0.14±.02†

Table 4.3. The effect of pindolol and alprenolol on the anxiogenic response to 8-OH-DPAT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls. † = $p<.05$ †† = $p<.01$ relative to 8-OH-DPAT alone after Tukey's test.

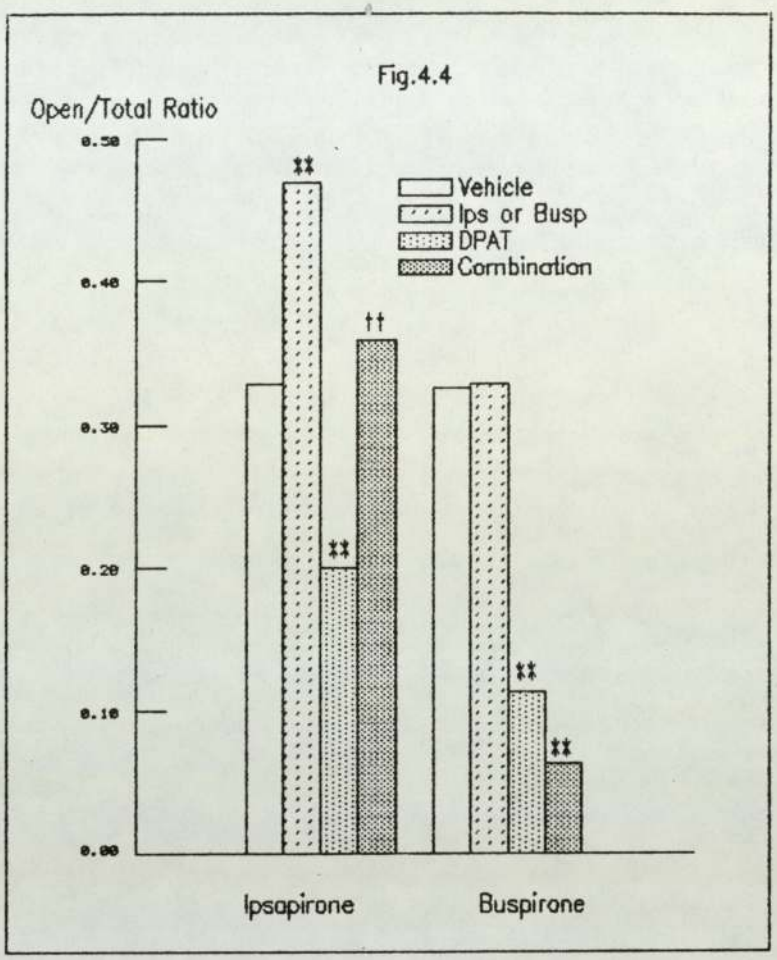


Fig.4.4. The effect of **ipsapirone** (1.0 mg/kg) and **buspirone** (0.05 mg/kg) on the response to **8-OH-DPAT** (0.05 mg/kg)(*DPAT*) on open/total entry ratio in the elevated X-maze. Statistical comparisons relative to vehicle controls were made on raw data using a Two-Way ANOVA followed by Tukey's test for unconfounded means. * = $p<.05$ ** = $p<.01$ relative to vehicle.

† = $p<.05$ †† = $p<.01$ relative to 8-OH-DPAT alone after Tukey's test.

Table 4.4.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	5.0±.57	15.4±1.8	0.33±.02
Ipsapirone 1.0mg/kg	8.8±.59**	19.2±1.7	0.47±.02**
8-OH-DPAT	4.0±.69	18.6±1.3	0.21±.02**
Ipsapirone + 8-OH-DPAT	9.0±1.2**	24.8±1.7*	0.36±.02††
Vehicle	5.3±.41	16.0±.79	0.33±.01
Buspirone 0.05mg/kg	5.8±.41	17.8±1.0	0.33±.02
8-OH-DPAT	2.0±.00**	18.8±1.8	0.11±.01**
Buspirone + 8-OH-DPAT	0.80±.40**	15.5±2.3	0.06±.03**

Table 4.4. The effect of ipsapirone and buspirone on the anxiogenic response to 8-OH-DPAT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls. †† = $p<.01$ relative to 8-OH-DPAT alone after Tukey's test.

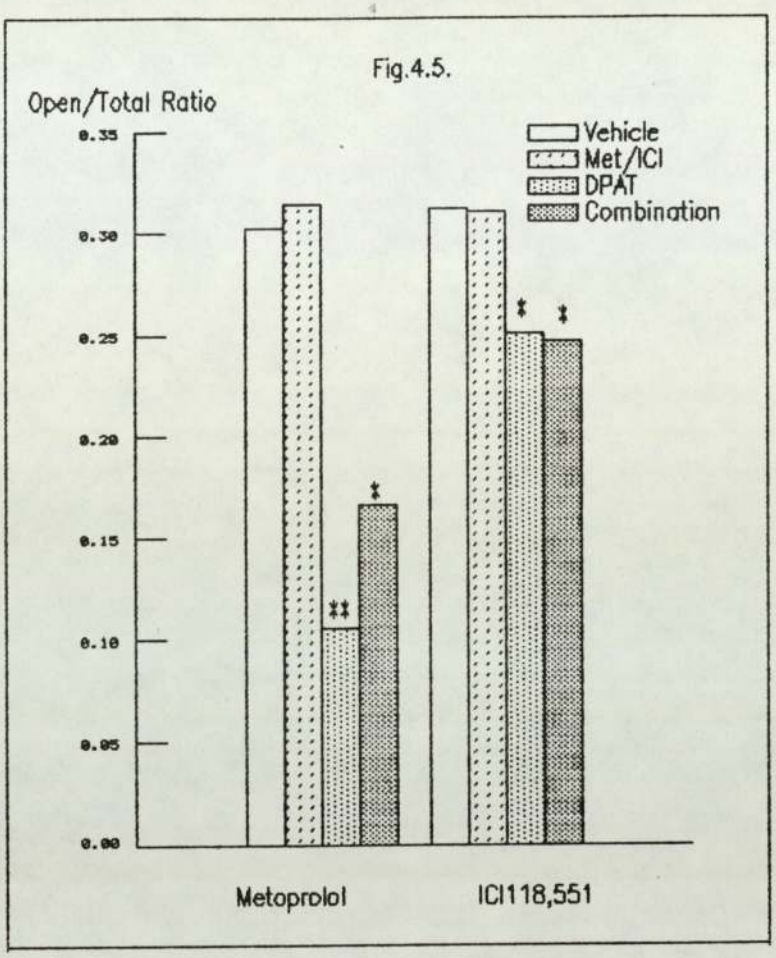


Fig.4.5. The effect of **metoprolol** (3.0 mg/kg) and **ICI118,551** (1.0 mg/kg) on the response to **8-OH-DPAT** (0.05 mg/kg)(*DPAT*) on open/total entry ratio in the elevated X-maze. Statistical comparisons relative to vehicle controls were made on raw data using a Two-Way ANOVA followed by Tukey's test for unconfounded means. * = $p<.05$ ** = $p<.01$ relative to vehicle.

Table 4.5.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	4.6±.36	15.2±1.2	0.30±.008
Metoprolol	4.8±.33	15.4±.80	0.31±.02
8-OH-DPAT	2.0±.61*	18.3±1.1	0.11±.03**
Metoprolol + 8-OH-DPAT	3.4±1.1	18.6±2.2	0.17±.05*
Vehicle	5.8±.72	18.4±1.6	0.31±.01
ICI118,551	5.6±.73	17.8±1.8	0.31±.009
8-OH-DPAT	6.6±.67	26.8±3.2	0.25±.01*
ICI118,551 + 8-OH-DPAT	6.8±.52	27.6±1.6	0.25±.008*

Table 4.5. The effect of metoprolol and ICI118,551 on the anxiogenic response to 8-OH-DPAT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls.

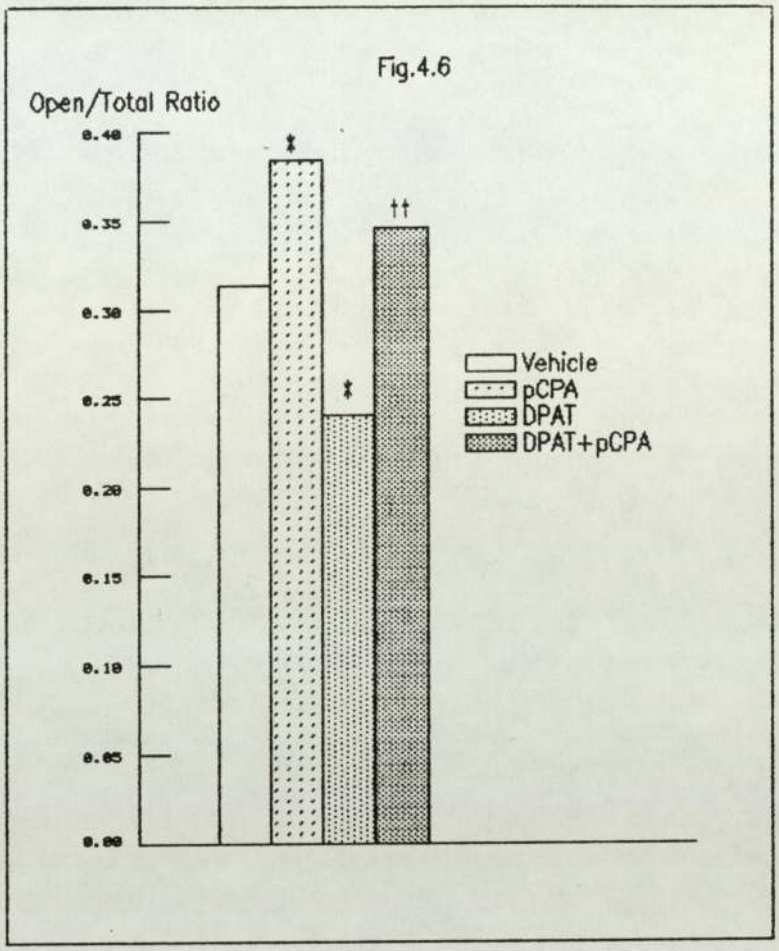


Fig.4.6. The effect of 3 days pretreatment with **para-chlorophenylalanine** (300 mg/kg) on the response to **8-OH-DPAT** (0.03 mg/kg) on the open/total entry ratio of the elevated X-maze. Statistical comparisons were made on raw data using a two-way ANOVA design followed by Tukey's tesat for unconfounded means.

* = $p<.05$ ** = $p<.01$ relative to vehicle. † = $p<.05$ relative to 8-OH-DPAT alone after Tukey's test.

Table 4.6.

Mean number of entries in 10 minutes (±sem).

Drug	Open	Total	Open/total
Vehicle	5.1±.49	16.2±1.3	0.31±.02
pCPA	5.1±.39	13.8±.97	0.38±.02*
8-OH-DPAT	4.7±.68	18.5±1.6	0.25±.02*
pCPA + 8-OH-DPAT	4.4±.45	12.6±1.1	0.35±.01††

Table 4.6. The effect of 3 days pretreatment with pCPA on the anxiogenic response to 8-OH-DPAT in the elevated X-maze. Statistical comparisons were made on raw data using an ANOVA. * = $p<.05$ ** = $p<.01$ relative to vehicle controls. †† = $p<.01$ relative to 8-OH-DPAT alone after Tukey's test.

CHAPTER 5

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

Introduction

In order to confirm the results obtained in the elevated X-maze another model of anxiety was sought and an attempt made to repeat some of the more important results already described. In selecting a model several factors were considered. As a result of the comments made earlier in the General Introduction concerning models employing suppression of rewarded responding such models were avoided. A model was needed which makes use of more physiologically relevant stimuli as does the X-maze. The Social Interaction model was therefore chosen as a suitable model since the parameters measured are, like those of the X-maze, part of the rats normal behavioural repertoire. The Social Interaction test was used as modified by Gardner and Guy (1984) from the original format as described by File and Hyde (1978). The modifications suggested by Gardner and Guy (1984) enable the anxiolytic actions of the benzodiazepines to be detected acutely. The anxiogenic-like effects seen with 8-OH-DPAT in the elevated X-maze are a crucial part of the investigation so far and it was therefore important to repeat the 8-OH-DPAT effect in another model of anxiety, along with other anxiogenic-like and anxiolytic-like compounds. Diazepam and βCCE were tested to establish the test under our laboratory conditions. Representative doses of 8-OH-DPAT, 5MeODMT and RU24969 were tested in the Social Interaction arena for their anxiogenic effect, and ipsapirone, pindolol, ritanserin and ketanserin were tested for their anxiolytic-like effects.

Results

5.1. The effects of diazepam and βCCE.

Diazepam produced a significant increase in time spent in social interaction at 1.0 mg/kg (Fig.5.1) without affecting either the number of walks or rears (Fig.5.2). At 0.5 mg/kg, time spent in social interaction showed a slight non-significant increase and there were no effects on walks or rears. At 2.0mg/kg there were no effects on social interaction and rats appeared somewhat sedated. No effects were seen on the number of walks but the number of rears was reduced.

βCCE at doses of 0.5 & 1.0 mg/kg produced a significant decrease in time spent in social interaction (Fig.5.3) whilst no changes in either number of rears or walks were seen with either dose (Fig.5.4).

5.2. The effects of 8-OH-DPAT.

8-OH-DPAT at a dose of 0.05 mg/kg produced a significant decrease in social interaction to almost 50% of the vehicle control (Fig.5.5). Number of rears was significantly reduced whilst at the same time the number of walks was significantly increased (Fig.5.6).

At the higher dose of 0.1 mg/kg 8-OH-DPAT again produced a significant decrease in the time spent in social interaction (Fig.5.5). The number of rears was still decreased significantly but the number of walks showed a non-significant increase (Fig.5.6).

5.3. The effect of ipsapirone.

Ipsapirone at a dose of 1.0 mg/kg produced a significant increase in time spent in social interaction to approximately 200% of vehicle control values (Fig.5.7) whilst producing no changes in the number of walks or rears (Table 5.1).

5.4. The effect of pindolol.

Pindolol at 0.1 mg/kg produced a significant increase in time spent in social interaction (Fig.5.7) but no change in either number of rears or walks (Table 5.1).

At 0.25 mg/kg pindolol produced a non-significant increase in social interaction and no change in either the number of walks or the number of rears.

5.5. The effects of ritanserin and ketanserin.

Ritanserin at a dose of 1.0 mg/kg produced a slight non-significant increase in social interaction (Fig.5.7). Rears or walks were not affected (Table 5.2).

Ketanserin 0.5 mg/kg had no effect on either social interaction (Fig.5.7) or walks or rears (Table 5.2).

5.6. The effects of RU24969 and 5MeODMT.

RU24969 at a dose of 0.5 mg/kg produced a pronounced fall in the time spent in social interaction (Fig.5.8) whilst producing a significant increase in the number of walks (Fig.5.10). At the higher

dose of 1.0 mg/kg, the fall in social interaction was still present (Fig.5.8) and the number of walks showed an even greater increase (Fig.5.10). No significant changes were seen in the number of rears (Fig 5.9) at either dose.

5MeODMT at 0.25 mg/kg produced a significant fall in social interaction (Fig.5.8) whilst producing a non-significant fall in the number of rears (Fig.5.9). At the higher dose of 1.0 mg/kg, time spent in social interaction fell drastically to only 10% of control values (Fig.5.8) as did the number of rears (Fig.5.9). No changes were seen with either dose in the number of walks (Fig.5.10).

Discussion

Overall, the results in the social interaction test were of a similar nature to those obtained in the elevated X-maze model. A parallel comparison of the results, particularly those with the 5-HT agonists and βCCE, showed a great similarity with the results in the elevated X-maze using similar doses, and therefore serve to verify the data so far obtained.

Diazepam exhibited an anxiolytic profile given acutely, since the modified version of the social interaction test was used for that specific purpose. However, only one dose of diazepam (1.0 mg/kg) produced a significant effect. The lower dose only produced a slight effect which could possibly be expected since this was the least effective dose in the X-maze. At the higher dose (2.0 mg/kg) the animals appeared somewhat sedated and showed a significant reduction in rearing activity which could explain the lack of effect, although no corresponding decrease in the number of walks was detected. As in the elevated X-maze βCCE showed a selective anxiogenic-like effect.

Although 8-OH-DPAT produced an anxiogenic-like response, it also decreased the number of rears and increased the number of walks. The former could be due to the presence of flat body posture thus making rearing difficult, although this component of the 5-HT syndrome was not seen to any extent at the lower dose. An increase in locomotor activity has previously been described for 8-OH-DPAT (*Tricklebank et al., 1985*) which probably explains the increase in

the number of walks. It is interesting to note that this effect was not apparent in the elevated X-maze (Chapter 2). It is possible that the overall aversiveness of the X-maze and its confined, novel, environment were sufficient to overcome this effect. The relatively large expanse of the social interaction arena compares more favourably to the chambers used in assesing locomotor activity. An additional factor to be noted here is the fact that the animals are pre-exposed to the arena whereas they are not pre-exposed to the X-maze.

The results with the 5-HT agonists RU24969 and 5MeODMT again mirror their effects in the elevated X-maze, producing an anxiogenic profile. However, as with the 8-OH-DPAT response, effects were seen on rears and walks. The drastic effect of the higher dose of 5MeODMT forced the use of a lower dose since it was felt that the presence of the flat body posture component of the 5-HT 'syndrome' could be a causative factor in the reduction in rearing activity and social interaction. The subsequent lower dose confirmed that the decrease in social interaction was independant from the 'syndrome' effects since no 'syndrome' component was detected. The number of rears was not significantly different from controls whilst time in active social interaction was still decreased. Similarly with RU24969, the reported tendency of the drug to increase locomotor activity (*Gardner and Guy, 1983*) manifested itself in an increase in the number of walks. At the lower dose, whilst this effect was smaller, it was still present. Again this is in contrast to the results on the elevated X-maze where no significant increase in

total entries was seen with this compound. It would appear that the hyperlocomotion seen with these compounds is seen to a greater extent where circumstances allow 'free running' to take place, as in the social interaction arena.

Although pindolol produced a complex dose-response curve on the X-maze, the two doses which were anxiolytic-like in this test both produced an increase in social interaction, although only one significantly so. Further doses of pindolol would indicate whether the same complex curve is apparent in the social interaction test but the purpose of the present experiments was to examine the doses which produced an anxiolytic-like effect in the X-maze.

The fact that the 5-HT_2 antagonists ketanserin and ritanserin showed no activity in social interaction underlies the doubts raised in earlier chapters as to the exact role of 5-HT_2 receptors in anxiety, since now it appears that not only will such compounds not antagonise the anxiogenic-like effects of 5-HT agonists, but they also have no activity in the social interaction test at doses anxiolytic in the X-maze.

The results in the Social Interaction test are therefore in agreement with previous results in the X-maze in that 8-OH-DPAT, 5MeODMT, RU24969 and βCCE indicate anxiogenic activity whilst diazepam, pindolol and ipsapirone indicate anxiolytic activity.

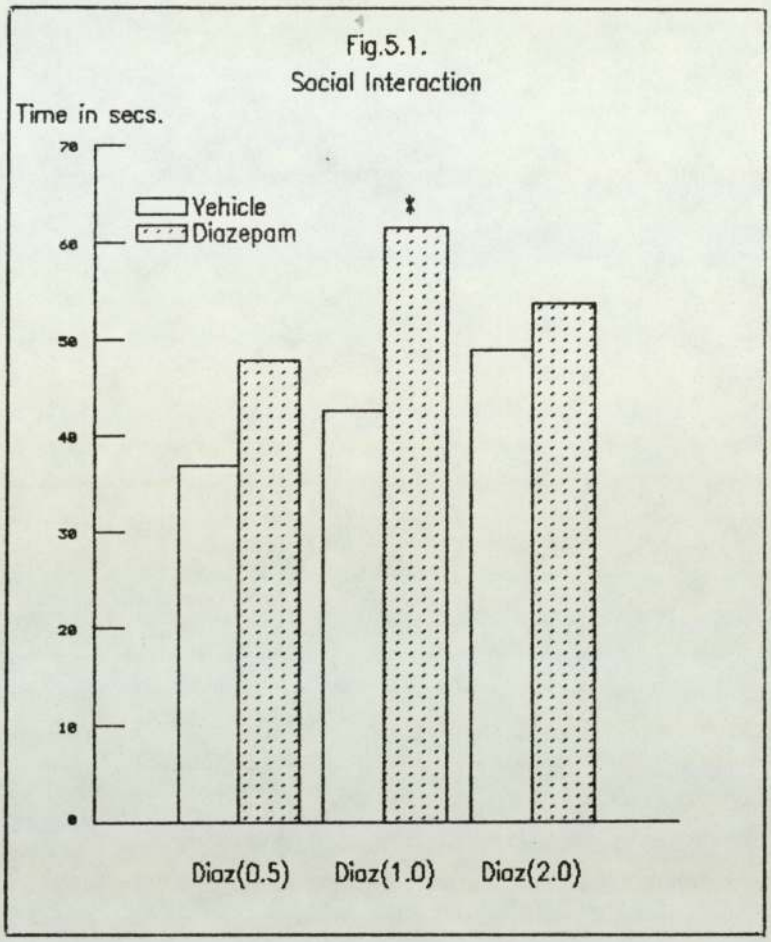


Fig.5.1. The effect of **diazepam** (.5, 1.0 & 2.0 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. ** = $p<.01$.

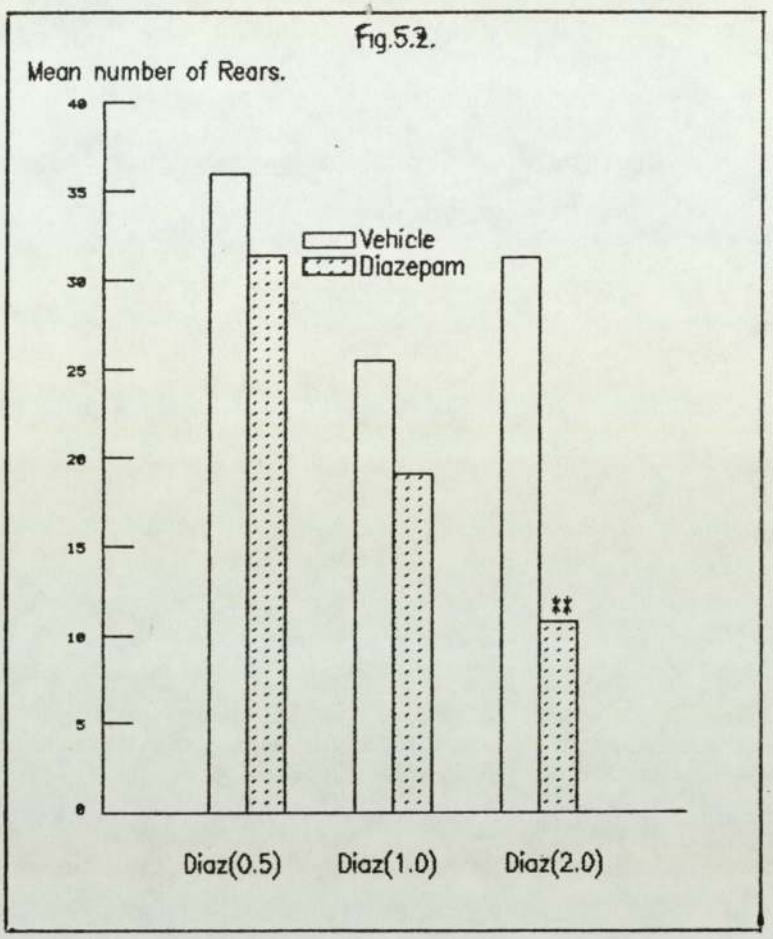


Fig.5.2. The effect of **diazepam** (.5, 1.0 & 2.0 mg/kg) on the number of rears made in the social interaction test compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. ** = $p<.01$.

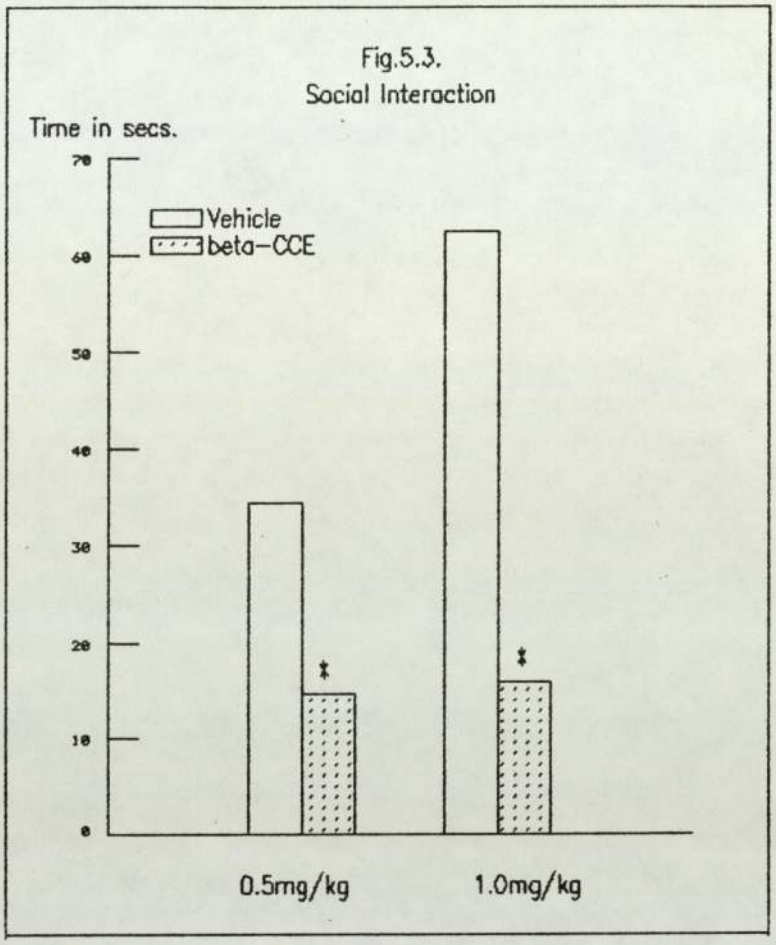


Fig.5.3. The effect of βCCE (0.5 & 1.0 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. ** = $p<.01$.

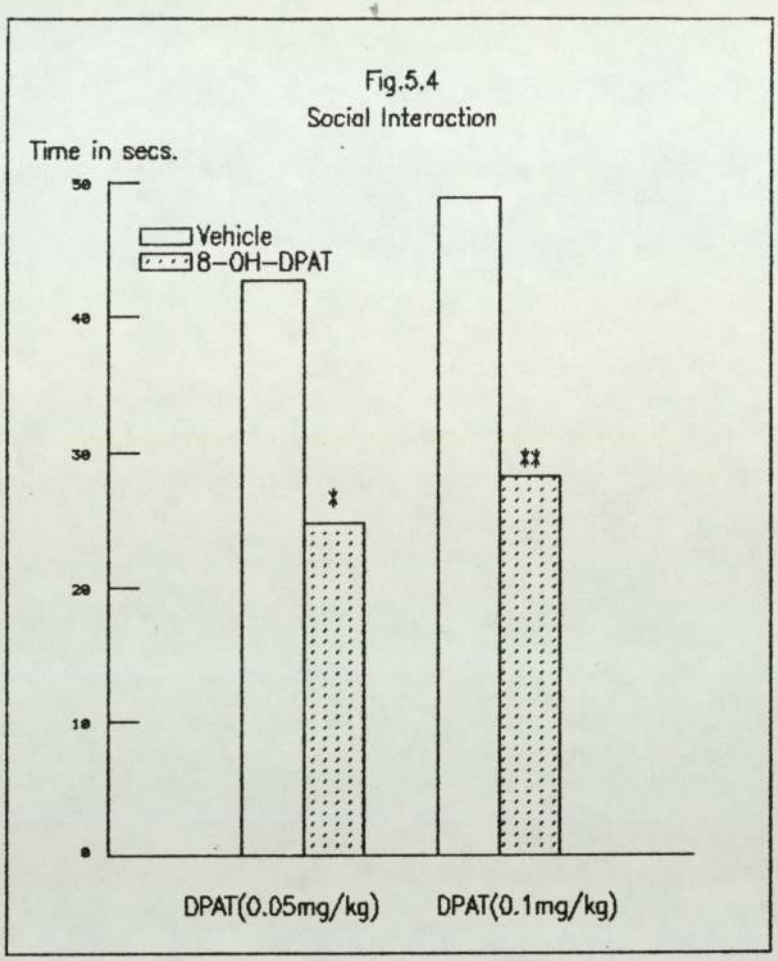


Fig.5.4. The effect of **8-OH-DPAT** (0.05 & 0.1 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

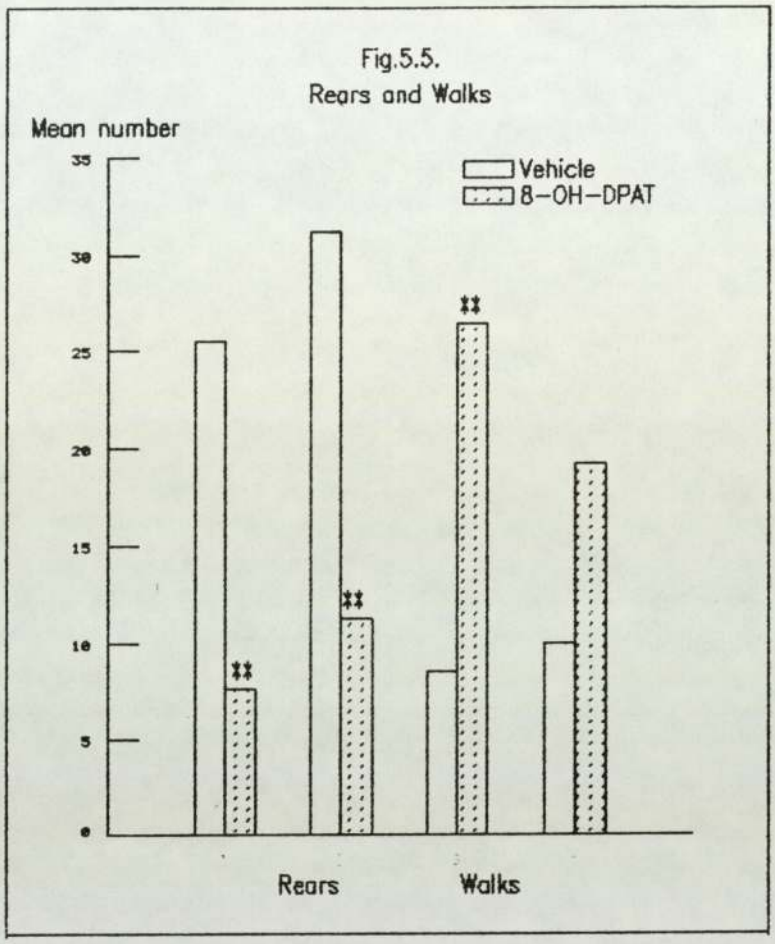


Fig.5.5. The effect of **8-OH-DPAT** (0.05 & 0.1 mg/kg) on the number of walks and rears in the social interaction test compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

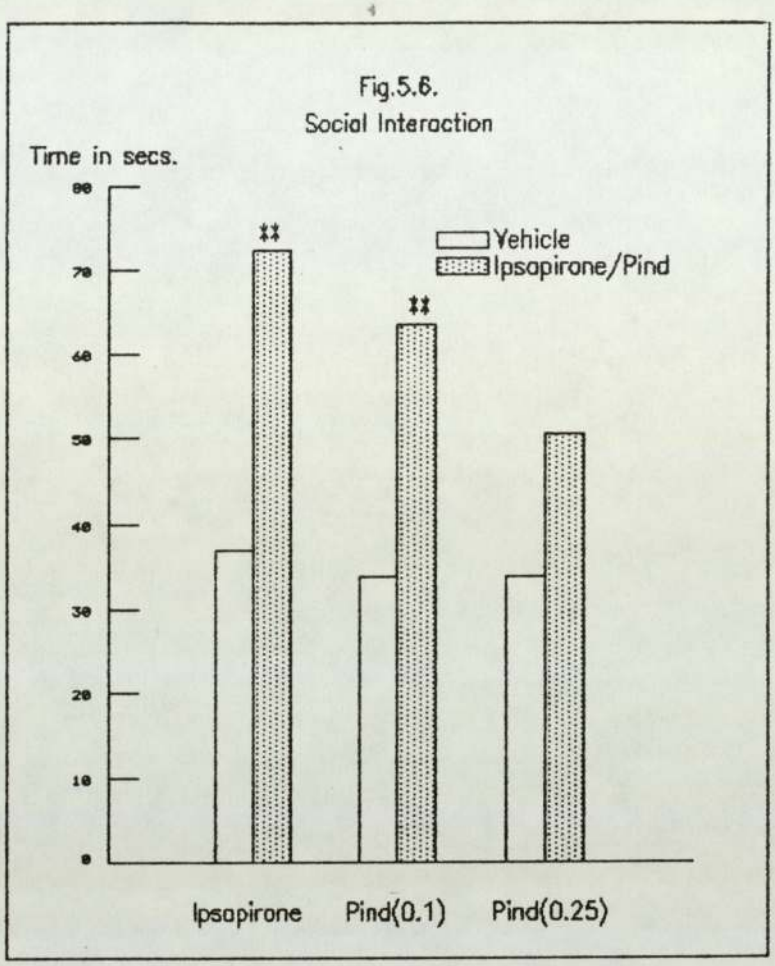


Fig.5.6. The effect of **ipsapirone** (1.0 mg/kg) and **pindolol** (0.1 & 0.25 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test.

** = $p<.01$.

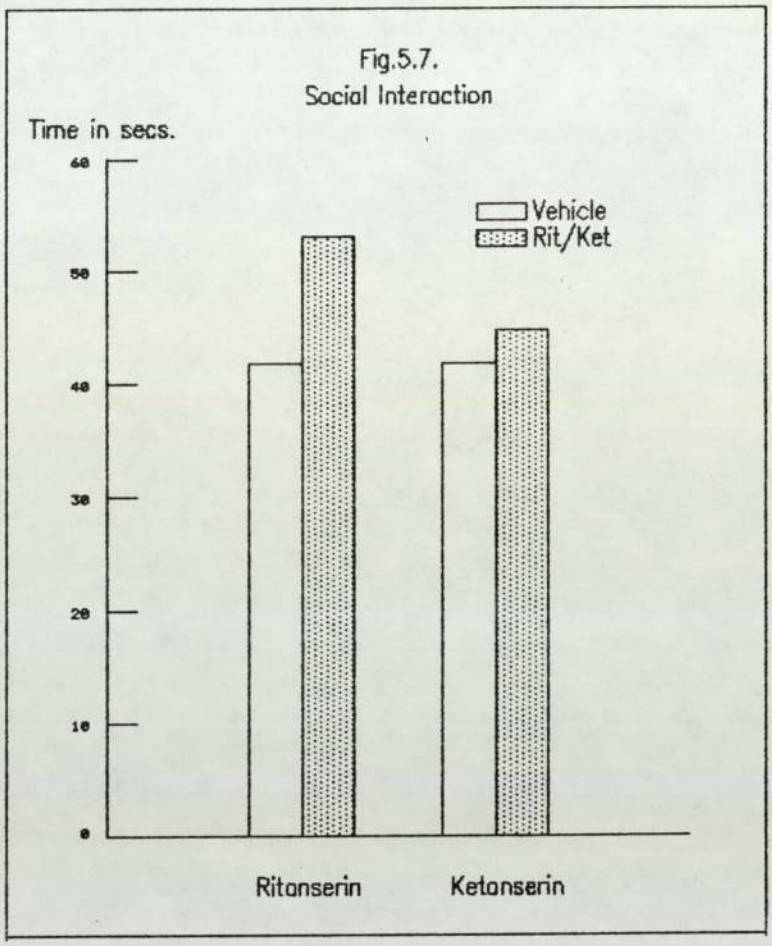


Fig.5.7. The effect of **ritanserin** (1.0 mg/kg) and **ketanserin** (0.5 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test.

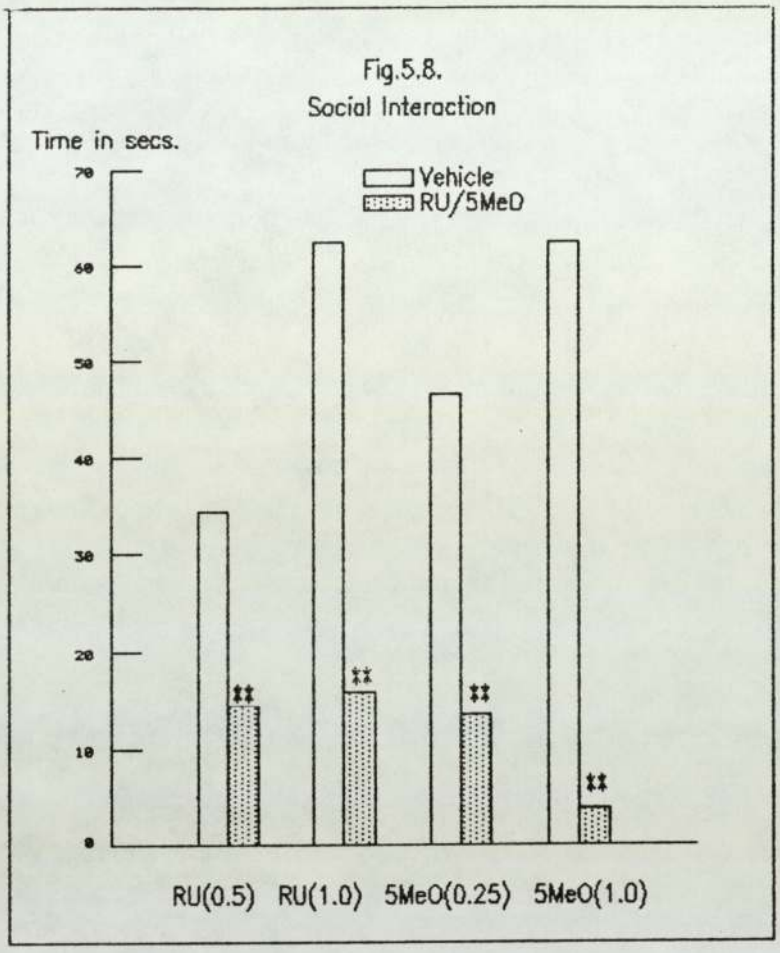


Fig.5.8. The effect of **RU24969** (0.5 & 1.0 mg/kg) and **5MeODMT** (0.25 & 1.0 mg/kg) on the time spent in active social interaction of pairs of male rats compared with concurrently run vehicle controls. Statistical comparisons were made on raw data using a t-test. ** = $p<.01$.

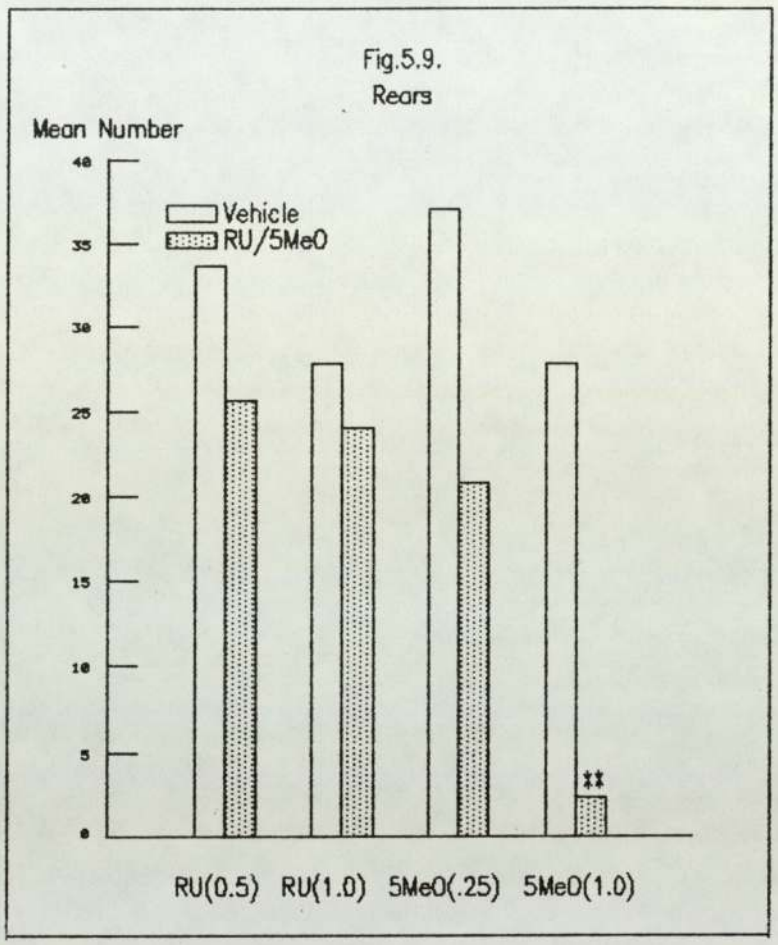


Fig.5.9. The effect of **RU24969** (0.5 & 1.0 mg/kg) and **5MeODMT** (0.25 & 1.0 mg/kg) on the number of rears in the social interaction test compared with vehicle controls. Statistical comparisons were made on raw data using a t-test. ** = $p<.01$.

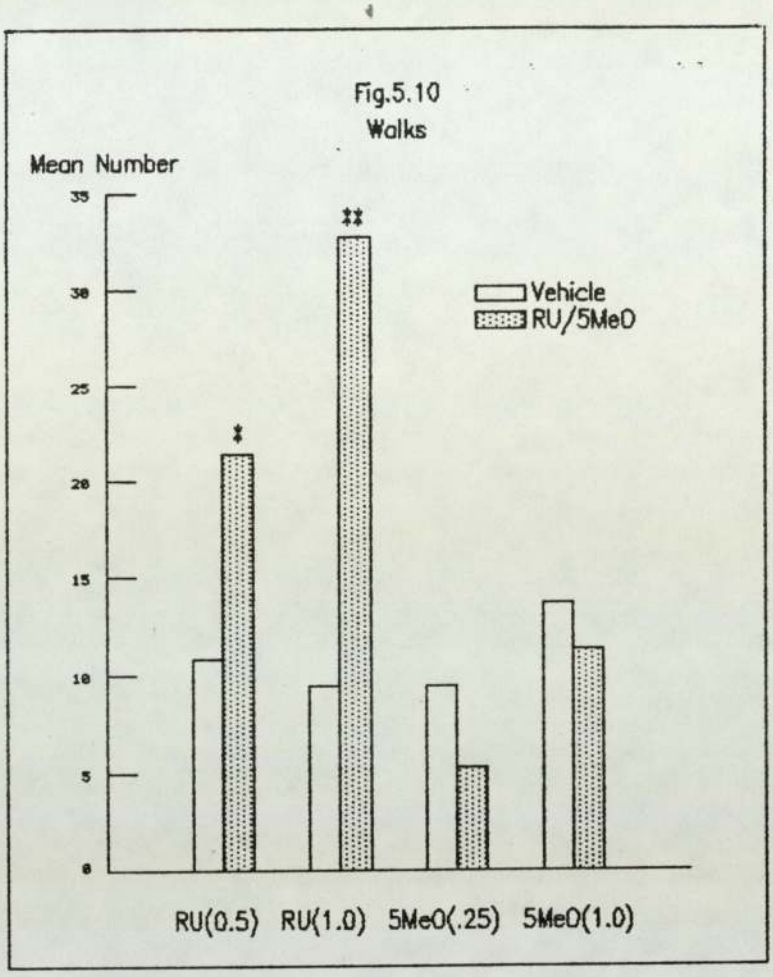


Fig.5.10. The effect of **RU24969** (0.5 & 1.0 mg/kg) and **5MeODMT** (0.25 & 1.0 mg/kg) on the number of walks in the social interaction test compared with vehicle controls. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ ** = $p<.01$.

Table 5.1.

Drug/dose	S.I.	Rears	Walks
diazepam			
0.5mg/kg	47.8±6.4	31.3±3.9	11.8±2.1
	(36.9±3.2)	(36.0±2.3)	(9.3±1.5)
1.0mg/kg	61.5±4.8*	19.0±2.3	11.2±0.8
	(42.6±5.2)	(25.5±2.3)	(8.5±1.0)
2.0mg/kg	53.7±10.1	10.7±2.8**	13.5±3.3
	(48.8±4.9)	(31.2±3.7)	(10.0±1.8)
βCCE			
0.5mg/kg	25.6±6.1*	33.0±2.1	15.3±2.1
	(46.5±4.8)	(37.0±2.8)	(13.7±1.6)
1.0mg/kg	18.4±4.1*	32.0±2.9	11.1±2.1
	(34.4±4.3)	(33.6±3.1)	(10.8±1.2)
8-OH-DPAT			
0.05mg/kg	24.8±5.5*	7.6±1.5**	26.4±3.4**
	(42.6±5.2)	(25.5±2.3)	(8.5±1.0)
0.1mg/kg	28.2±2.4**	11.2±2.1**	19.2±3.6
	(48.8±4.9)	(31.2±3.7)	(10.0±1.8)
ipsapirone			
1.0mg/kg	72.3±6.2**	24.6±3.9	6.3±0.30
	(36.9±3.2)	(36.0±2.3)	(9.3±1.5)
pindolol			
0.1mg/kg	63.6±6.9**	29.8±1.6	10.7±1.6
	(33.8±3.7)	(31.5±1.5)	(8.9±0.5)
0.25mg/kg	50.6±5.5	28.0±2.3	10.8±1.8
	(33.8±3.7)	(31.5±1.5)	(8.9±0.5)

Table 5.1. Mean time spent in social interaction (S.I., secs.), number of rears and number of walks in a 5 minute test session for pairs of male PVG rats. Vehicle control values in brackets. * = $p<.05$ ** = $p<.01$ t-test relative to vehicle controls.

Table 5.2.

Drug/dose	S.I.	Rears	Walks
ritanserin			
1.0mg/kg	53.0±8.3	37.4±3.6	8.8±2.5
	(41.8±6.5)	(33.9±1.3)	(10.3±2.0)
ketanserin			
0.5mg/kg	44.6±9.2	37.0±1.2	8.0±2.0
	(41.8±6.5)	(33.9±1.3)	(10.3±2.0)
5MeODMT			
0.25mg/kg	13.6±1.7**	20.8±3.3	11.3±0.5
	(46.5±4.8)	(37.0±2.8)	(13.7±1.6)
1.0mg/kg	3.8±1.6**	2.3±0.8**	5.3±1.5
	(62.4±5.8)	(27.8±1.7)	(9.4±1.3)
RU24969			
0.5mg/kg	14.6±3.9**	25.6±1.5	21.4±2.9*
	(34.4±4.3)	(33.6±3.1)	(10.8±1.2)
1.0mg/kg	15.9±2.7**	24.0±3.2	32.7±3.2**
	(62.4±5.8)	(27.8±1.7)	(9.4±1.3)

Table 5.2. Mean time spent in social interaction (S.I., secs.), number of rears and number of walks in a 5 minute test session for pairs of male PVG rats. Vehicle control values in brackets. * = $p<.05$ ** = $p<.01$ t-test relative to vehicle controls.

CHAPTER 6.

OPERANT CONFLICT MODELS OF ANXIETY.

Introduction.

Operant conditioning techniques, since their description by Skinner in the 1950's, have provided a basis for experimental analysis of anxiety in animals. The tests used vary widely from the simple to the extraordinarily complex and were employed in most of the early studies into the role of 5-HT in anxiety in the 1960's and early 1970's.

Recently questions have been raised as to the validity of studying anxious behaviour motivated by reward and punishment (see General Introduction). The use of such obviously 'un-natural' and physiologically non-relevant techniques as electric shock and strict training regimens can be questioned (See General Introduction, section 7.3). For these reasons new models have been developed using more physiologically relevant procedures. However, in a study such as the present it was felt that some examination of operant techniques was warranted, considering the vast amount of data already obtained as to the role of 5-HT in anxiety using such models. It was hoped that the results with the operant paradigms would shed further light on the results already obtained and perhaps help to clarify some of the problems previously described with these models.

One of the earliest, and most widely used models, was the Geller-Seifter test (*Geller and Seifter, 1960*) which was therefore examined here. As a result of studies undertaken previously in this

laboratory (*Mithani, 1984*) a wholly variable interval schedule was also examined. This schedule enables a direct comparison to be made between responding during each of the components of the schedule and facilitates the setting of baseline levels by titrating the level of lever presses against the duration and intensity of electric footshock.

The format of the experiments described here compares the responses made on the drug treatment day with the previous vehicle treatment day and also the subsequent vehicle treatment day. Shock level was set such that approximately 50% suppression was maintained. As usual diazepam was used as the standard anxiolytic agent and the effects of the 5-HT_2 receptor antagonists ritanserin, ketanserin and seganserin were examined. Several of the 5-HT agonists used in the elevated X-maze (8-OH-DPAT, RU24969 and 5MeODMT) were also examined here.

Results

6.1. The effect of diazepam on the variable interval schedule.

Diazepam (0.5 - 5.0 mg/kg) produced no changes in the number of lever presses in the unpunished component when compared with the previous vehicle control day, and the following vehicle control day (Fig.6.1).

On the punished responding component of the schedule diazepam significantly increased lever presses at all doses compared with vehicle control days (Fig.6.2). Diazepam also increased the number of lever presses in the extinction component of the schedule (Fig.6.3). See Table 6.1. for raw data. It is interesting to note that after the increase in responding on day 2, responding on the 3rd vehicle-injection day fell to below normal control levels.

6.2. The effect of ritanserin, ketanserin and seganserin on the variable interval schedule.

Ritanserin (0.1 - 5.0 mg/kg) overall produced a selective increase in punished responding (Fig.6.5). However, this effect was only significant at two of the doses used (1.0 & 5.0 mg/kg) with no effect seen at 2.5 mg/kg. Unpunished responding was also slightly increased at 0.5, 1.0 & 5.0 mg/kg (Fig.6.4). No significant changes were seen in extinction responding (Table 6.2).

Ketanserin (0.1 mg/kg) produced no change in either punished or unpunished responding but produced a small fall in extinction responding (see Table 6.3).

Seganserin (2.0 mg/kg) had no effect on either unpunished or punished responding but produced a slight increase in both at 1.0 mg/kg (Table.6.3.). No significant effects were seen on extinction responding (Table 6.3).

6.3. The effect of 8-OH-DPAT, RU24969 and 5MeODMT on the variable interval schedule.

8-OH-DPAT (0.03 - 0.1 mg/kg) produced a fall in the number of unpunished lever presses (Fig.6.5), whilst producing no effect in the responding during the punished segments (Fig.6.6) compared with vehicle control days. Fig.6.7 illustrates the effects of 8-OH-DPAT on extinction responding. At the two higher doses a slight elevation of lever pressing is seen.

RU24969 (0.5 - 1.0 mg/kg) had no effect on either unpunished or punished responding but produced an increase in extinction responding at the lower dose (0.5 mg/kg). see Table 6.4.

5MeODMT (2.0 mg/kg) had no effect on any of the parameters measured (Table 6.4).

6.4. Results with the Geller-Seifter test and the continuous reinforcement schedule.

Ritanserin (0.5 - 2.5 mg/kg) was largely inactive in both the Geller-Seifter test and in the continuous reinforcement schedule with the exception of one dose (0.1 mg/kg) in the former test where it produced a significant increase in punished responding (Table 6.5). In the latter test, punished responding was maintained at a very low level.

Discussion

Upon initial examination diazepam appears to have a selective effect upon the punished responding component of the schedule, which is what would be expected of an anxiolytic compound. However, responding during the extinction component also increased to a level comparable to that in the punished component. Such an effect with diazepam has previously been demonstrated both with a schedule identical to the one used here, and with other conflict schedules (*Mithani, 1984*). This phenomenon may possibly be explained by the factors discussed by Soubrié (1986a; 1986b) and Thiébot et al., (1985). Benzodiazepines were suggested to lessen the ability of the animal to tolerate a delay for a reward and would therefore produce an increase in responding when rewards are witheld as in the extinction component. This inability to wait may then override the tendency of the punishment to suppress responding. This is more likely to occur in non-continuous reinforcement schedules.

The effects of ritanserin and the other 5-HT_2 antagonists on this model are difficult to explain. As in the elevated X-maze model ritanserin seemed to possess the greater anxiolytic-like effect. However, in the conflict model the effect was not repeatable and did not appear to be dose related since there was a slight increase in punished responding at 0.1, 1.0 and 5.0 mg/kg, but not at 0.5 or 2.5 mg/kg. It also had some effect on unpunished responding; being increased at three doses. It is important to note, however, that the significant effects gained with ritanserin appeared to be largely

due to changes in control responding, the absolute values on ritanserin-injection days remaining unchanged. Also, this pattern is different from the pattern seen with diazepam since the extinction responding did not increase in similar manner. In fact at the three doses producing an increase in unpunished responding, extinction responding remained unchanged at one dose, increased at another and decreased at the third.

Ketanserin and seganserin were largely inactive with the only possible effects being an overall increase in activity with seganserin and a decrease with ketanserin. Together with the lack of effects on the Geller-Seifter test these results tend to agree with the numerous other studies indicating no consistent anxiolytic activity with 5-HT_1/5-HT_2 antagonists in conflict paradigms (see *Gardner, 1985*).

The continuous reinforcement schedule appears not to be suitable for such studies as these since the administration of footshock contingent upon every response suppressed responding to unacceptably low levels. The punished component of the Geller-Seifter test, of course, also contains continuous reinforcement, but the responding is not suppressed to such an extent. A possible explanation of this is that the presence of rewards upon every lever press in the unpunished component of the CRF schedule, decreases the drive of the rat to lever-press.

Little work has been carried out using 8-OH-DPAT in conflict tests with the exception of the studies of Engel and colleagues. Using a modified version of the punished drinking paradigm (*Vögel et al., 1971*) an increase in the number of shocks accepted was seen (*Engel et al., 1984*). No effect was seen on punished responding in the pesent study. Since the punished drinking schedule contains no unpunished component no parallel can be drawn with the decrease in responding seen in the present study.

The greatest fall in responding was seen at the highest dose studied (0.1 mg/kg), both in unpunished and extinction responding. This could be due to the disruption of behaviour by the presence of the behavioural syndrome. The dose of 0.1 mg/kg was the threshold dose for the appearance of the 'syndrome' with 8-OH-DPAT. However, at the lower doses, no 'syndrome' effects were seen but unpunished responding was still decreased.

RU24969 has previously been shown to possess little activity in conflict tests (*Shephard et al., 1982*), a fact with which the present study agrees. An anxiogenic-like reduction in punished responding has been observed with RU24969 (see *Gardner, 1985*) but hyperlocomotion may have disrupted behaviour. This may have been the case in the present study since the doses employed were the same as those used by Gardner, and increases in unpunished and extinction responding were observed. Likewise 5MeODMT showed little activity here and agrees with previous studies (*Shephard et al., 1982*) indicating a slight fall in unpunished responding.

The results described here indicate the danger of relying on tests which have been developed over the years because of their sensitivity to benzodiazepine-like compounds. Schedules without the extinction component would indicate that the model is selectively sensitive to anxiolytic actions of benzodiazepines. Such models run the risk of being unable to detect new classes of anxiolytic compounds. For this reason new models are being increasingly used in the investigations of novel putative anxiolytic compounds.

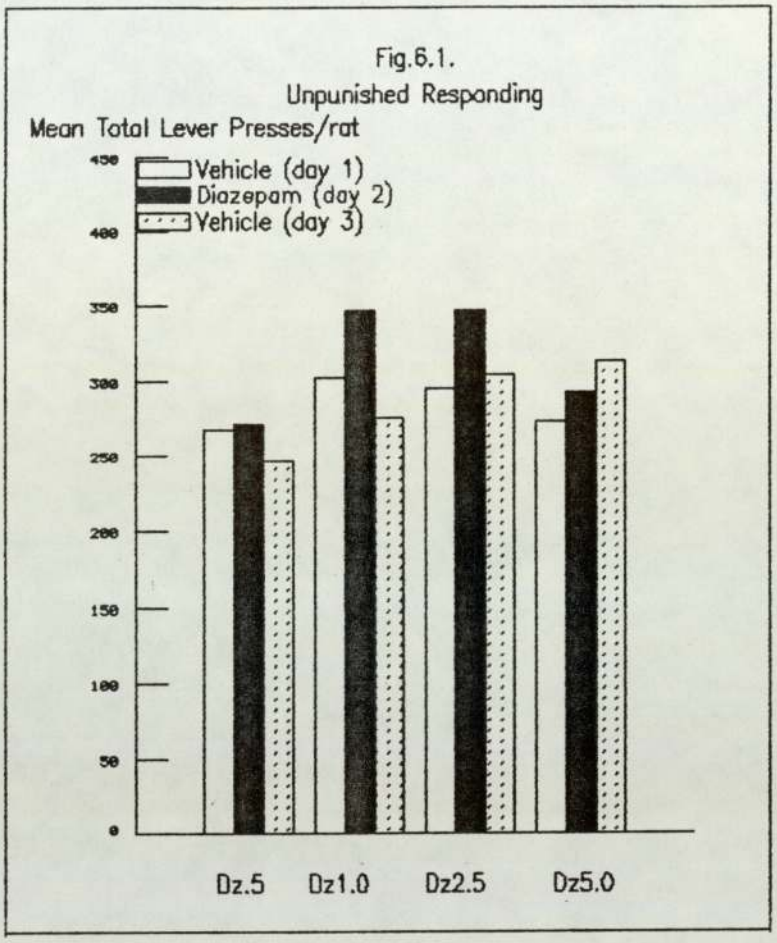


Fig.6.1. The effect of **diazepam** (0.5, 1.0, 2.5 & 5.0 mg/kg) on unpunished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA followed by Tukey's test for Unconfounded means versus day 1 control.

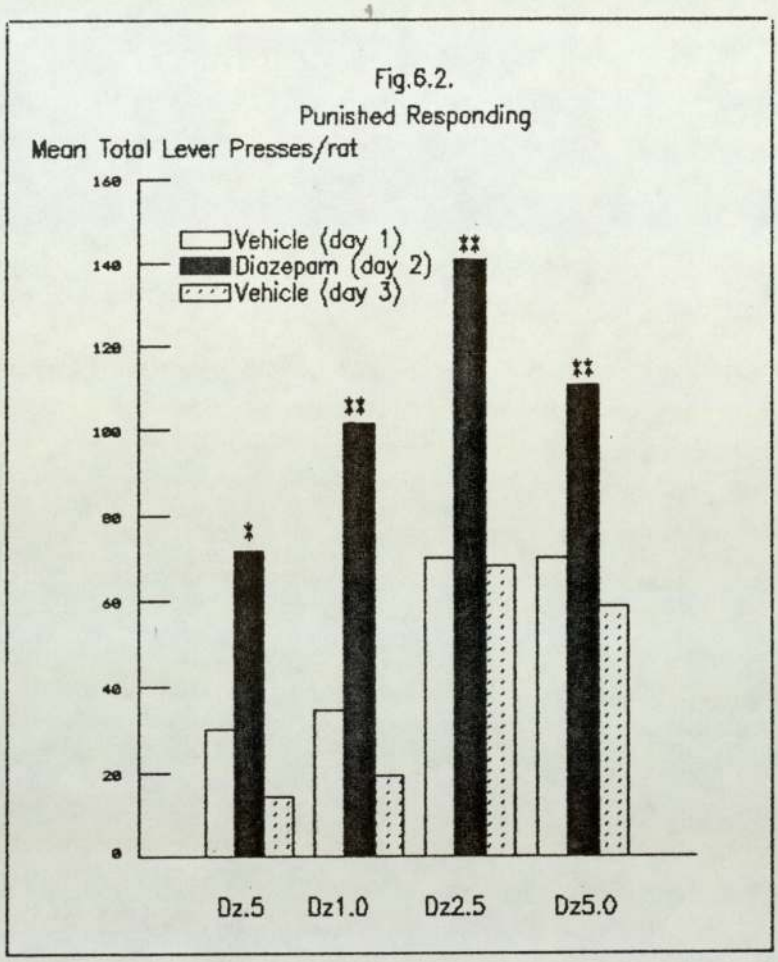


Fig.6.2. The effect of **diazepam** on punished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA followed by Tukey's test * = $p<.05$ ** = $p<.01$ versus day 1 control.

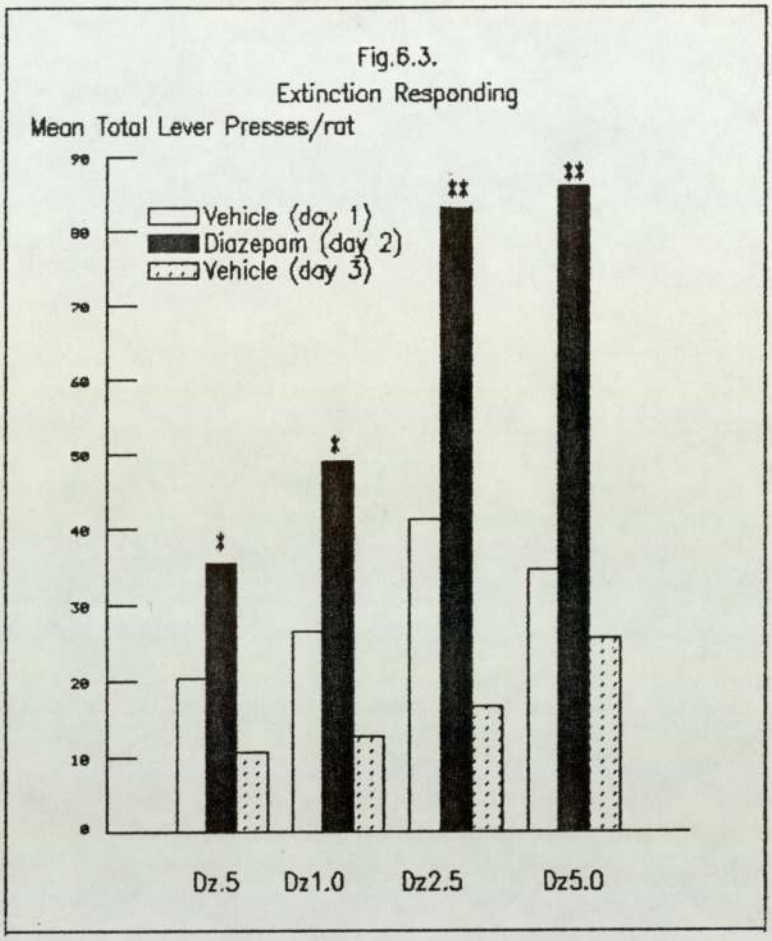


Fig.6.3. The effect of **diazepam** on extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA followed by Tukey's test * = $p<.05$ ** = $p<.01$ versus day 1 control.

Table 6.1.

Mean total lever presses per rat (±sem).

Treatment (dose mg/kg)	Unpunished Responding	Punished Responding	Extinction Responding
Diazepam			
vehicle(day 1)	267.0±41.3	30.3±8.8	20.4±4.6
diazepam(0.5)	270.6±40.7	71.7±12.7*	35.6±8.6*
vehicle(day 3)	246.8±17.5	14.0±4.3	10.6±3.1
vehicle	302.0±38.6	34.7±10.6	26.5±5.7
diazepam(1.0)	346.7±43.2	101.3±17.5**	49.0±9.7*
vehicle	275.3±24.9	19.3±4.3	12.7±2.4
vehicle	295.0±39.7	69.8±14.7	41.4±7.5
diazepam(2.5)	347.0±53.3	140.8±18.5**	83.0±11.8**
vehicle	304.0±35.8	68.0±13.9	16.6±4.7
vehicle	272.8±25.9	70.0±14.3	34.7±6.9
diazepam(5.0)	292.4±45.6	110.7±16.5**	86.0±10.2**
vehicle	313.3±46.5	58.6±14.1	25.6±5.5

Table 6.1. The effect of **diazepam** (0.5, 1.0, 2.5 & 5.0 mg/kg) on unpunished, punished and extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA followed by Tukey's test for unconfounded means. * = $p<.05$ ** = $p<.01$ versus day 1 control.

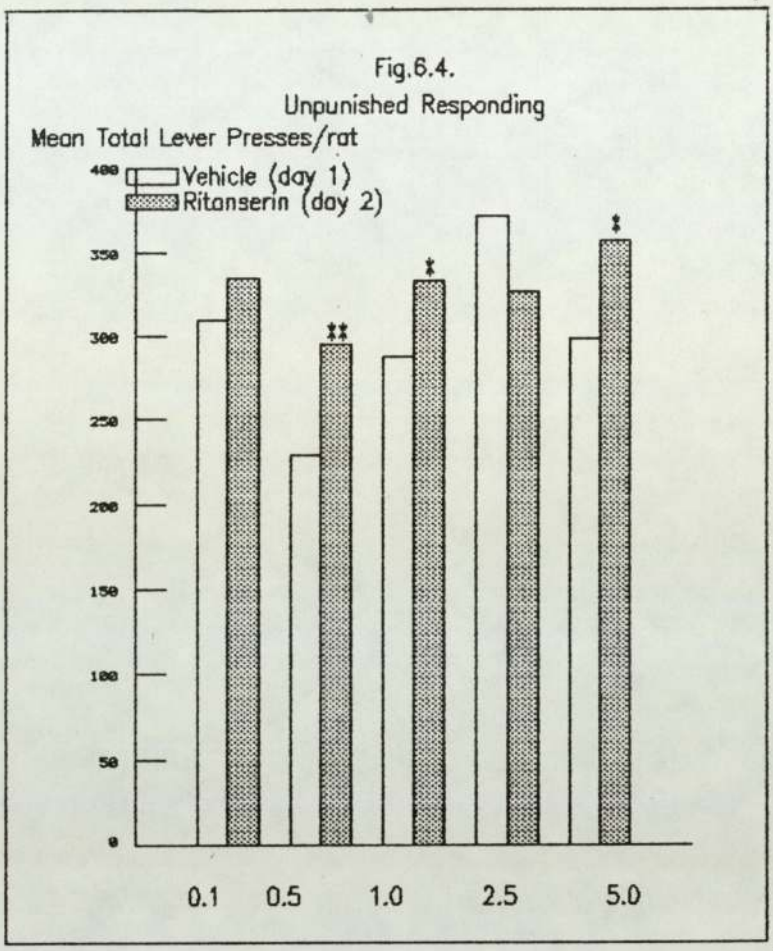


Fig.6.4. The effect of **ritanserin** (0.1, 0.5, 1.0, 2.5 & 5.0 mg/kg) on unpunished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA versus day 1 control.

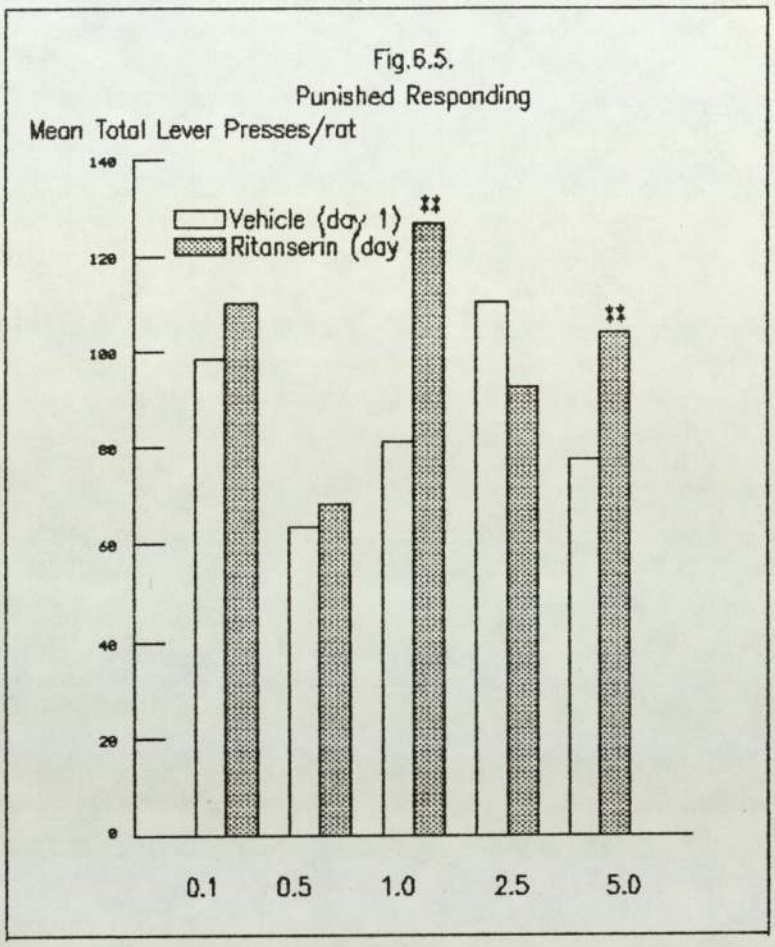


Fig.6.5. The effect of **ritanserin** on punished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA 1. * = $p<.05$ ** = $p<.01$ versus day 1 control.

Table 6.2.

Mean total lever preses per rat (±sem).

Treatment (dose mg/kg)	Unpunished Responding	Punished Responding	Extinction Responding
Ritanserin			
vehicle	310.0±24.5	96.6±9.4	22.6±3.2
ritanserin(0.1)	334.3±28.8	110.2±7.0	35.2±6.3
vehicle	352.8±33.7	120.5±13.0	40.4±5.1
vehicle	228.5±21.5	63.4±14.7	42.8±8.2
ritanserin(0.5)	295.4±33.7**	68.3±12.2	30.3±8.2
vehicle	242.0±27.0	42.8±15.3	10.3±2.2
vehicle	288.3±32.0	81.3±8.6	20.0±2.8
ritanserin(1.0)	332.7±36.3*	126.7±10.3**	36.4±6.7
vehicle	298.8±30.8	98.3±14.0	31.6±6.4
vehicle	371.8±53.9	110.3±18.1	29.5±4.0
ritanserin(2.5)	326.5±43.3	92.8±16.2	27.7±5.0
vehicle	295.7±48.0	85.3±17.5	26.1±3.9
vehicle	298.7±25.5	77.5±12.9	31.7±6.3
ritanserin(5.0)	356.7±51.7*	104.0±19.3**	32.4±6.4
vehicle	279.3±33.7	53.4±16.7	21.0±2.5

Table 6.2. The effect of **ritanserin** (0.1, 0.5, 1.0, 2.5 & 5.0 mg/kg) on unpunished, punished and extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA. * = $p<.05$ ** = $p<.01$ versus day 1 control.

Table 6.3

Mean total lever presses per rat (±sem)

Treatment (dose mg/kg)	Unpunished Responding	Punished Responding	Extinction Responding
Ketanserin			
vehicle	251.5±52.2	31.6±10.3	31.3±6.3
ketanserin(0.1)	223.0±39.4	35.8±10.9	18.0±3.7
vehicle	242.3±42.8	41.8±14.9	30.3±8.2
Seganserin			
vehicle	394.8±42.3	116.0±19.4	29.0±6.6
seganserin(1.0)	441.7±52.0*	182.1±15.1**	44.7±10.8
vehicle	325.0±44.0	119.8±18.8	27.1±3.6
vehicle	397.0±48.8	147.5±23.0	53.0±10.3
seganserin(2.0)	376.4±64.1	154.0±27.2	47.6±8.7
vehicle	412.1±61.2	151.1±23.5	43.3±8.1

Table 6.3. The effect of ketanserin (0.1 mg/kg) and **seganserin** (1.0 & 2.0 mg/kg) on unpunished, punished and extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA. * = $p<.05$ ** = $p<.01$ versus day 1 control.

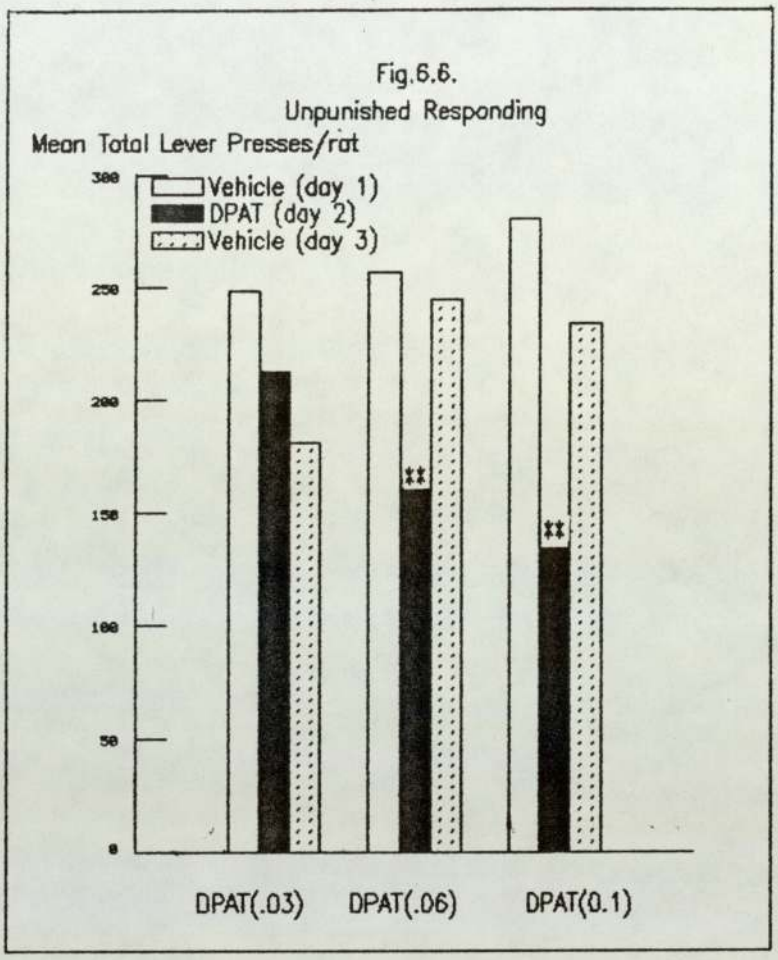


Fig.6.6. The effect of **8-OH-DPAT** (.03, .06 & .10 mg/kg) on unpunished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA. * = $p<.05$ ** = $p<.01$ versus day 1 control.

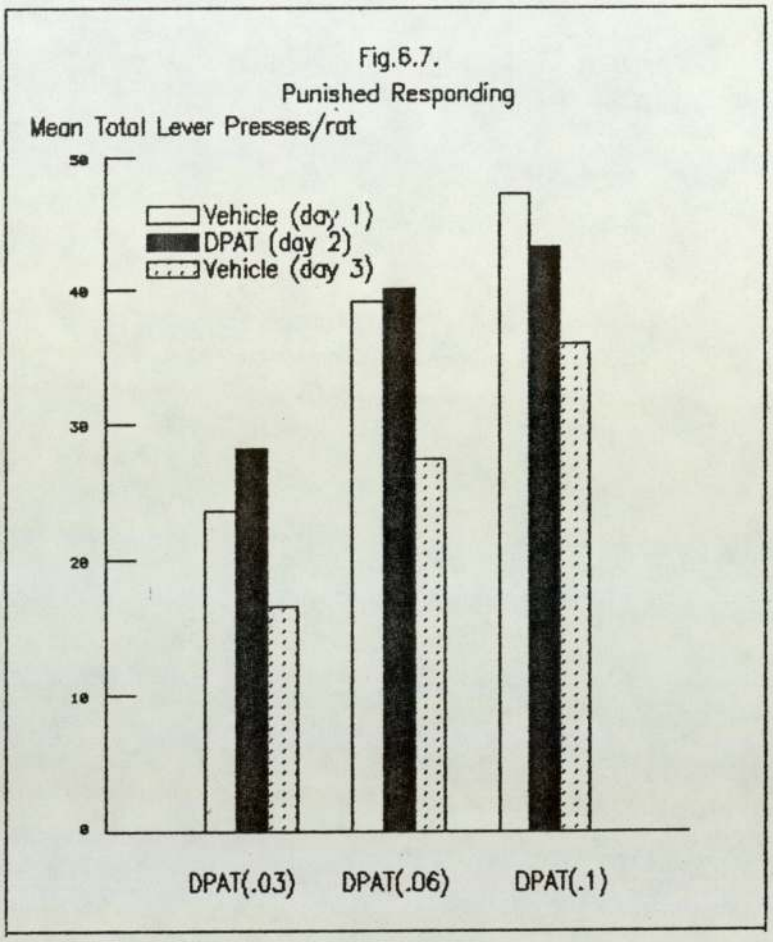


Fig.6.7. The effect of **8-OH-DPAT** on punished responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA versus day 1 control.

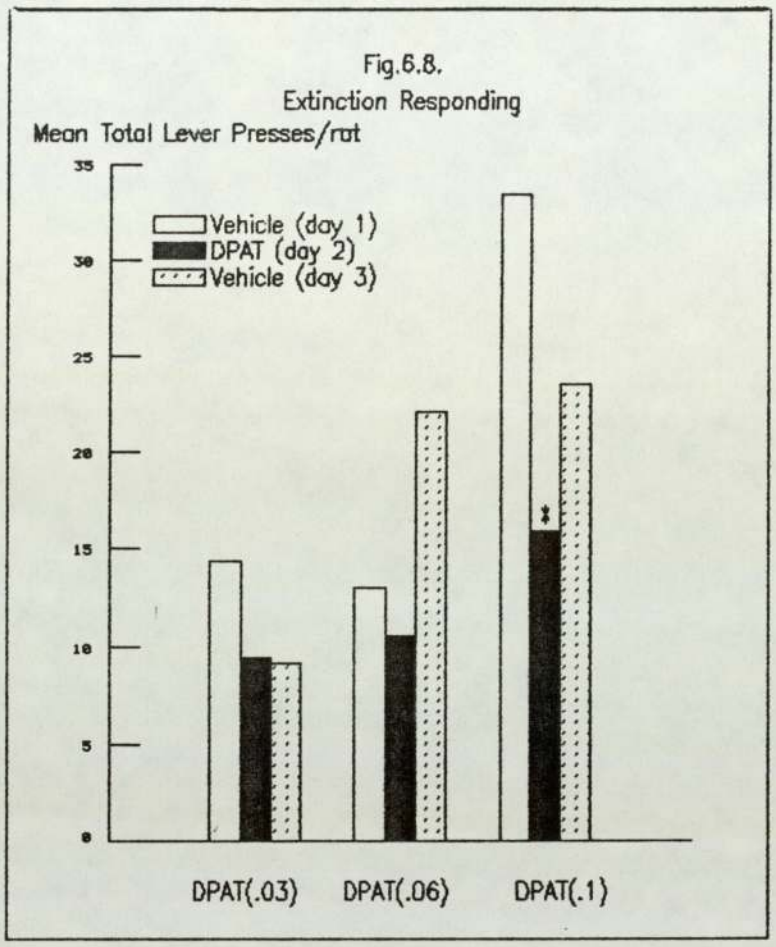


Fig.6.8. The effect of **8-OH-DPAT** extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA. * = $p<.05$ ** = $p<.01$.

Table 6.4. Mean total lever presses per rat (±sem)

Treatment (dose mg/kg)	Unpunished Responding	Punished Responding	Extinction Responding
8-OH-DPAT			
vehicle	247.8±27.5	23.6±8.5	14.4±5.2
DPAT(.03)	212.4±35.5	28.1±12.2	9.4±3.0
vehicle	181.1±30.9	16.5±8.5	9.1±3.9
vehicle	256.5±25.2	39.1±10.4	13.0±5.1
DPAT(.06)	160.8±32.2**	40.0±12.8	10.6±3.5
vehicle	244.3±27.9	27.3±9.7	22.1±6.0
vehicle	280.6±21.6	47.1±9.3	33.4±5.7
DPAT(.10)	133.4±23.7**	43.1±10.2	15.9±4.3*
vehicle	233.2±15.7	35.9±9.8	23.5±4.5
RU24969			
vehicle	380.8±40.4	53.5±9.6	42.7±11.9
RU24969(0.5)	354.4±42.5	49.4±17.5	82.8±23.3
vehicle	297.4±38.7	23.3±7.2	26.6±8.3
vehicle	397.3±41.9	126.8±14.5	81.8±20.1
RU24969(1.0)	460.3±35.5	171.3±20.4	89.8±14.9
vehicle	321.6±35.5	90.6±20.3	51.5±7.9
5MeODMT			
vehicle	436.2±43.6	128.3±25.5	86.8±26.9
5MeO(2.0)	301.2±42.3*	96.8±20.9	66.0±11.0
vehicle	380.2±30.0	107.6±16.3	45.8±10.0

Table 6.4. The effect of **8-OH-DPAT, RU24969 & 5MeODMT** on unpunished, punished and extinction responding in the variable interval punished schedule. Statistical comparisons were made on raw data using a 2-way ANOVA. * = $p<.05$ ** = $p<.01$ versus day 1 control.

Table 6.5.

Mean total lever presses per rat (±sem)

Treatment (dose mg/kg)	Unpunished Responding	Punished Responding
Geller-Seifter		
vehicle	660.0±65.8	34.2±7.5
ritanserin(0.1)	752.0±72.5	70.0±7.5*
vehicle	784.6±68.4	71.8±8.6
vehicle	427.5±34.8	43.3±11.4
ritanserin(1.0)	402.1±65.4	34.6±10.8
vehicle	372.8±31.8	30.6±8.9
CRF		
vehicle	114.5±17.3	8.9±1.0
ritanserin(0.1)	98.1±19.1	6.8±2.0
vehicle	109.0±15.3	9.0±1.5
vehicle	99.8±9.1	6.4±0.9
ritanserin(2.5)	59.8±6.9	1.9±0.5
vehicle	86.5±17.9	2.0±0.6

Table 6.5. The effects of ritanserin on punished and unpunished responding in the **Geller-Seifter** test and in the continuous reinforcement schedule **(CRF)**. Statistical comparisons were made on raw data using a t-test. * = $p<.05$ versus day 1 control.

CHAPTER 7

THE EFFECT OF DORSAL RAPHÉ NUCLEUS (DRN) LESIONS ON 5-HT LIGAND EFFECTS IN THE ELEVATED X-MAZE.

Introduction

The majority of 5-HT containing cells in the brain are contained within a group of nuclei in the brainstem called the raphé nuclei. Although there are nine of these nuclei (see Introduction section 2.) 80% of forebrain 5-HT is contained within just two of them, the dorsal raphé nucleus (DRN), and the median raphé nucleus (MRN) (*Azmitia, 1978; Dahlstrom and Fuxe, 1965*). Previous studies using selective lesions have produced varied results. Dorsal raphé lesions have been found to abolish the effect of chlordiazepoxide on punished responding (*Thiébot et al., 1982; Soubrié et al., 1981*) to increase punished responding (*Green and Hodges, 1986*) and to increase social interaction (*File et al., 1979*). However, dorsal raphé lesions have been reported to have no effect on behavioural inhibition, and to have no effect on the ability of diazepam to release punished responding (*Thiébot et al., 1984*), and combined dorsal/median raphé lesions had no effect on the Social Interaction test (*File and Deakin, 1980*).

The effect of DRN lesions on exploration in the elevated X-maze was examined. The anxiogenic response to 8-OH-DPAT and the anxiolytic response to ipsapirone were examined in DRN-lesioned and sham-operated rats.

The neurotoxin used, 5,7-dihydroxytryptamine, is relatively selective against 5-HT-containing neurones, although some action on noradrenergic systems cannot be discounted.

A total of 24 animals were used and divided into four experimental groups of 6; vehicle-sham, vehicle-lesion, drug-sham and drug-lesion. In the second and third experiments animals were used in the same order as on the first exposure to the maze i.e. vehicle-sham rats received vehicle on all exposures and 8-OH-DPAT-treated rats received ipsapirone on the second and yohimbine on the third exposure. Rats were tested in the X-maze 21 days after lesioning and biochemical analyses were made 14 days after first exposure to the maze.

Biochemical analysis of the striatal and hippocampal regions of the rat brains was carried out in order to verify the correct positioning of the lesions. The striatum is principally innervated by fibres originating in the DRN, and the hippocampus by fibres originating in the MRN (*Azmitia, 1978; Geyer et al., 1976b*). Selective lesions of the DRN should therefore produce a greater depletion in the striatum. Significant depletion in the hippocampus would suggest some damage to the MRN.

Finally, since there have recently been suggestions that 8-OH-DPAT may have some α_2-adrenoceptor antagonist properties (*Crist and Suprenant, 1987*), the effect of an α_2-antagonist, yohimbine, was examined in the lesioned rats.

Results

7.1. The effect of DRN lesions on the anxiogenic response to 8-OH-DPAT.

Vehicle-sham groups produced a 'normal' control open/total ratio of approximately 0.3, as did the vehicle-lesion group, indicating no effects on anxiety measures by DRN lesions alone. 8-OH-DPAT-sham groups produced the typical anxiogenic-like response normally seen with 8-OH-DPAT throughout this study (8-OH-DPAT dose = 0.1 mg/kg). However, in the 8-OH-DPAT-lesion group, the anxiogenic response was reversed to become an anxiolytic-like response in those rats shown to have a significant depletion of striatal 5-HT levels. Animals in which 5-HT levels were not significantly depleted produced a 'normal' anxiogenic response to 8-OH-DPAT (Fig.7.1, Table 7.1).

No effects were seen on total number of entries made in any of the four groups (Table 7.1).

7.2. The effect of DRN lesions on the anxiolytic response to ipsapirone.

Ipsapirone (1.0 mg/kg) produced an anxiolytic-like response, elevating the entry ratio in sham-operated animals, but this effect was abolished in DRN-lesioned animals (Fig.7.2., Table 7.2).

No significant effects were seen on total number of entries after either ipsapirone or DRN lesions (Table 7.2).

7.3. The effect of DRN lesions on the anxiogenic response to yohimbine.

Yohimbine (0.5 mg/kg) produced an anxiogenic-like fall in entry ratio in sham-operated rats, an effect which was present to an equal extent in the raphé lesioned group of rats (Fig. 7.3.). No changes in total entries were observed in either group (Table 7.3.)

7.4. The effect of DRN lesions on hippocampal and striatal 5-HT levels.

Overall, 5-HT levels were depleted by 63% in the striatum and 11% in the hippocampus. Of the 10 rats given 5,7-DHT 2 showed no depletion of 5-HT levels and 1 showed partial depletion in both striatum and hippocampus. If results from these rats were discounted the mean depletion figures were 73% for the striatum and 8% for the hippocampus.

Overall sham means were as follows:

Hippocampus - 837.4 (±36.6) ng/g

Striatum - 930.4 (±48.5) ng/g

Means for successfully lesioned rats were as follows:

Hippocampus - 769.5 (±53.3) ng/g

Striatum - 257.6 (±26.0) ng/g $p<.01$ (vs. sham striatum).

Discussion

Of the 12 5,7-DHT-treated rats 3 appeared to have been unsuccessfully lesioned since striatal 5-HT levels were not significantly depleted selectively when compared with the mean sham-operated control levels. Of the rats with significant striatal depletions none were depleted to an equal extent in the hippocampus, suggesting that the lesion was selective for the DRN as opposed to the MRN, since the striatum and hippocampus are principally innervated by the DRN and MRN respectively (*Azmitia, 1978; Geyer, 1976b*). When this is considered in conjunction with the data obtained in the X-maze it can be seen that in 8-OH-DPAT-treated rats which appeared to have selective lesions of the DRN, the anxiogenic-like effect of 8-OH-DPAT was not only abolished but actually reversed to become anxiolytic-like. In the single 8-OH-DPAT-treated rat which appeared not to be successfully lesioned, the anxiogenic-like response remained unchanged. It is to be noted that there was no effect of the lesions alone on exploration in the X-maze, whereas DRN lesions have previously been reported anxiolytic (*Green and Hodges, 1986*).

It is necessary here, therefore, to examine the possible roles of the DRN and the MRN in anxiety. The majority of lesion studies in the past have tended to concentrate on raphé nuclei lesions in general and not selective DRN or MRN lesions. Combined DRN-MRN lesions resulting in 90% striatal and hippocampal depletion produced an anxiogenic response in the Social Interaction test (*File and Deakin, 1980*), which may have been due to the general hypoactivity observed,

whilst selective lesions of the MRN produced no change (*File et al., 1979*). Lesions of the DRN which also produced partial lesion in the MRN produced an anxiolytic response in the same test (*ibid*).

A number of studies have shown that the MRN has a role in response suppression since MRN lesions tend to release punishment-suppressed responding (*Srebro and Lorens, 1975; Thornton and Goudie, 1978; see Willner, 1985* for review). A reduction in 5-HT levels with pCPA has much the same effect (see General Introduction), but such a depletion would not be selective for either DRN or MRN but would deplete both. Overall it seems that depletion with pCPA or MRN lesions produce increased responsiveness and a lack of behavioural inhibition, whereas DRN lesions have little or no effect on such behaviours (see *Willner, 1985* for review). Also the behavioural effects of pCPA-induced depletion and of MRN lesions are blocked by destruction of the hippocampus (*Jacobs et al., 1975*) thus further implicating the MRN. Little work appears to have been done using selective DRN lesions and examining their effects in a number of animal models of anxiety.

It could be suggested therefore that the MRN is responsible for the results seen in anxiety models involving response suppression whereas the effects seen in animal models such as the X-maze may be mediated via the DRN, since in the present study selective DRN lesions abolished the responses to both 8-OH-DPAT and ipsapirone. Before this tentative suggestion can be discussed further it is necessary to repeat the experiments in this chapter using MRN-

lesioned animals. However, the complete reversal of the 8-OH-DPAT response from anxiogenic to anxiolytic remains to be explained. It is possible since the DRN has been destroyed that 8-OH-DPAT has, in this situation, its main action on the MRN. Also, since File and co-workers produced an anxiolytic effect in partially DRN+MRN lesioned animals (*File et al., 1979*) and an anxiogenic effect in animals depleted by 90% (*File and Deakin, 1980*), it is possible that the level of depletion may be responsible for the effects seen. The mean overall depletion in the striatum in the present study was 73%.

The fact that the ipsapirone effect was not reversed but simply abolished cannot be explained. All our results on the maze and in 'syndrome' experiments point to an antagonist action of ipsapirone presumably at the same receptor at which 8-OH-DPAT has its effect. Therefore if the 8-OH-DPAT effect is reversed by DRN lesions one would expect the ipsapirone effect to be similarly reversed, unless the anxiolytic and anxiogenic effects are mediated by seperate mechanisms.

Finally, the last experiment carried out with the lesioned animals using yohimbine was performed in order to further investigate the suggestion that 8-OH-DPAT acts as an antagonist at α_2-adrenoceptors (*Crist and Suprenant, 1987*). Since the anxiogenic-like effect seen with yohimbine was unaffected by DRN lesions this would suggest that the anxiogenic-like effect seen with 8-OH-DPAT is not an α_2-mediated effect and that the yohimbine effect does not depend upon an intact DRN.

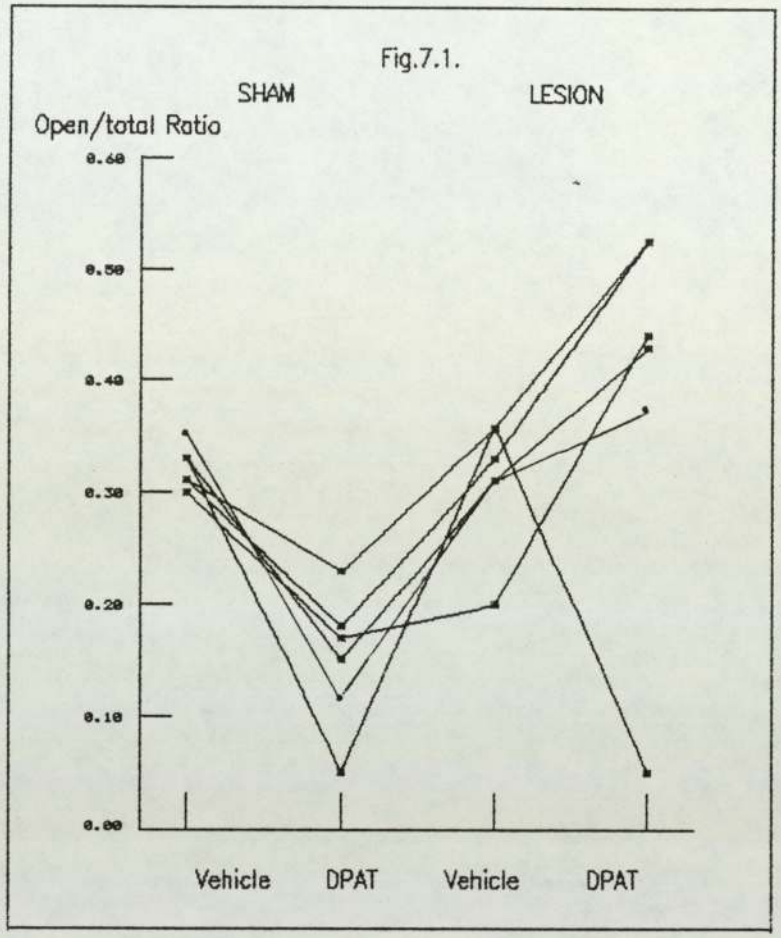


Fig. 7.1. The effect of DRN lesions on the response to 8-OH-DPAT (0.1 mg/kg) in the elevated X-maze. Each point represents the result from an individual animal.

Table 7.1

Number of entries in 10 minutes.

Group	Open	Total	Open/ Total	5-HT level hippocampus	5-HT level striatum
Vehicle-Sham					
	3	10	.30	731	990
	6	18	.33	719	632
	4	13	.31	767	998
	4	13	.31	876	1024
	5	15	.33	867	1111
	7	20	.35	876	888
Mean (±sem)		**14.8±1.4**	**0.32±.005**	**806±24.3**	**940±53.9**
8-OH-DPAT-Sham					
	2	12	.17	705	811
	1	17	.06	1108	1112
	3	13	.23	892	1194
	2	11	.18	1039	919
	3	20	.15	697	816
	2	17	.12	772	670
Mean (±sem)		**15.0±1.3**	**0.15±.02****	**869±56.4**	**920±64.1**
Vehicle-DRN					
	1	5	.20	500 (59%)	494 (53%)
	5	14	.36	893 (106%)	280 (30%)
	5	14	.36	693 (82%)	397 (42%)
	6	18	.33	967 (115%)	247 (26%)
	4	13	.31	747 (89%)	639 (68%)
	4	13	.31	1184(141%)	201 (21%)
Mean (±sem)		**12.8±1.6**	**0.31±.02**	**831±76.7**	**376±54.0**
8-OH-DPAT-DRN					
	4	9	.44	660 (78%)	371 (39%)
	1	17	.06	641 (76%)	773 (83%)
	10	19	.53	514 (61%)	205 (22%)
	10	19	.53	811 (97%)	276 (29%)
	10	23	.43	670 (80%)	154 (16%)
	7	19	.37	885 (105%)	187 (20%)
Mean (±sem)		**17.6±1.7**	**0.39±.05††**	**697±42.6**	**328±74.7**

Table 7.1. The effect of DRN lesions and Sham operations on exploration in the elevated X-maze and on hippocampal and striatal 5-HT levels. Results are expressed as per individual rat and 5-HT levels as ng/g. Figures in brackets are 5-HT levels as a percentage of mean sham controls. Statistical comparisons were made using a 2-way ANOVA. ** = $p < .01$ vs. vehicle-sham; †† = $p < .01$ vs. 8-OH-DPAT-sham.

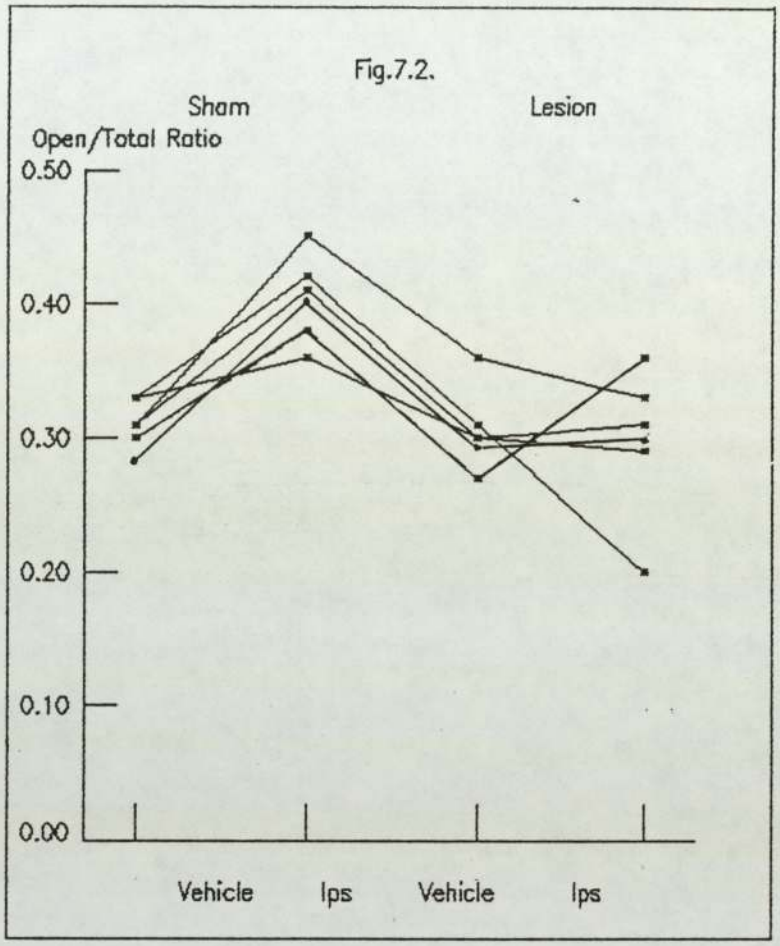


Fig.7.2. The effect of DRN lesions on the response to ipsapirone (1.0 mg/kg) in the elevated X-maze. Each point represents the result from an individual animal.

Table 7.2.

Number of entries in 10 minutes.

Group	Open	Total	Open/ Total	Hippocampal 5-HT level	Striatal 5-HT level
Vehicle-Sham					
	3	9	.33	731	990
	4	12	.33	719	632
	4	13	.31	767	998
	5	15	.33	876	1024
	3	10	.30	867	1111
	4	15	.27	876	888
Mean (±sem)		**12.3±.8**	**0.31±.008**	**806±24.3**	**940±53.9**
Ipsapirone-Sham					
	8	19	.42	705	811
	4	11	.36	1108	1112
	7	17	.41	892	1194
	5	11	.45	1039	919
	5	13	.38	697	816
	4	10	.40	772	670
Mean (±sem)		**13.5±1.2**	**0.40±.01****	**869±56.4**	**920±64.1**
Vehicle-DRN					
	4	13	.31	500 (59%)	494 (53%)
	3	10	.30	893 (106%)	280 (30%)
	3	10	.30	693 (82%)	397 (42%)
	4	11	.36	967 (115%)	247 (26%)
	4	15	.27	747 (89%)	639 (68%)
	4	14	.29	1184(141%)	201 (21%)
Mean (±sem)		**12.2±.7**	**0.31±.01**	**831±76.7**	**376±54.0**
Ipsapirone-DRN					
	2	10	.20	660 (78%)	371 (38%)
	4	14	.29	641 (76%)	773 (80%)
	4	13	.31	514 (61%)	205 (21%)
	4	12	.33	811 (97%)	276 (29%)
	4	11	.36	670 (80%)	154 (16%)
	3	10	.30	885 (105%)	187 (20%)
Mean (±sem)		**11.6±.53**	**0.30±.02††**	**697±42.6**	**328±74.7**

Table 7.2. The effect of DRN lesions and Sham operations on exploration in the elevated X-maze and on hippocampal and striatal 5-HT levels. Results are expressed as per individual rat and 5-HT levels as ng/kg. Figures in brackets are 5-HT levels as a percentage of mean Sham controls. Statistical comparisons were made using a 2-way ANOVA. ** = $p < .01$ vs. vehicle-sham. †† = $p < .01$ vs. ipsapirone-sham.

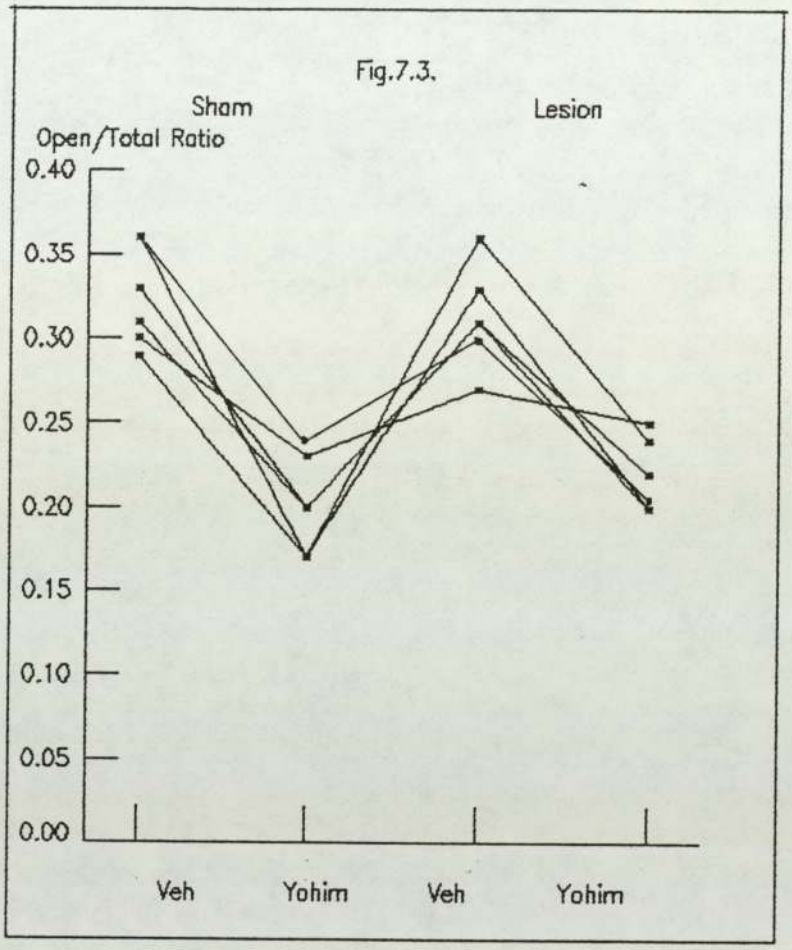


Fig. 7.3. The effect of DRN lesions on the response to yohimbine (0.5 mg/kg) in the elevated X-maze. Each point represents the result from an individual animal.

Table 7.3.

Number of entries in 10 minutes.

Group	Open	Total	Open/ Total	Hippocampal 5-HT level	Striatal 5-HT level
Vehicle-Sham					
	3	9	.33	731	990
	5	14	.36	719	632
	3	10	.30	767	998
	4	14	.29	876	1024
	4	13	.31	867	1111
	5	14	.36	876	888
Mean (±sem)		**12.3±.84**	**0.33±.01**	**806±24.3**	**940±53.9**
Yohimbine-Sham					
	1	5	.20	705	811
	2	12	.17	1108	1112
	3	13	.23	892	1194
	2	12	.17	1039	919
	3	15	.20	697	816
	4	17	.24	772	670
Mean (±sem)		**12.3±1.5**	**0.20±.01****	**869±56.4**	**920±64.1**
Vehicle-DRN					
	4	13	.31	500 (59%)	494 (53%)
	4	11	.36	893 (106%)	280 (30%)
	4	15	.27	693 (82%)	397 (42%)
	3	9	.33	967 (115%)	247 (26%)
	5	16	.31	747 (89%)	639 (68%)
	3	10	.30	1184(141%)	201 (21%)
Mean (±sem)		**12.3±1.0**	**0.31±.01**	**831±76.7**	**376±54.0**
Yohimbine-DRN					
	1	5	.20	660 (78%)	371 (39%)
	4	17	.24	641 (76%)	773 (83%)
	3	12	.25	514 (61%)	205 (22%)
	2	10	.20	811 (97%)	276 (29%)
	4	18	.22	670 (80%)	154 (16%)
	3	14	.21	885 (105%)	187 (20%)
Mean (±sem)		**12.7±1.8**	**0.22±.008****	**697±42.6**	**328±74.7**

Table 7.3. The effect of DRN lesions and Sham operations on exploration in the elevated X-maze and on hippocampal and striatal 5-HT levels. Results are expressed as per individual rat and 5-HT levels as ng/kg. Figures in brackets are 5-HT levels as a percentage of mean Sham controls. Statistical comparisons were made using a 2-way ANOVA. ** = $p < .01$ vs. vehicle-sham.

General Discussion

The purpose of this study has been to examine the effects of various ligands for 5-HT receptors on animal models of anxiety, with a view to revealing more information concerning the role played in anxiety by 5-HT. The effects of ligands varying in their affinity for the 5-HT receptor subtypes were examined in the **elevated X-maze** model of anxiety, the **Social Interaction** test and **operant conflict.** In each model the effects of standard anxiolytic and anxiogenic compounds were first examined in order to provide a standard for comparison. Diazepam as the standard anxiolytic agent increased the open/total entry ratio in the elevated X-maze, increased the amount of time spent in active social interaction and released punished responding in operant conflict tests. βCCE as the anxiogenic agent produced the opposite effects in the X-maze and Social Interaction.

The 5-HT agonists quipazine, 5MeODMT, RU24969 and 8-OH-DPAT all vary in their selectivity for the 5-HT receptor and its various subtypes. The present studies indicate an anxiogenic-like role for all four agonists in the X-maze and Social Interaction test. The agonists were tested in the X-maze over a wide dose range and the relative potency, in decreasing the entry ratio, closely resembles the suggested affinity of the agonists for the 5-HT_{1A} receptor subtype i.e. 8-OH-DPAT > RU24969 > 5MeODMT (*Tricklebank, 1985; Hamon et al., 1986*). Thus, taken alone these results tend to agree with the long-held belief that an increase in 5-HT neurone activity

results in anxiogenic effects. However, when the possible location of the various receptor subtypes involved is considered the picture becomes less clear. 8-OH-DPAT is a relatively selective agonist for 5-HT_{1a} receptors (*Middlemiss and Fozard, 1983*) and was the most potent anxiogenic-like agent studied in the X-maze. Its site of action has been suggested to be the autoreceptor located on the cell body of 5-HT neurones (*Verge et al., 1985*), possibly in the dorsal raphé nucleus (see *Dourish et al., 1986a*). Behavioural studies also suggest that 8-OH-DPAT acts presynaptically. Low doses elicit hyperphagia (*Dourish et al., 1985*) and produce an anticonflict effect (*Engel et al., 1984*) with both these effects being abolished by pretreatment with pCPA (*Engel et al., 1984; Dourish et al., 1986b;1986c*). 8-OH-DPAT is thus believed to depress neuronal firing by its action on somato-dendritic autoreceptors and therefore result in a decreased release of 5-HT from presynaptic terminals (*Dourish et al., 1986a*). This theoretically would produce an anxiolytic effect by virtue of the decrease in 5-HT neurone activity. The basis of such a suggestion is a global consideration of the results obtained in many studies. The suggestion is that a reduction in 5-HT neurone activity, via post-synaptic antagonists, lesions or depletion results in anxiolytic-like activity whereas enhancement of 5-HT function using agonists or electrical stimulation of the raphé results in anxiogenic-like activity (see Introduction; *Iversen, 1984; Chopin and Briley, 1987 and Traber and Glaser, 1987* for reviews). It must be pointed out that there are many exceptions to this rule but it serves a useful purpose as a working hypothesis. The effects seen with 8-OH-DPAT

in the present study are clearly at odds with such a hypothesis. A post-synaptic action of 8-OH-DPAT therefore remains possible as it has been demonstrated to bind to post-synaptic sites in the hippocampus and brainstem (*Gozlan et al., 1983*). An agonist action on post-synaptic sites would result in an increase in neuronal activity and would therefore explain the anxiogenic-like effects seen in the anxiety models studied in this thesis. However, results with pCPA pretreatment are incompatible with such a theory since the effect of pCPA, which effectively removes all presynaptic control, was to abolish the anxiogenic-like effect of 8-OH-DPAT thereby implicating a presynaptic action. Subsequent lesions of the DRN also abolished the 8-OH-DPAT effect thus strengthening this suggestion.

The two putative anxiolytics ipsapirone and buspirone have been shown to bind to 5-HT_{1A} receptors and have variously been suggested to be agonists (*Dourish et al., 1986a; Rowan and Anwyl, 1987*) antagonists (*Goodwin et al., 1986*a; *Reynolds et al., 1986*) or partial agonists (*Clague and Spedding, 1987; Hicks, 1987; Martin and Mason, 1987*) at such a receptor. Recently a buspirone analogue, BMY 7378, has been described as an antagonist at 5-HT_{1A} receptors (*Yocca et al., 1987*). Dourish et al., (1986a) suggested that both buspirone and ipsapirone along with 8-OH-DPAT, are agonists at presynaptic 5-HT_{1A} autoreceptors on the cell bodies of dorsal raphé neurones. Indeed both buspirone and ipsapirone, like 8-OH-DPAT, decrease dorsal raphé neurone firing (*Vandermaelen and Wildeman, 1984*) and increase plasma corticosterone secretion (*Koenig et al., 1986*) and they substitute for 8-OH-DPAT in drug discrimination studies (*Cunningham et al.,*

1987). Our studies indicate that ipsapirone has an opposite action on anxiety to that of 8-OH-DPAT in both the X-maze and the social interaction test, and ipsapirone antagonises the anxiogenic-like effect of 8-OH-DPAT in the X-maze, in a similar manner to pindolol and alprenolol. In unpublished observations we have shown the behavioural syndrome induced by 8-OH-DPAT to be abolished by pretreatment with ipsapirone, and to some extent with buspirone. Buspirone produced no significant effects in the X-maze, except a general depression of activity. Buspirone appears to be something of an oddity since although it shows potential clinical anxiolytic activity (*Goldberg, 1979; Goldberg and Finnerty, 1979*), it has proven difficult to detect in animal models of anxiety (*Goldberg et al., 1983; File, 1985; Gardner, 1986*). One factor possibly contributing to this facet could be the primary metabolite of buspirone, and of its analogue gepirone, (1-(2-pyridinyl)-piperazine) which appears to possess α_2-antagonistic activity (*Giral et al.,1987*). Since α_2-antagonists are anxiogenic on the elevated X-maze it is possible that the two compounds, buspirone and its metabolite, are producing mutually antagonistic effects. The fact remains, however, that a 5-HT_{1A} agonist, 8-OH-DPAT, produces an anxiogenic profile which is antagonised by another 5-HT_{1A} ligand, ipsapirone, which itself is anxiolytic. Despite the fact that most evidence suggests that ipsapirone behaves as an agonist at 5-HT_{1A} receptors our results here point to an antagonist action of ipsapirone.

The antagonism of the 8-OH-DPAT response by pindolol and alprenolol appeared to be mediated via 5-HT$_1$ receptors since metoprolol and ICI118,551 failed to have the same effect in controlled experiments, thus tending to discount a β-adrenoceptor involvement. At present such agents are the only compounds available which have antagonist activity at 5-HT$_1$ receptors (*Middlemiss, 1986a; Nahorski and Willcocks, 1983; Middlemiss et al., 1977; Middlemiss , 1984*). During an extensive study on β-adrenoceptors and 5-HT-mediated behavioural responses (*Costain and Green, 1978*), it was concluded that non-selective β-adrenoceptor antagonists (i.e. β_1 and β_2) block the 5-HT mediated responses (tranylcypromine with L-tryptophan-induced hyperactivity) whereas compounds selective either for β_1 or β_2 receptors had no effect. This and other work led to the conclusion that non-selective β-blockers act as antagonists at the 5-HT autoreceptor with (±)cyanopindolol being the most potent to date (*Middlemiss, 1986a*). Nevertheless as Middlemiss (1986a) points out the activity of cyanopindolol at 5-HT receptors is still 10 times weaker than its activity at β-adrenoceptors, hence the neccessity to determine the effects of metoprolol and ICI118,551 on the maze and on the 8-OH-DPAT response since these antagonists should be devoid of activity at 5-HT receptors (*Costain and Green, 1978; Middlemiss, 1986a*).

These results, taken on their own suggest the involvement of the 5-HT$_1$ or 5-HT$_{1_B}$ receptor in anxiety in the models studied. Traber and Glaser (1987) in their review also suggest that 5-HT$_{1_B}$ activity is an important factor in the activity of several putative anxiolytics.

Further studies in this area may address themselves to several crucial points. Firstly, and perhaps most importantly, recent evidence has come to light suggesting a possible α_2-antagonist effect for 8-OH-DPAT (*Crist and Suprenant, 1987*). Bearing in mind that α_2-antagonists should also produce an anxiogenic response on the elevated X-maze (*Handley and Mithani, 1984a*) and on punished responding (*Handley and Mithani, 1984b*) it is important to determine which receptor type is responsible for the anxiogenic effect seen with 8-OH-DPAT. An initial approach to this problem was to test the α_2-antagonist yohimbine in DRN-lesioned animals. The anxiogenic response produced in the X-maze was not abolished by the lesion, as in the case of the 8-OH-DPAT response. This therefore provides preliminary evidence that α_2-adrenoceptors may not be implicated in the anxiogenic response to 8-OH-DPAT, as seen in the elevated X-maze. Further studies using different approaches will be needed to confirm this suggestion.

This illustrates the neccessity for new selective ligands for 5-HT receptor subtypes. In particular 5-HT$_1$ antagonists are needed for further studies since the use of agents which are primarily active at β-adrenoceptors is not at all desirable. Spiroxatrine is a

promising new ligand suggested to be a 5-HT$_1$ antagonist (*Nelson and Taylor, 1986*), but its selectivity is already in doubt (*Alexander and Wood, 1987*). More recent still is another suggested 5-HT$_{1_B}$ antagonist MDL73005EF which has indicated potential anxiolytic activity (*Moser, 1987*).

Whilst the work so far summarised implicates the 5-HT$_1$ or 5-HT$_{1_B}$ receptor subtypes, the fact that 5-HT$_2$ antagonists appear to have some anxiolytic activity cannot be ignored. Ritanserin in particular produced an anxiolytic profile in the X-maze over a wide dose range and although no significant effect was seen in the social interaction test, a possible trend was present which may warrant further investigation using a wider dose-range. Slight but inconsistent anxiolytic activity was detected in operant conflict and ritanserin has also been reported to have anxiolytic properties in the rat open field test (*Colpaert et al., 1985*). In this test, where emergence from a small dark compartment into a large brightly lit arena is quantified, ritanserin was more effective than diazepam and chlordiazepoxide. However, the authors point out that the anxiolytic effects cannot be simply ascribed to action at 5-HT receptors since ritanserin was not particularly potent in antagonising 5-HT-induced hypothermia in rodents, or 5-HTP-induced head twitch. It should be pointed out here that ritanserin has a not inconsiderable affinity for α_2-adrenoceptors, a fact which must be taken into consideration. Clinical anxiolytic activity is not a general property of 5-HT$_2$ antagonists but ritanserin has been reported active in clinical trials (*Arriaga et al., 1984*; *Ceulemans et al., 1984*). Despite the

anxiolytic actions seen with ritanserin, other 5-HT_2 antagonists show less activity and in our antagonism studies the anxiogenic response to 8-OH-DPAT and 5MeODMT was not reduced by the anxiolytic response to ritanserin. The fact that anxiolytic responses to ipsapirone and pindolol antagonise the 8-OH-DPAT effect, but ritanserin does not, clearly indicates that ipsapirone and pindolol may be producing their anxiolytic effects via a different mechanism to that of ritanserin. An interesting factor here is that in clinical trials with ritanserin it has been suggested that the anxiolytic activity may be qualitatively different from such activity produced by the benzodiazepines (*Ceulemans et al., 1984; Arriaga et al., 1984*). If 5-HT_2 receptors are indeed involved in anxiety, their mechanism of action has not yet been satisfactorily explained.

The recent studies indicating a possible anxiolytic role for the 5-HT_3 receptor antagonists (*Jones et al., 1987; Costall et al., 1987; Tyers et al., 1987*) warrant their inclusion in such a study as this. Although only preliminary investigations were possible in the present study no anxiolytic activity was observed in the X-maze for four different 5-HT_3 antagonists. It must be stressed that these were only preliminary studies which need to be expanded. However, a very recent study (*Molewijk et al., 1987*) also indicates a lack of anxiolytic activity in the light/dark box model for the 5-HT_3 receptor antagonists. It remains to be seen therefore whether these compounds do indeed have a role to play in anxiety. At present it appears that their principle role is as anti-emetics.

As discussed in the Introduction and in Chapter 7 of this thesis, the vast majority of 5-HT neurones in the brain are contained within the dorsal and median raphé nuclei and a number of studies have looked at the effects of lesions of these areas on animal models of anxiety. Results have been conflicting however, and no systematic studies have been carried out using the same models of anxiety. Hence the results are based on several different models and variously employ lesions of the MRN and DRN together (*File and Deakin, 1980*),or either the MRN or DRN (*File et al., 1979; Green and Hodges, 1986*), or use *selective* lesions which upon examination of the biochemical data were not selective for one system at all (*File et al., 1979*). It appears that no single study has examined the effect of truly selective lesions of the DRN in an animal model and then compared the effects of lesions of the MRN in the same model with equal depletion in both lesion groups. Thus the DRN lesions performed here were intended to the initial step in an ongoing project to systematically evaluate the effects of DRN and MRN lesions in the X-maze and possibly the Social Interaction test. The results obtained in the present study are very interesting but without a comparison with MRN lesions in the same model are difficult to evaluate. The finding that DRN lesions block the effects of 8-OH-DPAT and ipsapirone tend to confirm the suggestion made in the light of the pCPA results that 8-OH-DPAT is acting presynaptically to produce its anxiogenic-like effect. As to what the results mean in terms of the role of the DRN is more speculative. Previous studies suggest that the MRN is important in the mediation of behavioural inhibition (see Chapter 7) and could therefore be

expected to be responsible for the results seen in models involving release of suppressed responding (e.g. the anticonflict effect of 8-OH-DPAT; *Engel et al., 1984*). The DRN could then be suggested to mediate an opposite effect and hence be responsible for the anxiogenic-like effect of 8-OH-DPAT in the X-maze. Destruction of the DRN would therefore transfer the main action of 8-OH-DPAT to the MRN with the resultant anxiolytic effect. However, if ipsapirone is indeed acting as an antagonist at the same receptor as 8-OH-DPAT, then one would expect its action also to be reversed. In rats with selective MRN lesions one would therefore expect the lesion to have no effect upon the results already seen in the X-maze.

It has been suggested in the past that two functionally opposing 5-HT systems may be controlling the behavioural effects of 5-HT manipulation (*Schenberg and Graeff, 1978; Gardner, 1985*). For example serotonin antagonists have been shown to block the forepaw treading, hindlimb abduction and head weaving induced by agonists, but to potentiate the hyperlocomotion (*Crow and Deakin, 1977; Green et al., 1984*) suggesting that it is possible that hyperlocomotion and the other 'syndrome' effects are mediated by two different systems. If these opposing systems do exist then they may have a role to play in anxiety. Schenberg and Graeff (1978) showed that electrical stimulation of the dorsal mesencephalic central gray is aversive, whilst stimulation of the ventral portion is rewarding. They also showed that whilst tryptamine antagonists (cyproheptadine and methysergide) tended to facilitate escape responding from brain electrical stimulation, chlordiazepoxide increased latencies of

escape. Therefore the existence of these opposing systems could explain some of the differing results obtained in various brain regions and with different animal models of anxiety, depending on which brain regions are modulated and on the particular pathways involved in each model (see also *Gardner, 1985*).

The results in Chapter 6 of this thesis lend support to the contention that operant conflict models of anxiety serve a limited purpose in the investigation of new non-benzodiazepine putative anxiolytics. Whilst such procedures were not studied exhaustively in the present study they do tend to agree with the arguments put forward by Thiébot, Soubrié and colleagues. They argue that whilst most studies have been concentrating on operant conflict models they may well have been in danger of missing several new classes of putative anxiolytic by virtue of the fact that the models were primarily designed to detect benzodiazepine-like anxiolysis. They suggested that the release of punished responding seen with benzodiazepines may simply be the expression of the benzodiazepine effect on impulsivity. The basis of the argument is that any compound or class of compounds which adversely effect the ability of the animal to tolerate a delay in presentation of a reward will cause a release of the response witholding caused by the punishment. Such a measurement, it is argued, may contamimate any anxiety related effect or may not be a measure of anxiety at all.

These suggestions may well correlate with the work of Gray (1981a; 1981b; 1981c) on the behavioural inhibition system (BIS). He proposed a septohippocampal system mediating behavioural inhibition. The system will act to inhibit ongoing behaviour and the anti-anxiety drugs will impair the function of the BIS to produce their classic release of behavioural inhibition.

The other factor compounding such problems with the operant conflict models is that these models by their very nature involve liquid or food rewarded behaviour. Any study undertaken with these models enquiring in to the role of 5-HT needs to take into account the effects that manipulations of the serotonergic system may have on satiety. It is well known that 5-HT plays a role in thirst and hunger (*Blundell, 1979; Dourish et al., 1985; Samanin et al., 1979; Soulairac and Soulairac, 1970*) and many of the ligands used in this study have been demonstrated to have an effect on food or liquid intake (*Dourish et al., 1985; 1986b*).

Whilst there is undoubtedly a large body of evidence implicating the involvement of 5-HT in the manifestation of anxiety, there are those who believe that the so-called 'serotonin theory of anxiety' has no more grounds for support than a theory implicating noradrenaline or GABA (*Panksepp and Cox, 1986; Pellow et al., 1986*). A superficial study into the literature soon reveals suggestions that neuropeptides may have a role in anxiety be it via an anxiogenic peptide (*Ferrero et al., 1984*) or a possible anxiolytic effect of an NMDA receptor antagonist (*Stephens et al., 1986*). Anxiogenic effects

have been seen in animals or humans with caffeine (*File and Hyde, 1979; Pellow et al., 1985*), amphetamine (*File and Hyde, 1979*), naloxone (*File, 1980*), yohimbine (*Charney et al., 1983*) and the 5-HT agonists (present thesis). In addition there are the effects of the numerous drugs acting on the benzodiazepine/GABA receptor complex (*Pellow and File, 1984*). It is most probable therefore, that no single transmitter system will be found to be responsible for mediating anxiety effects.

The work detailed throughout this thesis is part of an ongoing project and the results contained herein indicate several directions in which to continue the study. Perhaps most importantly initially is the examination of selective lesions of the median raphé nucleus (MRN) in order to clarify its role in anxiety in relation to the dorsal raphé nucleus (DRN). A number of the compounds used in the study have been used by other workers but have been administered centrally with varying results. Thus it would be desirable to administer such compounds as 8-OH-DPAT and ipsapirone centrally, possibly via in-dwelling cannulae in to the raphé nuclei or the hippocampus. The hippocampus is known to contain post-synaptic 5-HT_{1a} receptors (*Gozlan et al., 1983*) and would provide extremely useful information as to the cellular location of the action of 8-OH-DPAT. Since the suggestion that 8-OH-DPAT may have antagonist actions at α_2-receptors (*Crist and Suprenant, 1987*) it is important to determine the receptor at which the observed anxiogenic effect of 8-OH-DPAT originates. Preliminary evidence suggests that the effect is not α_2-mediated but this would need to be confirmed.

Other workers using the elevated X-maze tend to measure the time spent on each of the arms of the maze (*Pellow et al., 1985*) in addition to the entry ratio and have shown that certain drugs may be deemed active when time rather than entries is taken into account. A problem with this, however, is that in our experience animals spend a significant proportion of the time in the centre of the maze indulging in 'peeping' behaviour prior to executing an arm entry. In fact the behaviour observed on the maze could be categorized into three seperate types of behaviour: exploratory activity, 'home-cage' activity in enclosed arms (grooming, sniffing etc.) and the 'peeping' behaviour. It is worth considering therefore, whether it is possible to measure the dwell-time when the rat is hesitating and deciding which arm to enter. Although we had no way of quantifing the behaviour, a rat which has had an anxiolytic drug would be less hesitant about entering an arm, whereas an 'anxious' rat would half enter an arm first and then pause, after which it would either continue or retreat. Measurement of this dwell-time may provide useful information in addition to the entry ratio and may be more valid than simply measuring time spent on each arm of the maze.

Finally, since it was one of the aims of the present study to examine the effects of a number of compounds over several models, it would be desirable to extend the models used, possibly into the use of other species. In particular the light/dark box model (*Crawley and Goodwin, 1980; Crawley, 1985; Costall et al., 1987*) has

been demonstrated to be of value in studying the effects of some 5-HT ligands on anxiety.

In conclusion, although this study probably poses more questions than it answers, it has suggested that 5-HT ligands in general have definite effects upon animal models of anxiety. Furthermore it implicates the 5-HT_{1A} subtype as playing a significant role in anxiety, as measured by the elevated X-maze and the Social Interaction test since 5-HT_1/5-HT_{1A} agonists exhibited an anxiogenic profile which was antagonised by putative 5-HT_1 antagonists. Two of these agents, ipsapirone and pindolol were anxiolytic when given on their own. Whilst the above evidence implicates the 5-HT_1 receptor, at present a role for the 5-HT_2 receptor cannot be excluded.

Depletion and lesion experiments suggested that the 5-HT_1-effect was presynatically mediated and required the presence of an intact DRN. The exact neuronal mechanisms involved in the 5-HT_1 response remain to be explained after further studies have been carried out.

Finally the results indicate the usefulness of the elevated X-maze as an animal model of anxiety.

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