On the Modulation of the Effects of Some 5-HT-Related Agents in Anxiety Models

James Whiteford M^{c}Blane.

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

October 1992

The University of Aston in Birmingham.

On the Modulation of the Effects of Some 5-HT-Related Agents in Anxiety Models.

James Whiteford McBlane. Doctor of Philosophy.

Summary.

The results of an investigation into how stressors interact with the action of serotonergic agents in animal models of anxiety are presented.

Water deprivation and restraint both increased plasma corticosterone concentrations and elevated 5-HT turnover. In the elevated X-maze, water deprivation had a duration-dependent "anxiolytic" effect. The effect of restraint was dependent on the duration of restraint and was to inhibit maze exploration.
Water deprivation did not influence the action of diazepam or any 5-HT_{1A} ligand in the X-maze.
Restraint switched the "anxiogenic" effect of 8-OH-DPAT to either "anxiolytic" or inactive, depending on the time after the restraint when testing was performed. The Vogel conflict test detected an "anxiolytic" effect of buspirone which was additive with "anxiolytic" effects of pindolol and propranolol. Diazepam and fluoxetine were also active, but 8-OH-DPAT, ipsapirone, gepirone and yohimbine were inactive.
In the elevated X-maze, "anxiogenic" responses to picrotoxin, flumazenil, RU 24969, CGS 12066B, fluoxetine and 8-OH-DPAT were detected. Other 5-HT_{1A} ligands were inactive. Diazepam and corticosterone had "anxiolytic" effects.
Increasing light intensity did not change behaviour on the elevated X-maze, but was able to reverse the effect of 8-OH-DPAT to an "anxiolytic" action. This effect was attributed to a presynaptic mechanism, because it was abolished by pCPA.
The occurence of different behaviours in different regions of the maze was shown to be susceptible to modulation by "anxiolytic" and "anxiogenic" drugs.

These results are discussed in the context of there being at least two separate 5-HT mechanisms which are involved in the control of anxiety.

Keywords: 5-HT_{1A} receptor; 8-OH-DPAT; stress; anxiety; elevated X-maze.

Acknowledgements.

I would like to thank Dr. Sheila Handley for her continual support and intellectual guidance throughout all stages of the work described here.

I would also like to thank Mr. Martin Baxter and Dr. Martyn Critchley of the Wellcome Research Laboratories for their support of the entire project, but particularly experiments in Chapters 4 and 7.

I further wish to express my gratitude to Mr. M. Gamble and the staff of the animal house at the University of Aston, particularly Animal House Steve, for care of the rats used in these experiments; Prof. J.A. Blair for the use of the HPLC system with electrochemical detection; Dr. Mark Salter of the Wellcome Research Laboratories for the use of the HPLC system with flourescence detection; Mrs. E.A.M. Scott of the Chemical and Biological Defence Establishment for the camera used in Chapter 7.

I also wish to acknowledge the Science and Engineering Research Council and The Wellcome Foundation, without whose financial support the work could not have been undertaken.

Finally, I wish to thank my family for their sustained interest and support during the last three years.

List of Contents

LIST OF TABLES

LIST OF FIGURES

Page

Figure

LIST OF ABBREVIATIONS

1-PP (PmP)	1-(2-pyrimidinyl)-piperazine
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydoxytryptophan
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
AP 5	2-amino-5-phosphonovaleric acid
B-CCE	ethyl-beta-carboline-3-carboxylate
cAMP	cyclic adenosine monophosphate
DOI	(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
GABA	gamma-amino butyric acid
HPLC	high pressure liquid chromatography
LSD	lysergic acid diethylamide
mCPP	1-(3-chlorophenyl) piperazine
NMDA	n-methyl-d-aspartate
pCPA	para-chlorophenylalanine
TFMPP	1-[3-(trifluoromethyl)-phenyl] piperazine

Chapter 1 - General Introduction.

The use of anxiolytic drugs.

In 1990, over 18 million prescriptions for benzodiazepines were issued in the UK (King, 1992). Prescribing of benzodiazepines has declined from its peak of nearly 31 million in 1979 (King, 1992). Over the course of the last decade, it has been realised that the widespread use of benzodiazepines presented problems of its own which were unforeseen when these drugs were initially introduced. Although the benzodiazepines have real efficacy and are undoubtedly safer than the class of drugs they replaced, the barbiturates, their anxiolytic profile is tainted by unwanted side effects. Chief among these, in the eyes of the media at least (Observer, 20th March 1988), is the propensity to produce dependence on chronic use (Rickels et al., 1989), although amnesia is also a common side effect seen with the benzodiazepines. These factors have combined with the rather fortuitous discovery of the anxiolytic properties of the serotonergic ligand buspirone (Goldberg and Finnerty, 1979) to herald a new era in research to develop anxiolytics that are not associated with the dependence-inducing potential of the benzodiazepines. The focus of this research over the last decade has been the serotonergic system.

The anatomy of the central serotonergic systems.

5-HT was initially found in brain tissue by Twarog and Page (1953) but ten years elapsed before it was conclusively established that 5-HT is contained within specific neuronal pathways (Dahlstrom and Fuxe, 1964). Using a histochemical technique, nine clusters of serotonergic cell bodies were identified in or near the Raphé regions of the medulla and upper brain stem. These clusters were arbitrarily called B1 to B9. The caudal groups, B1 to B3, project to the spinal cord and form a descending serotonergic pathway. Clear delineation of the serotonergic projections from B4 to B6 is hampered by the fact that these areas, as well as containing cell bodies of serotonergic neurones, possess axons of cells whose soma lie elsewhere (Parent et al., 1981). The more rostral groups, B7 to B9, project extensively to the forebrain with B7, the dorsal Raphé nucleus and B8, the median Raphé nucleus providing the majority of the innervation (Imai et al., 1986). Geyer et al. (1976) established that the dorsal Raphé nucleus predominantly innervates the striatum, thalamus and cerebral cortex, whereas the median Raphé predominantly innervates the septohippocampal formation in addition to the hypothalamus and the cerebral cortex.

The differential structure of serotonergic neurones has been described, in which neurones arising from the dorsal Raphé are thought to have small granular varicosities whereas those arising from the median Raphé nucleus have

large spherical varicosities and have larger axonal diameters (Kosofosky and Molliver, 1987).

The classification of 5-HT receptors.

An early classification of receptors for 5-HT was established using the guinea-pig ileum preparation (Gaddum and Picarrelli, 1957). Two receptors were characterized : one was located on the smooth muscle cells and mediated a contraction of the ileum which was susceptible to antagonism by dibenzyline (phenoxybenzamine) and was called the 5-HT D receptor. The second receptor was located on the innervating neurones of the myenteric plexus and mediated the depolarization of, and subsequent release of acetylcholine from, these neurones which could be blocked by morphine and was called the 5-HT M receptor. This nomenclature remained unchallenged for approximately 20 years, until Peroutka and Snyder (1979), using the technique of radioligand binding, found two separate binding sites for [^{3}H]-5-HT which they labelled 5-HT_1 and 5-HT_2. The 5-HT_1 site had a high affinity for [^{3}H]-5-HT but a low affinity for [^{3}H]-spiroperidol ([^{3}H]-spiperone) and the 5-HT_2 site had the reverse profile. This receptor classification was, however, not compatible with the earlier classification of Gaddum and Picarrelli (1957) in that, although there were similarities between the 5-HT_2 receptor and the 5-HT D receptor, the 5-HT_1 receptor was not

similar to the 5-HT M receptor (Bradley et al., 1986). Subsequent work has demonstrated that the 5-HT_1 site is heterogeneous. Pharmacological characterization of the 5-HT_1 site into a 5-HT_{1A} and a 5-HT_{1B} site was based on radioligand binding experiments indicating differential displacement of [^{3}H]-spiperone binding by [^{3}H]-5-HT, with the 5-HT_{1A} site having a high affinity and the 5-HT_{1B} site a low affinity for [^{3}H]-spiperone (Pedigo et al., 1981). Another subtype of 5-HT_1 receptor was initially identified using radioligand binding studies and called the 5-HT_{1C} receptor (Pazos et al., 1984). This site has a high affinity for [^{3}H]-mesulergine which neither the 5-HT_{1A} nor the 5-HT_{1B} site possessed. The large preponderance of radioligand binding data that was available suggesting subtypes of 5-HT_1 binding sites at this time led Bradley et al. (1986) to suggest that, until a function for these differing sites had been identified, the receptor subtypes should be known as 5-HT_1-like receptors, rather than 5-HT_1 receptors. It was envisaged that once a functional response had been attributed to a 5-HT_1-like receptor, then it would be reasonable to call this site a 5-HT_1 receptor. Since this proposal was made, there has been further expansion in the number of recognized 5-HT_1 receptors to include a 5-HT_{1D} receptor (Heuring and Peroutka, 1987) and a 5-HT_{1E} receptor (Titeler and Herrick-Davis, 1988).

Biochemical responses to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors have been characterized and it is now largely accepted that these sites are distinct receptors (Gonzalez-

Heydrich and Peroutka, 1990).

The 5-HT_2 receptor initially identified by Peroutka and Snyder (1979) has been suggested to be heterogeneous (M^{c}Kenna and Peroutka, 1989), but it is also possible that only a single receptor exists, with this receptor having different affinity states, dependent upon the binding of an agonist (Peroutka et al., 1989).

The 5-HT M receptor of Gaddum and Picarrelli (1957) was renamed the 5-HT_3 receptor (Bradley et al., 1986) in the light of experiments revealing highly specific antagonists for this receptor in peripheral tissues (Richardson and Engel, 1986). 5-HT_3 receptors are also present in the brain (Kilpatrick et al., 1987; Barnes et al., 1990).

A central 5-HT receptor that could not be classified as any of the above on the basis of its pharmacology has been described by Dumuis et al. (1989) and has been labelled the 5-HT_4 receptor. An additional receptor for 5-HT has been found in peripheral tissues and has been labelled the 5-HTp receptor (Frazer et al., 1990).

As is evident from the foregoing discussion, the classification of 5-HT receptors is a complicated matter. The number of subtypes of receptor for 5-HT has increased rapidly over the past decade, although this expansion has virtually halted and a period of consolidation seems now to be underway. This expansion has largely been based on pharmacological definitions of receptors. Future trends in receptor classification are likely to be based on a molecular biological approach to group similar receptors on

the basis of their amino acid structure. For instance, this approach has suggested that the 5-HT_{1C} receptor is in fact more similar to the 5-HT_2 receptor than the other 5-HT_1 receptors (Hoyer, 1988), a fact which had been previously suggested on the basis of the pharmacological profile of these receptors (Hoyer et al., 1985).

The distribution of central 5-HT receptors.

There is a considerable amount of evidence available suggesting that 5-HT receptor subtypes are differentially distributed within the brain. Hoyer et al. (1986a) have described the distribution of 5-HT_1 sites in detail using the technique of autoradiography. The 5-HT_{1A} receptor is most concentrated in the septum and hippocampus and, as befits its function as a cell body autoreceptor, significant amounts of this receptor have been found in the Raphé nuclei. The dorsal Raphé nucleus is twice as densely populated with 5-HT_{1A} receptors as the median Raphé (Pazos and Palacios, 1985). The 5-HT_{1B} receptor is located primarily in the substantia nigra and basal ganglia in the rat (Pedigo et al., 1981) and in the human brain the 5-HT_{1D} receptor is primarily located in these areas (Waeber et al., 1988). Both these receptors function as the terminal autoreceptor in the species in which they occur (Hoyer and Middlemiss, 1989), but a postsynaptic location for the 5-HT_{1B} receptor has also been suggested (Weissman et al.,

1987). This species difference in 5-HT autorecptors has been noted (Heuring et al., 1986) and has direct relevance for the study of agents which act at the terminal autoreceptor. The 5-HT_{1B} and 5-HT_{1D} receptors are thought to be analogous receptors in different species (Adham et al., 1992; Hoyer and Middlemiss, 1989). The 5-HT_{1B} receptor has only been definitely identified in the rat and mouse, although recent findings from molecular biological studies indicate that a 5-HT_{1B} receptor might be expressed in humans (Peroutka, 1992). The terminal autoreceptor, the 5-HT_{1D} receptor, is present in species other than the rat and mouse, including the guinea-pig and human (Heuring et al., 1986).

The 5-HT_{1C} receptor has a wide distribution (Pazos et al., 1985). The highest concentrations of mRNA coding for this receptor are found in the hippocampus (Molineaux et al., 1989). It is also the most prominent 5-HT receptor in the choroid plexus (Hoyer et al., 1986b). The 5-HT_2 receptor is highly concentrated in cortical areas, but can be found in other areas (Hoyer et al., 1986b). 5-HT_3 receptors are differentially distributed in rodent (Kilpatrick et al., 1987) and human brain (Barnes et al., 1990) with higher concentrations in limbic areas and the area postrema (Barnes et al., 1989).

Cellular effects of 5-HT.

Only a brief summary of the cellular effects that arise from agonist action at 5-HT receptors is presented here. Activation of the 5-HT_{1A} receptor can both stimulate (Hamon et al., 1984) and inhibit (De Vivo and Maayani, 1986) the production of cyclic AMP. The precise effect which is observed is thought to be dependent on the ongoing activity of the adenylyl cyclase system (Boddeke et al., 1992). Activation of the 5-HT_{1A} receptor produces a hyperpolarization of neurones in the rat hippocampal slice preparation (Andrade and Nicoll, 1987a) and on the cell bodies of serotonergic neurones in the Raphé nuclei (Hjörth and Magnusson, 1988).

In neurochemical studies investigating the effects of 5-HT_{1A} ligands on the rate of turnover of 5-HT, 8-OH-DPAT and buspirone (Higgins et al., 1988) and ipsapirone (Vandermaelen et al., 1986) have been reported to decrease 5-HT turnover, indicated by the reduction of 5-HIAA levels alone, or by the reduction of 5-HIAA : 5-HT ratios. A reduction in the firing activity of serotonergic neurones results from agonist action at presynaptic 5-HT_{1A} receptors. This has been demonstrated for 8-OH-DPAT (Sprouse and Aghajanian, 1986), gepirone (Blier and de Montigny, 1987) and buspirone (Trulson and Araseth, 1986; Trulson and Trulson, 1986; Vandermaelen et al., 1986; Wilkinson et al., 1987).

Direct evidence for a reduction of 5-HT release in response

to 5-HT_{1A} ligands has been obtained using the technique of brain microdialysis. 8-OH-DPAT caused a reduction in the release of 5-HT in the hippocampus, both after intra-Raphé administration (Hutson et al., 1989) and systemic administration (Sharp et al., 1989; Hjörth and Sharp, 1991). 8-OH-DPAT also causes a reduction in extraneuronal 5-HT concentration in the hypothalamus after systemic administration (Wilkinson et al., 1991).

The 5-HT_{1B} receptor and the 5-HT_{1D} receptor also mediate presynaptic hyperpolarization (Engel et al , 1986; Hoyer and Schoeffter, 1988) and are linked in a purely negative fashion to the adenylate cyclase second messenger system (Fozard, 1987). The 5-HT_{1C} receptor and the 5-HT_2 receptor stimulate the turnover of phosphatidylinositols in cell membranes (Conn et al., 1986) leading to the mobilization of intracellular calcium. This effect may be excitatory or inhibitory to whole cell function. The 5-HT_3 receptor functions as a ligand gated Na^+ channel causing depolarization of the cell (Derkach et al., 1989). The 5-HT_4 receptor is known to stimulate adenylate cyclase in a cell culture generated from mouse embryo colliculi (Dumuis et al., 1989).

5-HT receptors have tended to be linked to one particular second messanger system as just descibed. However, it has recently been demonstrated that 5-HT_{1A} receptor activation can influence the inositol phosphate second messenger system under certain circumstances (Boddeke et al., 1992). Interpretation of effects of 5-HT receptor activation on

biochemical and electrophysiological processes is beyond the scope of this thesis.

Behavioural effects of 5-HT.

Agents that enhance the stimulation of 5-HT receptors in the rodent brain lead to the production of a characteristic abnormal behaviour pattern that is known as the 5-HT syndrome. The syndrome is characterized by reciprocal forepaw treading, head weaving, flat body posture, Straub tail, abducted hind limbs and resting tremor (Grahame-Smith, 1971) and certain components of the syndrome appear to be mediated by distinct receptor subtypes (Tricklebank, 1985). The induction of the 5-HT syndrome has been used to identify partial agonist effects at 5-HT_{1A} receptors. Thus, although the 5-HT_{1A} receptor agonists 8-OH-DPAT, buspirone and ipsapirone all produce flat body posture, only 8-OH-DPAT produces forepaw treading, head weaving and tremor (Smith and Peroutka, 1986). Furthermore these latter behaviours produced by 8-OH-DPAT can be antagonised by both buspirone and ipsapirone (Smith and Peroutka, 1986). The occurence of 5-HT syndrome in humans is more open to question (Insel et al., 1982). Given that the syndrome arises from agonist action at 5-HT receptors, it is surprising that there are no reports of the syndrome following 5-HT uptake inhibitors.

A much studied behavioural response to 5-HT agonists is the

mouse head-twitch response. This consists of a quick flick of the head and neck and is observed in response to a wide range of agents which promote activation of 5-HT receptors including the direct acting, but non-selective, agonists 5-methoxy-dimethyltryptamine (5-MeODMT), LSD, quipazine and (Bedard and Pycock, 1977; Corne and Pickering, 1967; Friedman and Dallob, 1979). The 5-HT_2 receptor agonist DOI is also potent at producing this effect (Heaton and Handley, 1989). In contrast, the rat does not exhibit head twitches after the administration of these agents. The wet dog shake in the rat is thought to be the homologous behavioural response to agonist action at 5-HT_2 receptors (Kennett and Curzon, 1991).

Interaction between 5-HT receptor subtypes.

Evidence is accumulating that there is a functional interaction between the 5-HT_{1A} receptor and the 5-HT_2 receptor. Goodwin and Green presented the first evidence that the function of the 5-HT_2 receptor is modulated by 5-HT_{1A} receptor agonists when they reported that 8-OH-DPAT reduced the occurence of head twitches in mice given carbidopa and 5-hydroxytryptophan (Goodwin and Green, 1985). Subsequently, Arnt and Hyttel (1989) reported that the 5-HT_{1A} agonist 8-OH-DPAT inhibits head shakes induced by the 5-HT_2 receptor agonist DOI and this effect has been detected for several other 5-HT_{1A} receptor ligands (Dursun

and Handley, 1991). This evidence indicates that agonist action at 5-HT_{1A} receptors is inhibitory to the function of agonists at 5-HT_2 receptors. Further investigations into this phenomenon have indicated that this effect at the 5-HT_{1A} receptor is mediated presynaptically because it is abolished by pCPA pretreatment (Handley and Dursun, 1991). Backus et al. (1990) have reported that ritanserin, a 5-HT_{1C} and 5-HT_2 antagonist, potentiates the expression of 5-HT_{1A} mediated forepaw treading, indicating that antagonism of 5-HT_2 receptors can influence responses mediated by 5-HT_{1A} receptors.

The concept of stress.

In scientific terms, there is no accepted definition of stress. The concept of stress was first investigated by Cannon (1929) and Selye (1956), the former of whom developed the notion that stress is defined by the presence of an increase in the plasma catecholamine concentration; Selye promoted the idea that stress is associated with an increase in plasma glucocorticoid concentration (Selye, 1956). There is no right or wrong to distinguish between these two definitions which are not mutually exclusive, but many authors have concentrated on the increase in the plasma glucocorticoid concentration as the definitive characteristic of stress. Green and Curzon (1968) stated "stress...being for our purposes defined as a situation in

which there is increased pituitary-adrenal activity".

The word "stress" is used to mean both the mechanism by which an adverse force or influence is produced (a stressor) and the response of the subject to that influence (a stress reponse). Implicit in the concept of stress is the harmful nature of the influence and that influence must be able to produce changes in a measurable variable.

Despite this confusing state about the strict definition of stress, it is intuitively usually clear what stress is and what is not. The more common stressors in animal experiments represent quite severe physical onslaughts and include electric shock applied to the feet (Bliss et al., 1968; Thierry et al., 1968), restraint or immobilization (Dickinson et al., 1985; Morgan et al., 1975; Tanaka et al., 1982), food deprivation (Knott and Curzon, 1974), injection of chemicals such as histamine (Gaillet et al., 1991) and tail-pinching (Kalén et al., 1989b). In humans, stressors are usually of a more subtle nature eg poor housing conditions or marital discord (Deakin, 1991b). Psychiatric illness, including anxiety disorders, is known to be more likely to develop in individuals who experience these stressful situations (Cooke and Hole, 1983).

The biology of stress.

The biochemistry of the stress response has been extensively studied. In the rat, corticosterone is secreted

from the adrenal gland as the result of a short sequence of hormonal events following exposure to a stressful incident (Hodges and Jones, 1963). A crucial biochemical step in the stress response is the release of corticotrophin releasing factor (CRF) from the median eminence of the hypothalamus (Vale et al., 1981). CRF then passes into the portal venous system supplying the anterior pituitary, where it regulates the secretion of adrenocorticotrophic hormone (ACTH)(Reisne et al., 1986). ACTH is responsible for stimulating the synthesis and release of corticosterone from the adrenal cortex. Stress stimulates the pituitary-adrenal system causing the release of ACTH from the anterior pituitary and thus there is a rise in plasma corticosterone concentration.

The neurochemical changes in response to stress have been widely studied in animals. All manner of stressors including electric shock (Bliss et al., 1968; Thierry et al., 1968), restraint (Morgan et al., 1975) and food deprivation (Knott and Curzon, 1974) increase 5-HT turnover. These situations also increase the turnover of noradrenaline (Stone, 1979; Stone and Platt, 1982; Tanaka et al., 1982) and of dopamine (Reinhard et al., 1982). In general, "anxiolytic" drugs are able to block the increase in plasma corticosterone concentrations that are observed in rats subjected stressors (Lahti and Barsuhn, 1974; Le Fur et al., 1979).

Stress and the serotonergic system.

There is a complex and poorly understood interaction between the serotonergic system and the hypothalamo - pituitary - adrenal axis. Agonists at different 5-HT receptors can stimulate secretion at various points in the axis. An interesting effect of the 5-HT_{1A} ligands is their ability to stimulate the secretion of corticosterone in the rat. 8-OH-DPAT, buspirone, gepirone and ipsapirone have all been shown to be able to stimulate the hypothalamic-pitutary-adrenal axis resulting in increased plasma concentrations of corticosterone (Gilbert et al., 1988; Haleem et al., 1989; Owens et al., 1990; Prezgalinski et al., 1989; Urban et al., 1986). This effect, at least of 8-OH-DPAT, is susceptible to blockade by pindolol (Haleem et al., 1989). Additionally, the ability of restraint stress to increase plasma corticosterone was also completely blocked by pindolol (Haleem et al., 1989). It is also likely that agonists at 5-HT_{1c} and/or 5-HT_2 receptors can stimulate the secretion of ACTH (Bagdy et al., 1989; King et al., 1989; Koenig et al., 1987). Calogero et al. (1990) suggested that 8-OH-DPAT and DOI each directly stimulate the axis at the hypothalamic, the pituitary and the adrenal level, further complicating the connections between the axis and the serotonergic system.

Evidence also exists which suggests that corticosterone directly interacts with the function of the brain serotonergic system. Azmitia and M^{c}Ewen (1969) showed that

the activity of tryptophan hydroxylase, the enzyme responsible for producing 5-hydroxytryptophan from tryptophan and the rate limiting step in the synthesis of 5-HT, is reduced in adrenalectomized rats (Azmitia and M°Ewen, 1969). Administration of corticosterone restored the acitivity of the enzyme to normal. Cortisol produces an increase in the rate of synthesis of 5-HT in whole mouse brain (Necker and Sze, 1975). 5-hydroxytryptophan produces myoclonus in guinea-pigs without inducing any signs of the 5-HT syndrome (Jacobs, 1976), an effect which is probably mediated by 5-HT_{1A} receptors (Heal et al., 1992). Chronic administration of cortisol causes a sensitization to this response (Nausieda et al., 1982). In apparent contrast, the chronic administration of corticosterone to rats caused a reduction in the intensity of 5-HT_{1A}-mediated behavioural responses (Dickinson et al., 1985). Thus, there appears to be some interaction between 5-HT systems and corticosterone in rodents. The importance of this association is not understood at present, but one suggestion has been that in humans, chronic hypercortisolaemia, perhaps arising as a consequence of continual stressors, impairs the expression of hippocampal 5-HT_{1A} receptors, which contributes to the development of depression (Deakin and Greaff, 1991).

Animal models of anxiety are also known to produce an increase in the concentration of corticosterone in plasma. This has been demonstrated for the elevated X-maze (Pellow et al., 1985), the social interaction test (File and Peet, 1980) and the Vogel conflict test (File et al., 1988).

Recently, the effects of animal models of "anxiety" on the release of 5-HT has been investigated by microdialysis. Experiments of this sort have already indicated that the elevated X-maze is associated with an increase in extraneuronal 5-HT (Rex et al., 1991) and this technique seems likely, at the present time, to be of great value in investigating neurochemical changes that occur in different behaviours in the same animal in the future.

Animal Models of Anxiety.

The extent to which animal models can ever mirror the state of mind of the anxious patient is a point of some controversy (Abbot, 1987). Pharmacological validation of animal models of "anxiety" can be confirmed by reference to the action of drugs known to be effective anxiolytics in humans. Physiological validation, such as the measurement of corticosterone and / or catecholamines in plasma, is less widely considered but has a useful role to play in providing evidence regarding how stressful the model is. Animal tests for "anxiety" are dependent upon the objective measurement of animal behaviour presumed to be indicative of "anxiety". Data can be accumulated from the observation of the behaviour of animals from which the state of mind of the animal at the time of these behaviours is inferred, but the extent to which the model produces the same subjective feelings that are characteristic of human anxiety is

impossible to know. The more common animal models used to screen potential anxiolytic candidates are discussed below.

The Geller-Seifter test.

This was probably the earliest animal model specific for "anxiety" to be reported (Geller and Seifter, 1960). The test initially involves the training of an animal to press a lever in order to obtain a food reward, which is presented at irregular intervals. After acquisition, responding is punished by an electric shock at certain times within the session, which are indicated by a light switching on in the experimental chamber. During this stage responding still produces a reward, but the rate of lever pressing drops due to the experience of the shock. This results in the withholding of responding by the animal while the light is on but a return to a high rate of lever pressing when the light is off. Punished and unpunished responding is measured within the same session. An "anxiolytic" effect is detected as an increase in responding during the punished stage, without an increase in the unpunished stage and can detect both the acute and chronic "anxiolytic" effects of benzodiazepines (Geller and Seifter, 1960; Gardner, 1986).

The Vogel conflict test.

The Vogel conflict test was initially described by Vogel et al. (1971) and is based on the suppresion of drinking at a water spout in water-deprived animals by the delivery of an electric shock to the floor of the experimental chamber. Unlike the Geller-Seifter test, animals do not have to be trained to express this behaviour, and, therefore it is a much quicker procedure than the Geller-Seifter test to use on a routine basis. It is discussed in greater depth in chapter 4 of this thesis.

The elevated X-maze test.

The concept of the elevated X-maze test arose from work carried out by Montgomery (1955) in which the behaviour of rats in Y-shaped mazes was assessed with particular regard to the exploration of arms that were either open or enclosed. Handley and Mithani (1984) adapted Montgomery's Y-mazes into an X-shaped maze to make the test symmetrical and thus allow direct comparisons between exploration of the two types of arms. This test has been used extensively for the work in this thesis, and it is discussed further in chapters 5 to 8.

The potentiated startle response.

The practical utility of the potentiated startle response as an animal model of anxiety has been recently reviewed (Davis, 1992). When presented with an unexpected stimulus, such as a loud noise, rodents emit a startle response, the magnitude of which can be quantified. The magnitude of the startle response can be increased by the prior association of a neutral stimulus, such as a light or a tone, with a subsequent aversive and unavoidable punishment with the result that there is a greater startle response in the presence of the signal. The conditioned response is a state of fear, induced by anticipation of punishment.

One of the benefits of the test is that, unlike other animal models of anxiety, an "anxiolytic" effect is detected as a reduction in the size of the response being measured. Thus, "anxiolytics" decrease the amplitude of the startle response and "anxiogenic" drugs increase it. The test is reported to be selective for the identification of "anxiolytic" drugs (Davis, 1979b; Mansbach and Geyer, 1988), although the test is also responsive to morphine (Davis, 1979a).

The social interaction test.

The social interaction test measures various aspects of behaviour that are expressed by two naive male rats and

which can be influenced by changes in the immediate environment (File, 1978). The test measures the time spent in different behaviours including grooming, sniffing or following each other, or boxing and wrestling (File, 1978). Under bright light intensity and in unfamiliar surroundings, interactions between two naive rats are far less than those in familiar surroundings and low light intensity. This suppression of social interaction induced by environmental alterations can be attenuated or abolished by benzodiazepine "anxiolytics" (File and Hyde, 1978), can be increased by "anxiogenic" agents such as ACTH (File and Vellucci, 1978) and has been extensively validated by physiological means (File, 1978).

The mouse light aversion test.

Crawley et al. (1980) described a simple animal model of "anxiety" which makes use of the fact that mice will spend more time in the dark side of a differentially lit two compartment chamber than the light side. Mice naturally explore a novel environment, but when there is a differential in the light intensity of two areas, there is preferential exploration of the darker compartment (Crawley et al., 1980). The test measures the proportion of time spent in the dark and the light areas. "Anxiolytic" drugs increase the time spent on the light compartment without increasing the number of transitions between the two

compartments (Crawley, 1981).

Stimulation of the dorsal periaqueductal gray region.

Electrical stimulation of the dorsal periaqueductal gray region (DPAG) of the human brain elicits feelings of intense fear, impending doom and a feeling that one is going to die (Nashold et al., 1969). Graeff (1990) has indicated the similarities between the symptoms of panic attacks in humans and the effects of DPAG stimulation and on this basis has suggested that panic attacks result from over-activity in this region.

Stimulation of this region in animals elicits changes in behaviour, such as either freezing or uncontrolled fleeing, that are consistent with a fear reaction (Greaff, 1990). In recent years, responses to DPAG stimulation have been extensively studied in animals with a view to assessing the potential of this technique as a model of "anxiety".

5-HT itself and the non selective agonist 5-MeODMT increase the threshhold of electrical stimulation that is required in order to produce changes in behaviour indicative of anxiety, consistent with an "anxiolytic" effect (Schütz et al., 1985).

Other animal models of "anxiety".

The above tests are currently routinely used by many laboratories involved in assessing the mechanism of action of "anxiolytic" drugs and the detection of new drugs with anxiolytic properties in humans. There is a large number of less widely used tests, some of which are discussed below. Boissier et al. (1968) used electrifiable metal plates that form the floor of the test environment to inhibit spontaneous locomotion of animals exposed to this environment. The animal, usually a mouse, receives a footshock when it crosses from one plate to the next. Once the mouse has experienced this, there is a marked suppression of spontaneous locomtor activity. The effect of "anxiolytic" drugs in this test is to enhance locomotion and so reverse the effect of the punishment (Boissier et al., 1968).

Nolan and Parkes used an open field with holes in the floor of the enclosure (1973). The number of times that the animal poked its head through the holes in the floor was taken as a measure of "anxiety".

In the shock probe conflict test, an animal is placed in a novel environment containing a probe. Exploration of the environment is reduced when the probe is electrified and when the animal experiences the shock. This suppression of exploration can be reversed by the benzodiazepine group of "anxiolytics" (Meert and Colpeart, 1986a).

Gardner (1985a) has described a test which involves the

measurement of ultrasonic vocalizations in pre-weanling rat pups that are separated from their mother. "Anxiolytics" reverse the ultrasonic vocalisations caused by the separation of the pups from the mother.

The purpose of the foregoing presentation of different animal models of anxiety is to indicate the range of possible choices that exist for the experimenter wishing to select two or three tests that will be able to detect new "anxiolytic" drugs, or to investigate the mechanism of action of established "anxiolytic" drugs.

The classification of animal models of "anxiety".

The classification of "anxiety" is a complex topic. In the past, some models have been described as conflict tests, such as the Geller-Seifter test, because they operate on the principle that what the drug modulates is a state of conflict induced by two opposing drives and the animal experiences conflict between the two opposing drives. In the Geller-Seifter test, one drive is promoting responding - this may be hunger or could be the rewarding nature of the favoured food received on responding. A second drive inhibits responding - electric shock is aversive which leads to reduced responding. The actual behaviour which is measured, the number of lever presses in the Geller-Seifter test, is the outcome of these competing drives. In the

elevated X-maze conflict exists between the drive to avoid an innately aversive environment (the open arms) with the desire to explore the environment.

Alternatively, tests have been labelled as consummatory and non-consummatory. The Geller-Seifter and Vogel tests are in the former category; the elevated X-maze and social interaction tests are in the latter category. Finally, tests may be based on exploration (social interaction, elevated X-maze, mouse light aversion) or not based on exploration (Geller-Seifter, Vogel conflict test). Classification of this sort may give a true reflection of the nature of the tests, but is merely descriptive and does not contribute any new knowledge to "anxiety" tests. Such a classification does not help to explain the very confusing actions of serotonergic "anxiolytics" in animal models of "anxiety", for instance.

One method of classification has attempted to describe models in a fashion that has predictive value, at least for drugs acting through serotonergic mechanisms (Handley, 1991). This classification system assess both the nature of the stimulus (innate or learned [see Gray, 1982]) and the nature of the response (behaviour expressed or withheld). Such a classification system does help to reveal similarities in drug action across different tests. Its test will come with the generation of new data which will need to be incorporated in the existing framework of the classification.

The classification of clinical anxiety disorders.

Anxiety is a natural emotion, one which is commonly experienced by every individual at certain occasions which is also a useful mechanism for enhancing performance, or achieving goals which would otherwise not be realized. In its more extreme forms, anxiety is manifested as a diffuse and vague feeling of apprehension and is usually accompanied by a difficulty in relaxation and symptoms of autonomic activation, such as a pounding heart and sweaty palms, of differential intensity. The distinction between normal and pathological anxiety is not clear cut, but depends both on whether the presence of anxiety is appropriate for a particular situation, and on an assessment concerning the impairment of the normal functioning of the patient. If the disruptive effects can easily be accomodated within the lifestyle of the patient, then, quite simply, there is no disruption. Pathological anxiety can, however, mean that patients cannot live a normal life because they know they must avoid certain situations with which they cannot cope, or there is an ever present state of heightened anxiety.

There are two principal classification systems for clinical anxiety disorders; the revised third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IIIR) is the classification used in the United States whereas practitioners in the United Kingdom use the International Classification of Mental Diseases (ICD-10).

The more common classification used in research reports is the DSM-IIIR classification, and this is summarised below. DSM-IIIR recognizes several different anxiety disorders. These can be identified by particular clinical syndromes which may be of use in predicting clinical responses to pharmacological and/or behavioural therapy.

Generalized anxiety is characterized by excessive worrying for no apparent reason which lasts for at least six months and is accompanied by symptoms of autonomic anxiety. Obsessive-compulsive disorder is characterized by the intrusion of ideas or impulses into the patient's mind, but the patient is able to recognize the irrational nature of the compulsion. This produces changes in behaviour to cope with the compulsion that are expressed as rituals. Failure to carry out the ritual markedly heightens anxiety.

Post-traumatic stress disorder arises after experiencing a stress which would be considered to be extremely traumatic for almost any individual eg combat experience, being held hostage or experiencing a plane crash. The symptoms of this disorder are manifested as the re-experiencing of the traumatic events in dreams and waking thoughts, an emotional blunting to subsequent events and poor concentration and cognitive impairments and thus represents a failure to recover from the initial horrific experience.

Simple and social phobias are the irrational fear which results in the conscious avoidance of the feared object, which is maintained by the desire to avoid humiliation or embarrassment in front of other people.

Panic disorder - this is a state where the patient does not experience anxiety most of the time, but does suffer from unexpected acute attacks of intense anxiety, both psychological and physical symptoms being present. Agoraphobia may or may not be present. This is differentiated from phobic reactions because of the unpredictability of occurrence and because there is no association between fear of the reaction of other people and the attack.

There are noted differences in the treatment of these differing anxiety states which have helped in the identification of these different anxiety disorders. Treatments for each anxiety disorder have been summarised by Tyrer (1989), who has also emphasised the fact that most patients with anxiety also have symptoms of other disorders, most notably depression. Deakin (1991a) has also commented that most anxiety states occur in the presence of other disorders, notably depression, and accordingly, the perceived value of specific models for particular disorders may not be as great as might, at first sight, be thought.

Most scientists are reluctant at present to suggest that any one animal model of "anxiety" is a representation of one particular clinical disorder. It is probably true to state that all accepted animal models are responsive to benzodiazepines. This is so because detecting an "anxiolytic" response to benzodiazepines is still seen as the defining characteristic of animal models of "anxiety". As benzodiazepines are not effective in certain classes of

anxiety, it would seem that there are no appropriate models for these anxiety states, the phobic disorders or obsessive-compulsive disorder. Lister (1990) has made some attempt to assess the possibility that different animal tests are able to model different human anxiety disorders. One difficulty in attributing particular animal models to particular clinical anxiety syndromes is that clinical diagnoses are under continual review. The above description is an accurate account of clinical thinking regarding anxiety disorders at the present time.

The involvement of 5-HT in anxiety.

(1) Initial evidence.

A link between 5-HT and stress responses has already been reviewed, and the idea has been broached that animal models of "anxiety" could be considered to be stressors in their own right. The association between 5-HT and anxiety has been extensively investigated in animals over the past 25 years, but surprisingly little attention has been directed to the 5-HT system in clinical anxiety states. There is a particular dearth of information in generalized anxiety disorder (Nutt and George, 1990), although greater attention has been directed to obsessive-compulsive disorder (Lesch et al., 1990) and panic disorder (Sheehan et al., 1989) and central 5-HT.

A link between 5-HT and "anxiety" has been suspected for some time since early studies revealed that a reduction in the function of serotonergic systems can have "anxiolytic" effects and that the benzodiazepines can reduce the activity of serotonergic neurones (Stein et al., 1973). It was also appreciated that serotonergic systems are involved in mediating certain aversive responses (Cook and Davidson, 1973). More recently, the role of 5-HT in "anxiety" has been re-evaluated in the light of evidence that a group of ligands, selective for the 5-HT_{1A} receptor, are either clinically effective anxiolytics (Goa and Ward, 1986) or are active in animal models of "anxiety" (reviewed in Handley, 1991).

Initial impetus that gave rise to the suggestion that 5-HT was involved in "anxiety" came from the work of Robichaud and Sledge (1969), who reported that the tryptophan hydroxylase inhibitor (pCPA) has anti-conflict effects in the Geller-Seifter test. The 5-HT precursor 5-HTP was able to reverse the effect of pCPA (Tye et al., 1979) suggesting that serotonergic neurones are involved in the anti-conflict effect.

Depletion of 5-HT from central neurones with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), which specifically destroys serotonergic neurones, produces an "anxiolytic"-like release of punished responding (Stein et al., 1975). This effect was corroborated by Cook and Sepinwall (1975) using the less selective neurotoxin 5,6-dihydroxytryptamine (5,6-DHT). This group observed an increase in drinking

during the punished phase of a punished drinking test. In the social interaction test, micro-injections of 5,7-DHT into the dorsal Raphé nucleus produced changes similar to those seen with chronic benzodiazepine administration (File et al., 1979). Thiébot et al. (1982) reported that chlordiazepoxide could reverse the suppression of responding in a food reward motivated conflict test and also that 5,7-DHT given into the dorsal Raphé nucleus could inhibit the action of chlordiazepoxide, suggesting that serotonergic mechanisms were involved in the "anxiolytic" action of the drug.

There is evidence, too, from agonist and antagonist studies that 5-HT is involved in "anxiety". Many of the so called classical antagonists of 5-HT, characterized before the rapid expansion of different 5-HT receptor subtypes took place, have been reported to be "anxiolytic" in a range of models for "anxiety". Methysergide and cinanserin, both antagonists at 5-HT_1 and 5-HT_2 receptors, were reported to produce a release of punished behaviour in a food motivated conflict test (Graeff and Schoenfield, 1970). Metergoline and pizotifen were found to have mild "anxiolytic" properties in a conflict procedure using an electrified probe (Meert and Colpeart, 1986b) and cyproheptadine has also been reported to be "anxiolytic" in a conflict test (Graeff, 1974). These results are difficult to interpret because of the lack of selectivity of the drugs used. Pizotifen, for example, has dopamine antagonist properties, whilst cyproheptadine has antihistaminergic, noradrenergic

α_1 antagonist and calcium channel blocking properties (Peroutka, 1985).

There is relatively little evidence from studies with 5-HT agonists. However, quipazine, a non-selective 5-HT agonist (Hoyer and Schoeffter, 1988), has some "anxiogenic" activity in the X-maze test (Handley and Mithani, 1984). There is a large body of evidence, therefore, in favour of an involvement of 5-HT in anxiety. This can be summarized by saying that "anxiety" is associated with an elevation of serotonergic function and an "anxiolytic" effect results from a reduction of 5-HT (Iversen, 1984). However, this simple theory does not account for many other findings that are not consistent with the proposed role for 5-HT in anxiety states. In addition, some of the initial findings that first generated the interest in the topic have been questioned - for example Söderpalm et al. (1989) have suggested that 5-HTP has "anxiolytic"-like effects in the X-maze test. Blakely and Parker (1973) found that pCPA had no effect on punished bar pressing and was therefore devoid of anti-conflict properties. The actions of neurotoxins specific for serotonergic neurones have also been reported to be inconsistent with anti-conflict effects being mediated by 5-HT (Thiébot et al., 1984). These researchers found that 5,7-DHT neither affected the suppression of responding, induced by punishment, nor the ability of diazepam to reinstate responding during punishment (Thiébot et al., 1984). Studies with classical 5-HT antagonists have also produced conflicting results. Thiébot et al. (1984)

reported that a reduction in serotonergic tone was neither sufficient nor necessary for "anxiolytic" effects when metergoline failed to alter the anti-conflict effect of chlordiazepoxide. Furthermore, metergoline appears inactive in a modified social interaction test (Gardner and Guy, 1984) as does methysergide (ibid.). In punished drinking tests, metergoline and cyproheptadine have both produced no effect (Kilts et al., 1981; Petersen and Lassen, 1981; Commisaris and Rech, 1982). Clinical evidence with 5-HT antagonists or pCPA has not favoured an association between reduced 5-HT function and "anxiolytic" actions. Methysergide worsens anxiety in normal humans (Graeff et al., 1985;) and pCPA does not relieve anxiety in patients (Engelman et al., 1967).

(2) Recent evidence.

With the expansion in the knowledge of the existence of different receptors for 5-HT that occured in the 1980s came optimism that the conflicting data regarding animal models of "anxiety" could be explained on the basis of actions of non-selective ligands at different populations of receptors. It is not an exaggeration to say that all identified 5-HT receptors have been implicated in anxiety mechanisms, with the exceptions of the recently classified 5-HT_4 receptor and the 5-HT_{1E} receptor.

(a) - 5-HT_{1A} receptors :

5-HT_{1A} receptor ligands have only weak and inconsistent effects in the Geller-Seifter test or modifications thereof (Barrett and Gleeson, 1991). Several authors do report "anxiolytic" effects for buspirone (Geller and Hartman, 1982; Young et al., 1987) in either the rat or monkey. However, there are also reports of inactivity in this test (Brocco et al., 1990; Sanger, 1990). In contrast to these weak effects in mammals, in pigeons buspirone (Barrett et al., 1989; Witkin and Barrett, 1986; Witkin et al., 1987), ipsapirone (Gleeson and Barrett, 1990), 8-OH-DPAT (ibid.) and BMY 7378 (ibid.) produce a selective increase in punished responding at doses that do not influence unpunished responding. This difference in the sensitivity of pigeons to 5-HT_{1A} receptor agonists in this test has been a consistent finding (Barrett and Gleeson, 1991).

In the acoustic startle response, where the response is not signal-potentiated, 5-HT_{1A} ligands have an "anxiogenic" effect, that is they increase the startle magnitude. This has been demonstrated for buspirone (Eison et al., 1986; Kehne et al., 1988), gepirone (Eison et al., 1986; Kehne et al., 1988) and 8-OH-DPAT (Nanry and Tilson, 1989), although an opposite, "anxiolytic", effect has also been reported for 8-OH-DPAT (Davis et al., 1986).

The effects of 5-HT_{1A} receptor ligands in the Vogel conflict test are presented in detail in chapter 4, as are those for the elevated X-maze test in chapter 5 and therefore are not

discussed in detail here. However, a brief summary is appropriate here to indicate the full scope of responses to 5-HT_{1A} ligands in animal models.

Most reports using the Vogel test detect "anxiolytic" effects of buspirone (Brocco et al., 1990; Eison et al., 1986; Gower and Tricklebank, 1988; Moser et al, 1990; Oakley and Jones, 1983; Schefke et al., 1989; Wada and Fukuda, 1991). Fewer reports attest to "anxiolytic" effects of ipsapirone (Chojnacka-Wojicik and Prezgalanski, 1991) and gepirone (Eison et al., 1986). The Vogel test, in which it is very difficult to show "anxiogenic" effects because the baseline at which the test operates is usually so low that further decrements in responding are not possible, is particularly suscepible to "anxiolytic" effects of 5-HT_{1A} receptor ligands.

The elevated X-maze, unlike the Vogel conflict test, is readily able to detect "anxiolytic" or "anxiogenic" effects and both have been commonly reported for the same drug. Thus, 8-OH-DPAT is "anxiogenic" in some hands (Critchley and Handley, 1987; Critchley et al., 1992; Klint, 1991; Kshama et al., 1990; Moser et al., 1990), inactive in others (Pellow et al., 1987) and "anxiolytic" in yet different hands (Dunn et al., 1989; Luscombe et al., 1992; Söderpalm et al., 1989). It is possible to cite such a range of effects for buspirone and ipsapirone, although fewer reports exist for other 5-HT_{1A} ligands.

This apparent disarray in the actions of these drugs in this test lead Wright et al. (1992) to conclude that the

elevated X-maze is simply not able to detect the "anxiolytic" activity of these agents which is demonstrated in other animal models and in humans. Identifying the reasons for the disparate effects in the elevated X-maze forms one theme of the present thesis.

"Anxiolytic" effects of 5-HT_{1A} receptor ligands have been reported in other tests. These include the rat pup isolation call test (Hård and Engel, 1988) and isolation-induced aggression in mice (McMillen et al., 1987). When the startle response is potentiated by cueing with a signal to indicate that the aversive stimulus is imminent, 5-HT_{1A} ligands generally produce a reduction in startle magnitude indicating an "anxiolytic" effect (Davis, 1992). This has been amply demonstrated for buspirone, ipsapirone and gepirone (Davis et al., 1988; Kehne et al., 1988; Mansbach and Geyer, 1988). 8-OH-DPAT is inactive according to Davis et al. (1988).

The social interaction test detects "anxiolytic" and "anxiogenic" effects of agents acting at 5-HT_{1A} receptors. "Anxiolytic" effects of buspirone, ipsapirone and gepirone have been reported after systemic administration (Dunn et al., 1989) and after injection into the dorsal Raphé nucleus (Higgins et al., 1988), indicating a possible presynaptic location. However, other groups, using a modified social interaction test which resulted in a high baseline to allow both "anxiolytic" and "anxiogenic" effects, have reported that 8-OH-DPAT is "anxiogenic" (Critchley et al., 1987) and Gardner detected a biphasic

effect of buspirone (Gardner, 1985).

Costall et al. (1988, 1989) have identified "anxiolytic" effects of buspirone in the mouse light aversion test after both systemic and dorsal Raphé administration. An "anxiolytic" effect has also been demonstrated for ipsapirone and indorenate in this model and been attributed to action at 5-HT_{1A} receptors (Fernández-Guasti and Lòpez-Rubalcava, 1990).

A recent study suggested that 8-OH-DPAT administered into the DPAG reduced the intensity of the "anxiogenic" responses to the excitatory amino acid D,L-homocysteic acid (Beckett et al., 1992), consistent with an "anxiolytic" action.

How do 5-HT_{1A} receptor ligands influence anxiety ?

Dourish et al. (1986) provided a reasonable explanation for the involvement of 5-HT_{1A} receptors in anxiety states. This explanation is based on the knowledge that both benzodiazepines and 5-HT_{1A} receptor agonists inhibit the activity of serotonergic Raphé neurones and so inhibit 5-HT turnover. 5-HT_{1A} receptors are located on the cell soma (Pazos et al., 1985) and agonist action inhibits 5-HT release in terminal areas (Sharp et al., 1989). "Anxiolytic" action arises because of the reduction in 5-HT release in terminal areas (Dourish et al., 1986) and is thus consistent with the long-held opinion that reducing

5-HT tone is "anxiolytic".

This theory does not explain why the therapeutic effects of 5-HT_{1A} agonists take two to three weeks to develop or why the initial experience of these agents is either neutral or anxiogenic (Goa and Ward, 1986; Newton et al., 1986) and for these reasons it seems unlikely that it provides the true explanation of "anxiolytic" activity with 5-HT_{1A} receptors.

A more attractive theory which accounts for the delayed onset of clinical action has been developed from electrophysiological experiments using repeated dosing (reviewed by De Montigny and Blier, 1992). Repeated treatment with a number of 5-HT_{1A} agonists causes desensitization of 5-HT_{1A} autoreceptors on serotonergic Raphé neurones without changing the responsivity of postsynaptic 5-HT_{1A} receptors in the hippocampus. The effect of this is that in the early stages of treatment there is an insufficiency in serotonergic tone, which, with chronic treatment, develops into augmented action at postsynaptic 5-HT_{1A} receptors (Blier and De Montigny, 1987).

As the time course of this effect approximately parallels the time course of the onset of "anxiolytic" action, it is considered to be a reasonable explanation for the clinical effects of these drugs. However, it also implies that effects seen in animal models after single dosing do not arise from the same mechanism as the clinically beneficial effects.

To date this mechanism of induction of presynaptic 5-HT_{1A}

receptor desensitisation has been found with all effective classes of antidepressant drugs (de Montigny and Blier, 1992) and is consistent with the view of Deakin (1991), arguing from a different perspective, that antidepressant action arises through augmented 5-HT_{1A} receptor function in hippocampal structures.

(b) - 5-HT_{1B} / 5-HT_{1D} receptors :

The 5-HT_{1B} receptor has been the focus of a modest amount of research regarding "anxiety" mechanisms. Winslow and Insel (1991) reported that the 5-HT_{1B} agonist CGS 12066B (Neale et al., 1987) and TFMPP, a mixed 5-HT_{1B} / 5-HT_{1C} agonist (Hoyer, 1988), was "anxiogenic" in the rat pup ultrasonic isolation call. In addition, the mixed 5-HT_{1A} / 5-HT_{1B} agonist, RU 24969, is "anxiogenic" in the elevated X-maze test (Critchley and Handley, 1986) and TFMPP is "anxiogenic" in the shock probe conflict test (Meert and Colpaert, 1986b). As this receptor has been thought to be absent from the human brain (Hoyer et al., 1986a), it has not received a great deal of investigation with regard to anxiety mechanisms. However, as it has been recently suggested to exist in human brain (Peroutka, 1992), it may attract greater attention in the future.

The 5-HT_{1D} receptor has not yet been linked to anxiety modulation in humans, but experiments using the guinea-pig, which has the 5-HT_{1D} receptor, in the elevated X-maze are underway at the present time (Rex et al., 1991).

(c) - 5-HT_{1C} receptors :

Antagonism at 5-HT_{1C} receptors has been proposed to promote an "anxiolytic" effect (Kennett, 1992). mCPP, which binds to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} (Hoyer and Schoeffter, 1991) receptors as well as possessing antagonist activity at 5-HT_2 receptors (Hoyer and Schoeffter, 1991) is anxiogenic in humans (Charney et al. 1987) and in animals (Kennett et al., 1989). The "anxiogenic" effect of mCPP and TFMPP (Kennett et al., 1989), in the social interaction and light / dark exploration tests is abolished by drugs with antagonist action at 5-HT_{1C} receptors, but is not affected by agents without affinity for 5-HT_{1C} receptors (Kennett et al., 1989). This sugests that the "anxiogenic" effect of mCPP in the social interaction test is due to its 5-HT_{1C} agonist properties. Additionally, mianserin, an antagonist at 5-HT_{1C} receptors amongst others (Hoyer et al., 1988), could prevent the "anxiogenic" effect.

It has been suggested that antagonist action at hippocampal 5-HT_{1C} receptors gives rise to "anxiolytic" effects. This suggestion was based on findings of "anxiogenic" actions of intrahippocampal mCPP in the social interaction test (Whitton and Curzon, 1990). This is consistent with the concept that reducing 5-HT activity promotes anxiolysis (Iversen, 1984) and that reduction of hippocampal serotonergic activity also reduces anxiety (Gray, 1982).

In contrast to the results from experiments just described, the "anxiogenic" action of mCPP has been attributed to

action at 5-HT_{1B} / 5-HT_{1D} receptors (Graeff and Deakin, 1991). In this scheme, it is envisaged that the "anxiogenic" action of mCPP arises due to the inhibition of release of 5-HT in the periaqueductal gray region of the brain by agonistic action at terminal 5-HT autoreceptors. As tonal 5-HT in this region is thought to inhibit the genesis of panic attacks (Deakin and Graeff, 1991), the "anxiogenic" action of mCPP arises because it removes the inhibitory 5-HT tone.

(d) - 5-HT_2 receptors :

An association between antagonism at 5-HT_2 receptors and anxiety has developed independent of findings relating to actions at 5-HT_{1C} receptors (Sturtzman et al., 1990). Ritanserin, ketanserin and seganserin, which all have 5-HT_2 receptor antagonist properties, all produced "anxiolytic"-like effects in the elevated X-maze (Critchley and Handley, 1987). The selectivity of these ligands is particularly poor between 5-HT_{1C} and 5-HT_2 receptors, with the exception of ketanserin (Hoyer, 1988) and the conclusion that these effects are mediated through 5-HT_2 receptors is based largely on the ketanserin finding. Other authors have detected "anxiolytic" effects from ritanserin in the elevated X-maze (Tomkins et al., 1990) although it has also been reported to be "anxiogenic" in the elevated X-maze (File et al., 1987). The 5-HT_2 agonist DOI has both "anxiolytic" (Heaton et al., 1988) and "anxiogenic"

(Tomkins et al., 1990) effects in this one test.
The association between 5-HT_2 receptors and anxiety is sustained by clinical findings with ritanserin. This agent has some weak anxiolytic activity in patients with mixed anxiety and depression (Ceulemans et al., 1985).

(e) - 5-HT_3 receptors :

Some reports suggest that antagonism at 5-HT_3 receptors produces "anxiolytic"-like effects in animals (reviewed by Costall and Naylor, 1992). These effects are not seen in test that rely on the reinstatement of behaviour suppressed by punishment, but appear in tests that were classified as response suppression by innate fear stimulus by Handley (1991). Thus, in the elevated X-maze (Costall et al., 1987a; Upton and Blackburn, 1991), the monkey fear test (Jones et al., 1988) and the mouse light aversion test (Jones et al., 1988; Costall et al., 1990) "anxiolytic" effects are usually detected (but see File and Johnston, 1989 for negative findings in the elevated X-maze).
Initial clinical reports of anxiolytic activity in humans of the 5-HT_3 receptor antagonist ondansetron indicate some beneficial activity in generalised anxiety disorder (Lader, 1991a).

(f)- 5-HT uptake inhibitors :

The 5-HT uptake inhibitors paroxetine and indalpine have

both been found to be "anxiogenic" in the X-maze test (*en passant*, Chopin and Briley, 1987), which is consistent with a theory holding that an increase in serotonergic transmission has the potential for "anxiogenic" effects. Chronic studies have suggested that on continued exposure to 5-HT uptake inhibitors, there is an "anxiolytic" effect in the elevated X-maze (Cadogan et al., 1992) and in the social interaction test (Lightowler et al., 1992). This pattern resembles the effects of 5-HT uptake inhibitors in clinical use, where there is often initial exacerbation of symptoms including heightened anxiety but, on chronic use, an anxiolytic effect develops (reviewed by Nutt and Glue, 1989).

(3) 5-HT mechanisms and anxiety.

Deakin and Graeff (1991) have suggested that 5-HT has a dual function in anxiety and have detailed the anatomical and pharmacological characteristics of how brain 5-HT systems may be involved. According to this theory, it is envisaged that panic attacks arise due to spontaneous activation of neurones located within the dorsal periaqueductal grey area (DPAG). 5-HT inputs from the dorsal Raphé are thought to be inhibitory to neurones involved in initiating a panic attack, and thus panic attacks or "anxiogenic" effects, arise from any drug action which reduces the action of 5-HT in the dorsal PAG. This is

the effect expected to result from activation of 5-HT_{1A} receptors in the dorsal Raphé and may be detected as "anxiogenic" effects observed with these compounds.

A second prong of the Deakin and Greaff theory anticipates that activation of dorsal Raphé neurones, through outputs in the striatum, lead to an inhibition of approach behaviour. Reduction of 5-HT in these synapses after 5-HT_{1A} receptor activation reduces the inhibitory input into the striatum, hippocampus and other brain areas and leads to a reduced capacity to inhibit approach behaviour. In animal models this may be detected as "anxiolytic" activity.

A final prong of the Deakin and Greaff theory attributes hippocampal 5-HT_{1A} receptors a crucial role in antidepressant therapies. However, this aspect of the ideas of Deakin and Graeff (1991) are beyond the scope of the present discussion.

This complex integration of different 5-HT systems in the control of both anxiety and depression may be able to explain the opposite results obtained with 5-HT_{1A} receptor ligands in anxiety models of anxiety and can also account for "anxiolytic" activity of 5-HT_2 receptor antagonism.

Summary.

In summary, the involvement of 5-HT in anxiety is not clearly understood. The classical view that reductions in serotonergic transmission are always automatically "anxiolytic" and increases are "anxiogenic" is being

refined to accomodate the vast complexity of data which associates 5-HT with anxiety.

Other actions of 5-HT_{1A} ligands.

(1) Drug discrimination studies.

In drug discrimination studies, buspirone and ipsapirone can substitute for 8-OH-DPAT (Tricklebank et al., 1987), but quipazine, a non-selective agonist at 5-HT_1 and 5-HT_2 receptors (Hoyer, 1988), and RU 24969, a mixed 5-HT_{1A} / 5-HT_{1B} ligand (Hoyer, 1988), could not (Tricklebank et al., 1987). Under conditions in which ipsapirone was the training drug, generalization to 8-OH-DPAT, buspirone and 5-MeODMT was observed although there was no generalization from ipsapirone to diazepam, pentobarbitone, quipazine or RU 24969 (Spencer and Traber, 1987). These results support an action of 8-OH-DPAT, ipsapirone, and buspirone at the same type of receptor. Intriguingly, 8-OH-DPAT and ipsapirone do generalize to yohimbine (Winter, 1988), an agent with "anxiogenic" properties in both animals (Handley and Mithani, 1984) and humans (Holmberg and Gershon, 1961).

(2) Hypothermic effects.

Hypothermic responses to 8-OH-DPAT have been a consistent finding (Goodwin et al., 1987; Moser, 1991) and have been

characterized as a 5-HT_{1A} mediated effect (Goodwin et al., 1985b). In the rat, it is probably a postsynaptic effect (O'Connell et al., 1992), whereas in the mouse it appears to be presynaptically mediated, as it abolished by both pCPA and 5,7-DHT lesions (Goodwin et al., 1985a). Anderson et al. (1990) reported that gepirone had a hypothermic effect in humans. To date, this is the only published finding of a 5-HT_{1A} receptor ligand reducing body temperature in humans.

(3) Hyperphagia.

An increase in food intake in satiated animals after 5-HT_{1A} receptor agonist administration has been reported (Hutson et al., 1986). 8-OH-DPAT, for instance, has a marked hyperphagic effect (Dourish et al., 1988) and the destruction of serotonergic neurones can abolish this effect (Bendotti and Samanin, 1987), suggesting a presynaptic mechanism of action. Infusion of 8-OH-DPAT into the dorsal Raphé nucleus produces hyperphagia (Hutson et al., 1986), again supportive of a presynaptic effect. Gepirone, buspirone and ipsapirone also have this hyperphagic effect (Fletcher and Davis, 1990) strengthening the claim that this effect can be produced by the activation of 5-HT_{1A} receptors.

(4) Endocrine effects.

The capacity of 5-HT_{1A} agonists to stimulate the secretion of ACTH and of corticosterone has already been mentioned. Plasma prolactin concentrations also rise in response to 5-HT_{1A} receptor agonist action (Nash and Meltzer, 1989). However, there is doubt about whether this effect reflects 5-HT_{1A} activity; dopaminergic effects have been heavily implicated in the case of buspirone (Nash and Meltzer, 1989; Urban et al., 1986). In addition, activation of 5-HT_{1A} receptors increases plasma concentrations of adrenaline and noradrenaline (Baudrie and Chaouloff, 1991; Chaouloff et al., 1990) and also inhibits insulin release (Chaouloff and Jeanrenaud, 1987).

(5) Effects on repeated dosing with 5-HT_{1A} receptor agonists.

There is some confusion regarding the effects of repeated dosing of agents acting at 5-HT_{1A} receptors. There is evidence that second doses of 5-HT_{1A} agonists produce smaller effects than initial doses, at least with regard to effects arising from actions at presynaptic receptors. The ability of 8-OH-DPAT to reduce brain 5-HIAA concentration is reduced if there has been pretreatment with 8-OH-DPAT one day earlier (Beer et al., 1990), whereas induction of the 5-HT syndrome is not (ibid.). This finding has, however, been questioned (Hjörth, 1991). Binding of 8-OH-

DPAT in Raphé nuclei but not in the frontal cortex or hippocampus is reduced one day after a single dose of 8-OH-DPAT (Beer et al., 1990). The electrophysiological work of Blier and de Montigny demonstrating that chronic dosing of 5-HT_{1A} receptor agonists (and all other antidepressant drugs tested) produces pre-, but not post-, synaptic desensitization (Blier and De Montigny, 1987, 1990) has already been mentioned. However, the opposite profile of postsynaptic desensitization with an unchanged presynaptic sensitivity has also been recorded (Larsson et al., 1990), thus generating much confusion.

Clinical profile of the 5-HT_{1A} receptor ligands.

Buspirone was the first novel agent to be introduced for the treatment of anxiety since the introduction of the benzodiazepines. The benefit of the use of buspirone in the treatment of anxiety is that, unlike the benzodiazepines, it is currently thought not to produce any signs of withdrawal symptoms after medium to long term use (Lader, 1991b). In addition, buspirone does not produce significant psychomotor impairments, does not interact with alcohol and is safe in overdose (Robinson, 1990).

Many studies have been conducted for the purpose of assessing the value of buspirone for the treatment of generalised anxiety disorder (GAD) and buspirone is consistently found to be as effective as diazepam and

better than placebo after four weeks of treatment (eg Jacobson et al., 1985; Goa and Ward, 1986). The effects of buspirone are of gradual onset (ibid.) taking three to four weeks to reach their maximal effect. A beneficial effect of ipsapirone (Kuemmel et al. 1988), gepirone and tandospirone (Feighner and Boyer, 1989; Taylor, 1990) in GAD has also been reported, although more studies are required for an unequivocal anxiolytic action of these compounds to be established. With the exception of buspirone, none of these compounds is yet available to the medical profession for general clinical use.

In addition to GAD, buspirone has been tried in other anxiety disorders. For instance, efficacy for buspirone in obsessive-compulsive disorder has been reported (Napoliello and Domantay, 1991). Buspirone does not reduce the incidence of panic attacks in panic disorder, despite an improvement in general anxiety (Sheehan et al., 1990).

The $5\text{-}HT_{1A}$ ligands have also been investigated for antidepressant activity in humans. Buspirone alleviated major depression with associated anxiety (Schweizer et al., 1986). An improvement in depressive symptoms has been reported for gepirone (Amsterdam, 1992) although in this study much of the improvement was found in those patients whose illness was less severe. There are some reports that buspirone is of use in other psychiatric or neurological disorders, for instance tardive dyskinesia (Neppe, 1989) or anorexia nervosa (Price and Di Marzio, 1988), although a full scientific evaluation has not been carried out.

Comments on receptor selectivity and antagonism.

The selectivity of 8-OH-DPAT, buspirone, ipsapirone and gepirone for the 5-HT_{1A} receptor has been well characterized. 8-OH-DPAT possesses impressive selectivity for the 5-HT_{1A} receptor, being essentially inactive at 5-HT_2, 5-HT_3, dopamine D_1, histamine H_1 and H_2 receptors and having a several hundred-fold weaker affinity for 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, α_1 adrenoceptors and dopamine D_2 receptors than at 5-HT_{1A} receptors (van Wijngaarden et al., 1990). Marginal binding to the 5-HT uptake site is evident with 8-OH-DPAT (van Wijngaarden et al., 1990). Buspirone has weaker affinity for dopamine D_2 receptors than for 5-HT_{1A} receptors (van Wijngaarden et al., 1990) but is still able to produce effects in animals and humans through these receptors (Peroutka, 1985). The profile of ipsapirone is similar, but the selectivity between these two receptors is greater than for buspirone (van Wijngaarden et al., 1990). At concentrations that are several hundred times higher, buspirone and ipsapirone bind to 5-HT_{1B}, 5-HT_{1C} (not ipsapirone), 5-HT_{1D}, 5-HT_2 receptors, α_1 and α_2 adrenoceptors (buspirone not α_2) and histamine receptors. Gepirone binds to 5-HT_{1A} receptors, without acting at dopamine receptors (Eison et al., 1986; M^{c}Millen et al., 1987). Muscarinic, benzodiazepine and calcium channel acceptor sites are also insensitive to these ligands (Peroutka, 1985).

To date, there are no widely available drugs which have

antagonist activity at 5-HT_{1A} receptors without also having sufficient affinity for other receptors which limit the utility of the compound as a 5-HT_{1A} antagonist. In addition, these agents tend to have some weak agonist activity at 5-HT_{1A} receptors. This is the case for BMY 7378 (Sharp et al., 1990), spiroxatrine (Barrett et al., 1989) and NAN-190 (Claustre et al., 1991; Gruel and Glaser, 1992), which have all been proposed to be 5-HT_{1A} antagonists.

The reason why it is so difficult to identify compounds that have no agonist activity at presynaptic 5-HT_{1A} receptors but which retain the good postsynaptic antagonist activity of these compounds, has been attributed to the relative reserve of 5-HT_{1A} receptors at these sites (Meller et al., 1990). Presynaptic 5-HT_{1A} receptors are present at concentrations that are greatly in excess of that which are needed to regulate 5-HT synthesis (Meller et al., 1990) and presumably 5-HT cell firing. In consequence, a compound with very low efficacy, but with reasonable affinity, is still able to produce a maximal response because the full response is triggered when there is only fractional receptor occupancy. At postsynaptic receptors, there is no receptor reserve, with the consequence that a low efficacy, high affinity agent acts as as a partial agonist (Meller et al., 1990).

Very recently, WAY 100135 has been reported (Bill et al., 1993) to have antagonist properties at both pre- and postsynaptic 5-HT_{1A} receptors with no indication of any agonist activity at presynaptic 5-HT_{1A} receptors. Similarly,

SDZ 216,525 has also been claimed to have 5-HT_{1A} antagonist properties (Hoyer et al., 1991), although at presynaptic receptors there are still signs of agonist activity (Gurling et al., 1993). These newer compounds look likely to replace the current standard 5-HT_{1A} receptor antagonists, the ß-adrenoceptor antagonists pindolol and propranolol.

Principal Aims.

Iversen (1984) proposed that a reduction in serotonergic activity promotes anxiolysis. There is evidence that the response to 5-HT_{1A} ligands in the elevated X-maze is very variable (see Introduction p 49 and Introduction to chapter 5) and, in apparent contrast to the view expressed by Iversen (1984), lesion studies have shown that "anxiogenic" responses to one 5-HT_{1A} ligand, 8-OH-DPAT, in the elevated X-maze are presynaptically derived (Critchley et al., 1992). Evidence from the Vogel conflict test suggests that the "anxiolytic" effect of 8-OH-DPAT in this test (Engel et al., 1984) is also presynaptically mediated. The principal original aim of the present investigations was to assess the possibility that the variable responses to 5-HT_{1A} ligands in the elevated X-maze originate from more than one mode of action of these compounds on "anxiety"- mechanisms, but one promoting anxiety, the other reducing anxiety. In contrast to the variability reported in the elevated X-maze, 5-HT_{1A} ligands have generally been reported to be

"anxiolytic" in the Vogel conflict test (see Introduction to chapter 4), a test with the integral stress of water deprivation. It was therfore of interest to question whether water deprivation played any role in generating the more reliable "anxiolytic" profile in the Vogel test. The principal proposed experiments were :

(1) to compare responses to 5-HT_{1A} ligands in the Vogel test with responses in the elevated X-maze

(2) to assess the effects of water deprivation in the elevated X-maze

(3) to assess the effects of water deprivation on the response to 5-HT_{1A} ligands in the elevated X-maze

(4) to compare the effects of water deprivation with those of a well-studied stress, namely restraint, on behaviour on the elevated X-maze, responses to 5-HT_{1A} ligands in the elevated X-maze and on biochemical indices of 5-HT turnover

(5) to identify and characterize any further factors which could contribute to the variability in responses to 5-HT_{1A} ligands in the elevated X-maze.

Chapter 2

Experimental Methods

Contents

2.1. Animals and animal husbandry.

Experiments reported in this thesis were carried out using male Wistar rats supplied by Charles River with the exception of a group of hooded PVG rats obtained from Bantin and Kingman Limited for a particular experiment as described. Rats were kept in groups of between 5 and 10 in two different animal houses at an ambient temperature of 21 ± 1°C and under a 12 hour light / dark cycle (lights on 0800 hrs) and were maintained on a conventional 41B cube diet suplied by Pilsbury Limited with tap water ad libitum. The weight of animals used in studies involving 48 hour water deprivation were kept within the range 150 - 180g, in accordance with Home Office guidelines. Rats used in other experiments were between 150 and 230 g.

2.2. General experimental conditions.

Experiments for this thesis were conducted either at the University of Aston or the Department of Pharmacology, Wellcome Research Laboratories. All Vogel conflict experiments were conducted at the Wellcome Research Laboratories, whereas elevated X-maze experiments and biochemical analyses were conducted at both centres.
Animals used in the Vogel conflict test were housed in

groups of eight to ten in a quiet room for at least four days prior to the start of water deprivation. Animals used for the X-maze experiments were kept in groups of six for at least one week prior to the start of the experiment. Animals used for the biochemical experiments were also kept in groups of six for at least a week before being used.

2.3. Methods used in biochemical experiments.

2.3.1 Preparation of brain tissue.

Rats were killed by cervical dislocation, decapitated and their brains were removed, frozen at -70°C and stored until used, which was within four months. For most experiments, the cerebellum was discarded and the remainder of the brain was homogenized in 4 ml of 0.1 M perchloric acid and 0.4 mM sodium metabisulphite, containing 500 ng/ml of an internal standard, N-methyl-5-hydroxytryptamine (N-methyl-5-HT). The exception to this was the study investigating regional effects of water deprivation, where the brain was dissected into cerebral cortex, hypothalamus and hippocampus. The samples were centrifuged for 10 minutes at 1000 x g. The supernatants were kept on ice prior to analysis by HPLC with electrochemical detection (for conditions see 2.3.2).

2.3.2 Measurement of brain 5-HT, 5-HIAA and tryptophan.

Two methods of measurement of indoles extracted from brain tissue were employed in this study. Except for the study into the effects of water deprivation on 5-HT mechanisms in discrete brain areas, electrochemical detection was used. Separation of 5-HT, 5-HIAA and N-methyl-5-HT was achieved by reversed phase HPLC using an LDC Milton Roy 10 micron ODS-3 reverse phase column (25 cm x 4.6 mm). The column and precolumn (5 micron Whatman precolumn) were kept at a constant 30°C by a heat block. The mobile phase was pumped through the system at a rate of 1.2 ml/min. An LDC Milton Roy electrochemical monitor with a Spark Holland electrode was used, with the working electrode set at a potential of +0.650 V. Signals were recorded on a chart recorder with the peak height being used to calculate the concentration of the indoles with reference to standard samples, and the internal standard being used to correct for inter-sample injection error. For the discrete brain area study, samples were prepared as before and injected automatically by a Beckman System Gold Autosampler 507. Separation of tryptophan, 5-HT and 5-HIAA was achieved as above using the same mobile phase and flow rate. A Shimadzu fluorescence HPLC monitor was used to detect signals with excitation and emission wavelengths set at 285 nm and 345 nm respectively. Signals were analysed by a Beckman Gold Integration system with the peak area being used to calculate the concentration of the indoles with reference to standard

samples. In both cases, the mobile phase was 0.1 M citric acid, 180 mM sodium dihydrogen phosphate, 1.8 mM sodium octyl sulphonic acid, set to pH 5.2 using sodium hydroxide and phosphoric acid. Finally, methanol was added to a concentration of 11 %. This method is based on those published by Shibata et al. (1988) and by Kumar et al. (1990). Preliminary studies with electrochemical detection confirmed that there was a linear relationship between the peak height and the sample concentration of 5-HT or 5-HIAA or N-methyl-5-HT. Retention times of 5-HIAA, 5-HT and N-methyl-5-HT were 6min 50s, 20min 40s and 26min 0s respectively. When using fluorimetric detection, studies demonstrating linearity between indole concentration and peak area were not performed. Retention times of 5-HIAA, tryptophan and 5-HT were 2min 25s, 4min 15s and 5min 35s respectively. Typical traces are depicted in Figure 2.1 and Figure 2.2.

2.3.3 Measurement of plasma corticosterone.

Corticosterone was obtained by the following method. Rats were killed by cervical dislocation and decapitated. Trunk blood was collected into centrifuge tubes, centrifuged at 2500 x g for 10 minutes and the resulting plasma was stored at -70 °C until measurement. On the day of measurement, the plasma was defrosted and a 1 ml fraction was taken. 1 ml of an internal standard solution containing either equilenin or dexamethasone dissolved in methanol was added. 8 ml of

dichloromethane was added and the mixture was shaken for 15 minutes by a mechanical shaker. The solution was centrifuged at 2500 x g for 10 minutes and the organic phase removed into a glass tube. The organic phase was evaporated to dryness by maintaining at 80°C for 30 minutes. The residue was dissolved in 100 µl of methanol and 20 µl of this was injected onto the chromatograph. Corticosterone was analysed by HPLC and detected by measuring the absorption of light at a wavelength of 254nm. Separation of corticosterone and equilenin was achieved by reversed phase high pressure liquid chromatography using an LDC Milton Roy 10 micron ODS-3 reverse phase column (25 cm x 4.6 mm). The column was kept at room temperature. The mobile phase was pumped through the system at a rate of 1.0 ml/min. Signals were recorded on a chart recorder with the peak height being used to calculate the concentration of the indoles with reference to standard samples, and the internal standard being used to correct for inter-sample injection error. Preliminary studies confirmed the presence of a linear relationship between peak height and concentration for equilenin and corticosterone. Initially, the column was eluted with a mobile phase of the following constitution : 80% water, acidified with 0.1% triflouro-acetic acid and 20% acetonitrile, acidified with 0.1% trifluoroacetic acid. A second mobile phase was used subsequently, consisting of 70% 1 M potassium hydrogen phosphate titrated to pH 3.2 with phosphoric acid and 30% acetonitrile. The mobile phase was pumped through the

column at 1 ml/min. The method is derived from that of Kabra et al. (1979). A typical trace of corticosterone and equilenin is depicted in Figure 2.3. Retention times were 7min 0s for equilenin and 9min 40s for corticosterone.

2.4. Stressors.

Two stressors were chosen for the work to be undertaken. For water deprivation stress, rats were deprived of access to water for up to 48 hours by either removing the drinking bottles from the home cage or by fitting a cap over the drinking spout in the home cage. Food was available during all periods of water deprivation. Restraint was also used. For this, rats were wrapped in a soft cloth such that the limbs were held firmly against the torso. Neither the head nor the tail was restrained. This cloth was secured by a second cloth to prevent escape. Restrained rats were kept in a small cage individually, for the period of restraint. For restraint studies, experiments were planned such that rats were killed between 9.30 and 11.00 am.

2.5. Administration of drugs.

Drugs were injected intraperitoneally (ip), except where otherwise stated, by inserting a 25 g needle through the abdominal wall upwards towards the diaphragm. An injection volume of 1 ml/kg body weight was used in all cases. Drug solutions were made up in saline (0.85 % NaCl) or distilled

water with the appropriate vehicle used as control. Where more than two injections were given, opposite sides of the abdomen were used.

Buspirone was administered chronically for three weeks in the drinking water at a concentration of 0.4 mg/ml to rats of initial weight 110 g. Rats drank a mean of 25-35 ml/day in this experiment (see Figure 7.1b), resulting in the delivery of 10-14 mg of buspirone per day to each rat.

2.6. Methods used in behavioural experiments.

2.6.1 The Vogel conflict test.

The procedure was a modification of the technique described by Vogel et al. (1971). Water, but not food, was removed from the cage 24 or 48 hours prior to the test, as specified for each experiment. Approximately five hours before the test all rats were subjected to a pretest which involved placing the rat in the experimental chamber (dimensions 25 x 25 x 25 cm) where water was available via a spout connected to a water bottle clipped to the outside of the chamber. The number of licks of the spout made in the first five minutes after being placed in the chamber was counted by computer to a maximum of 300 licks in 48 hour water-deprived rats and to a maximum of 500 licks in 24 hour water deprived rats. Rats failing to reach the required number of licks in the first five minutes were not included in the drug test. Rats were then replaced in their

home cage until the test.

In the test, rats were allowed 20 licks before the punished schedule was initiated. On every subsequent 20^{th} lick the rat received a direct current electric shock, through the spout, of variable duration and variable current intensity, as specified in the results section, and the number of licks of the spout made was recorded over the next five minute period. Latency was recorded as the time taken to the first lick after being placed in the chamber at the start of the test.

These experiments were controlled by a Compaq Deskpro 386 / 20 e computer, using a Spider system (Paul Fray Eurobeeb computers). The drinkometers and shock generators were from Campden Instruments (Drinkometer 453; Shock source 52K).

2.6.2 The elevated X-maze test.

The elevated X-maze used in these experiments was a wooden platform in the shape of an X raised 1 m above the floor (Handley and Mithani, 1984). The maze had four arms each of length 35 cm and width 10 cm. All four arms were covered with a wire grid which had a mesh size of approximately 1 x 1 cm. Two opposing arms had sides 10 cm high thus forming an enclosed arm, whilst the remaining two were open. The sides of the maze were detachable and were made of aluminium, painted with grey matt paint in order to block reflections.

Most experiments were conducted in a windowless room of

dimensions 6m x 2m, which also served as the home cage room for these animals. The maze was positioned approximately central in the room, some four feet from a table, upon which the camera (Ferguson Videostar) used to record the experiments was stood.

Injections of drugs were 30 minutes (10 minutes for 8-OH-DPAT and 45 minutes in the case of flumazenil) prior to being placed on the X-maze. Rats were placed in the centre of the X-maze facing an enclosed arm and the behaviour of the rat was recorded on video for 10 minutes. The number of entries made into the open and the enclosed arms was recorded by an observer sitting in an adjacent room via a video camera. An entry was defined as all four feet being placed in the entered arm. The ratio of open to total entries was calculated for each rat. The time spent in each arm was also recorded.

2.6.3 Characterization of behaviour expressed on the elevated X-maze.

The tapes from a number of experiments were re-analysed in order to characterize the behaviours that were expressed on the maze. For behavioural characterization, behaviour was divided into five mutually exclusive behaviours and the occurence of each behaviour was timed in each of the three areas of the maze, namely the open arms, the enclosed arms and the centre square. The time spent occupied in each behaviour was then expressed as a percentage of the total

time spent in that area. The behavioural categories were as follows :

grooming - the animal licks itself, or licks its paws and then wipes itself with its paws;

rearing -the animal stands on its hind feet and lifts its body into an upright posture;

peering - the animal is positioned at the boundary between two areas of the maze and looks towards other areas of the maze;

sniffing - normal posture, with sniffing the only behaviour present and expressed away from a boundary;

locomotion - the animal is travelling from one point of the maze to another.

2.7. Measurement of light intensity.

Light intensity was measured at the centre of the elevated X-maze by placing the photometer (Vivitar 45 exposure meter) on the flat surface of the centre square of the elevated X-maze pointing in the direction of the main light source. The photometer measured incident light. The exposure value (EV) was read from the photometer and converted into photopic lux using the formula

$$lux = 2 \times EV^{2.5}.$$

The brightest light intensity was achieved by shining a

standard daylight balanced photographic photoflood lamp (150 W) at the centre of the maze. Other lighting conditions were set by varying the number of wall-lights in the experimental room that were switched on.

2.8.1. Statistical methods.

Except where otherwise stated, the groups were composed of 6 measures. A number of different statistical tests were employed to analyse results from experiments for this thesis. To compare a single group with another group, Student's unpaired t-test was used. To compare a single group against two or more groups, a one way analysis of variance (ANOVA) was first performed. If this was significant, each group was compared to the control group using Dunnett's t-test. In experiments where there were potentially two factors interacting, a two way ANOVA was performed. Subsequent comparisons between groups were performed using Tukey's U test for unconfounded means.

2.8.2. Presentation of graphs.

In graphs and tables, the symbols * and ** indicate $P < 0.05$ and $P < 0.01$ respectively, compared to the control group. In experiments where drug treatment may interact with another factor, the symbols $^{\infty}$ and $^{\infty\infty}$ indicates comparisons between drug treated animals only at $P < 0.05$ and $P < 0.01$ respectively.

2.9. Drugs and vehicles used.

Drugs were obtained from the following sources :

DRUG	SOURCE
DL-aminoglutethimide	Sigma Chemical Co.
buspirone hydrochloride	Troponwerke or Sigma Chemical Co.
chlordiazepoxide hydrochloride	Sigma Chemical Co.
CGS 12066B dimaleate	Research Biochem. Inc.
corticosterone	Sigma Chemical Co.
diazepam (Valium)	Roche Products Ltd.
equilenin	Aldrich Chemical Co.
ethyl-beta-carboline-3-carboxylate (ß-CCE)	Research Biochem. Inc.
flumazenil (RO 15-1788)	Roche Products Ltd.
fluoxetine hydrochloride	Eli Lilly
gepirone hydrochloride	Bristol Meyers
8-Hydroxy-2-(di-n-propyl-amino) tetralin HBr (8-OH-DPAT)	Research Biochem. Inc.
5-Hydroxyindole acetic acid	Sigma Chemical Co.
5-hydroxytryptamine creatinine sulphate complex	Sigma Chemical Co.
ipsapirone	Troponwerke
DL-p-chlorophenylalanine methyl ester (pCPA)	Sigma Chemical Co.

5-methoxy-3(tetrahydro-pyridin-4-yl)1H-indole (RU 24969)	Roussel Uclaf
N-methyl-5-Hydroxytryptamine oxalate	Sigma Chemical Co.
pindolol	Sigma Chemical Co.
DL-propranolol hydrochloride	Sigma Chemical Co.
yohimbine hydrochloride	Research Biochem. Inc.

Drugs were dissolved in saline with the following exceptions:

corticosterone - dissolved in 1 ml HPLC grade methanol and diluted with saline.

flumazenil - suspended in saline with the addition of two drops per 10 ml of decon 90.

fluoxetine - dissolved in distilled water.

yohimbine - dissolved in distilled water.

2.10. Reagents used in biochemical assays.

Chemicals used in biochemical experiments came from the following sources :

CHEMICAL	SOURCE
acetonitrile	Fisons
ammonium acetate	Fisons
citric acid	Fisons
Decon 90 detergent	Fisons

dichloromethane (HPLC Grade)	BDH
methanol (HPLC Grade)	Fisons
1-Octane sulphonic acid sodium salt	Fisons
perchloric acid	Fisons
phosphoric acid	BDH
sodium dihydrogen orthophosphate	Fisons
sodium hydroxide	BDH
trifluoroacetic acid	Fisons
water (HPLC Grade)	Fisons

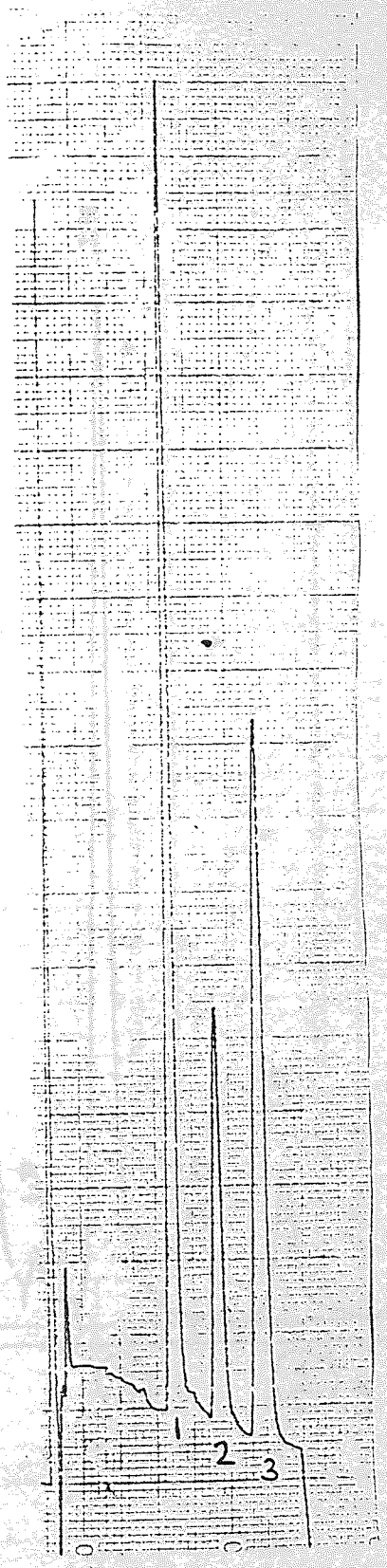


Figure 2.1.

Chromatogram of 5-HT, 5-HIAA and N-methyl-5-HT.

1 - 5-hydroxyindole acetic acid (5-HIAA).
2 - 5-hydroxytryptamine (5-HT).
3 - N-methyl-5-hydroxytryptamine.

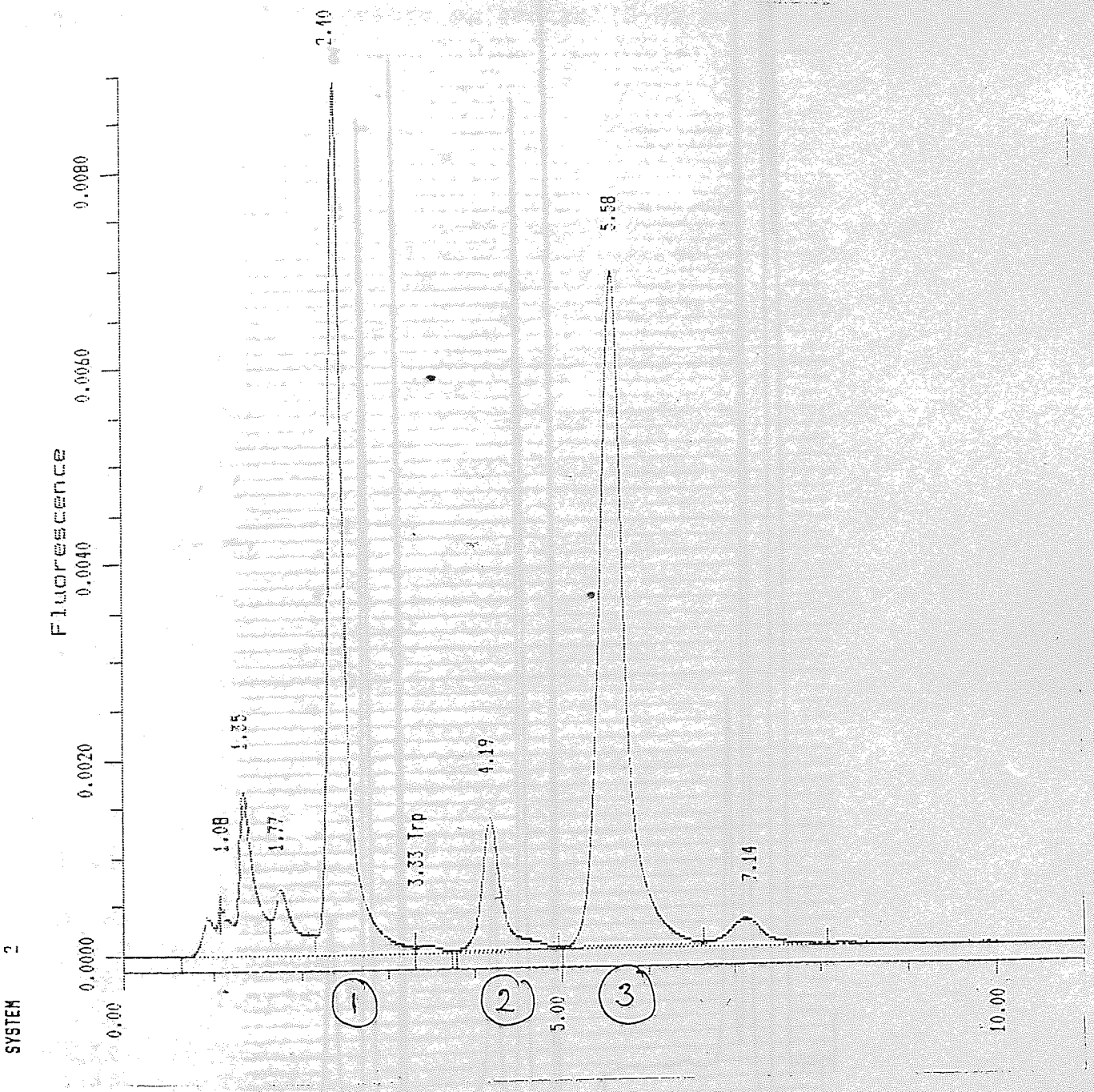


Figure 2.2.

Chromatogram of tryptophan, 5-HT and 5-HIAA.

1 - 5-hydroxyindole acetic acid (5-HIAA).
2 - tryptophan
3 - 5-hydroxytryptamine (5-HT).

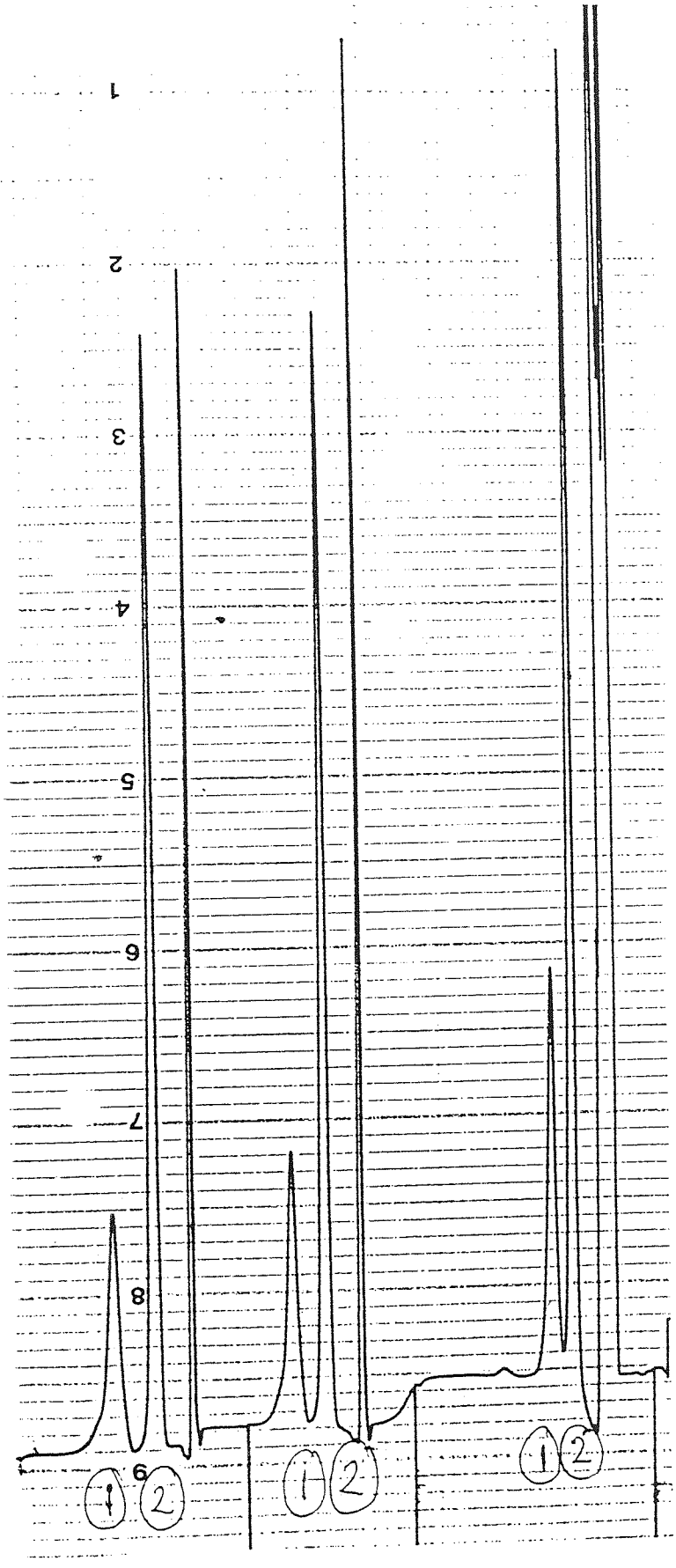


Figure 2.3.

Chromatogram of corticosterone and equilenin.

1 - corticosterone.
2 - equilenin.

Chapter 3

The effects of stressors on central 5-HT mechanisms.

Contents.

3.1 Introduction.

Studies in animals have shown that stressors increase the turnover of 5-HT in the brain. For example, footshock increased the rate of synthesis of 5-HT (Bliss et al., 1968; Thierry et al., 1968). This stress also increased the rate of metabolism of 5-HT, as well as that of dopamine and noradrenaline in rat brain (Bliss et al., 1968). Knott et al. (1973) established that food deprivation elevated brain tryptophan concentrations and that food deprivation stimulates brain 5-HT turnover (Knott and Curzon, 1974). Restraint has been extensively studied and much emphasis has been placed on alterations in the function of serotonergic neuronal function induced by this stress (eg Kennett and Joseph, 1981; Knott et al., 1973; Mitchell and Thomas, 1988; Morgan et al., 1975). Restraint also stimulates noradrenergic (Tanaka et al., 1991) and dopaminergic (Reinhard et al., 1982) systems in the brain. The rise in plasma corticosterone concentration in response to restraint is partly mediated by an increase in central 5-HT turnover, which is itself associated with an increased uptake into the brain of its precursor, tryptophan (Kennett and Joseph, 1981; Joseph and Kennett, 1983a, b). The increase in plasma corticosterone seen in response to restraint is completely abolished by treatment with the 5-HT_{1A} receptor antagonist pindolol (Haleem et al;., 1989), suggesting that the rise is brought about by actions on the 5-HT_{1A} receptor. Together this evidence suggests that during

restraint, the increase in tryptophan uptake is converted to an increase in 5-HT release, although there is no increase in 5-HT concentration (Adell et al., 1988). Released 5-HT acts on 5-HT_{1A} and on 5-HT_2 receptors to set in chain the events which lead to synthesis and release of corticosterone from the adrenal gland.

Agonists for 5-HT receptors are known to be able to stimulate the secretion of ACTH and to increase plasma corticosterone (Fuller, 1981). The 5-HT_{1A} receptor agonists buspirone, ipsapirone and gepirone all increase plasma ACTH concentration by a pindolol-sensitive mechanism (Gilbert et al., 1988), which implicates the 5-HT_{1A} receptor in this response. Stimulation of 5-HT_2 and of 5-HT_{1c} receptors has also been found to elevate plasma corticosterone concentrations in the rat (King et al., 1989; Koenig et al., 1987).

Serotonergic neurones from the dorsal Raphé nucleus have been found to innervate the paraventricular nucleus (Liposits et al., 1987) where CRF is synthesised (Mezey et al., 1983). This combination of evidence favours the interpretation of a functional connection between stress and activation of brain 5-HT systems.

Benzodiazepine "anxiolytics" have been known to ameliorate the effects of stress on corticosterone responses for a considerable period of time (Lahti and Barsuhn, 1974; Keim and Sigg, 1977) and it is established that benzodiazepines can decrease 5-HT turnover (Wise et al., 1973). It is not clear, however, whether the "anxiolytic" actions of

benzodiazepines and their ability to antagonize the increase in serotonergic processes are in fact related (Lista et al., 1989). "Anxiolytics" also decrease the elevated turnover of dopamine and noradrenaline (Thierry et al., 1976; Tanaka et al., 1982) which stressors can cause. The hippocampus is one region of the brain that has been studied with particular regard to the regulation of activity of the hypothalamic-pituitary axis (Sapolsky et al., 1991). Destruction of the hippocampus results in chronic hypersecretion of glucocorticoids (Sapolsky et al., 1991) and the hippocampus seems to be involved in terminating the glucocorticoid response to stress (Sapolsky et al., 1984). Lesions of the hippocampus also produce behavioural changes that are similar to those seen after administration of benzodiazepine "anxiolytics" (Gray, 1982).

A single study has investigated changes in 5-HT concentration in the pituitary in response to prolonged water deprivation (Piezzi and Wurtman, 1970) and a reduction in 5-HT concentration was found in some parts of the pituitary. Water deprivation increases the turnover of dopamine (Glick and Carlsson, 1989); however, the effects of water deprivation on the dynamic measure of central 5-HT turnover are unknown. The purpose of the experiments described in this chapter was to investigate the effects of water deprivation on central 5-HT biochemistry and to compare results with those of restraint.

3.2 The effect of water deprivation on body weight.

Water deprivation caused a reduction in body weight, the magnitude of which was related to the duration of deprivation. 12 and 24 hours water deprivation resulted in a loss of 6.0 % and 8.2 % respectively, while control groups gained 2.1 and 3.5 % of their initial body weight (Table 3.1). For 36 and 48 hours water deprivation, these figures were 13.1 % and 14.6 % loss in the water deprived groups, with the controls gaining 6.2 and 7.2 % (Table 3.1). Body weights were measured from rats used for the experiment described in section 6.2 to avoid unnecessarily stressing animals used in biochemical experiments. There is a difference in the initial weight ~~difference~~ between the 12 and 36 hour test groups and their respective control groups because these animals originated from consecutive batches of deliveries. Redistribution of rats into groups of equal mean weight was considered but was not done because it would have imposed a further stress.

3.3 The effect of water deprivation on plasma corticosterone concentration.

In each case, water deprivation for 12, 24, 36 and 48 hours resulted in a significant increase in the concentration of plasma corticosterone compared to the concurrently run

control group (unpaired t test, all $P < 0.01$) (Figure 3.1). The magnitude of this rise was not correlated with the duration of deprivation. Rats were killed as described in the methods between 9.30 and 11.00 am.

3.4 The effect of water deprivation on brain 5-HT concentration.

Water deprivation for 12, 24, 36 and 48 hours did not result in any significant changes in the concentration of brain 5-HT (unpaired t tests, $P > 0.05$), with the exception of the repeat experiment investigating the effect of 36 hour water deprivation, where water-deprived rats demonstrated a significantly (unpaired t test, $P < 0.05$) greater concentration of 5-HT (Table 3.3). There was a considerable degree of variation in the brain concentration of 5-HT between these separate experiments which were conducted over the space of four months, although within experiments this was not a notable feature.

3.5 The effect of water deprivation on brain 5-HIAA concentration.

The concentration of 5-HIAA in the brain did not vary systematically with the duration of water deprivation. Thus, there was no difference between the concentration of 5-HIAA between control and water-deprived rats for the periods 24 or 48 hours (unpaired t tests, $P > 0.05$),

although there was significantly greater 5-HIAA concentration in water-deprived rats for the periods 12 and 36 hours water deprivation (unpaired t tests, $P < 0.05$ [12 hours] $P < 0.01$ [36 hours]) (Table 3.3). Again, there was a considerable degree of variation in the brain concentration of 5-HIAA between these separate experiments, although within experiments this was not a notable feature.

3.6 The effect of water deprivation on brain 5-HT turnover.

Water deprivation for 12 and 24 hours did not result in any significant changes in the turnover of 5-HT (unpaired t tests, $P > 0.05$) (Figure 3.2). In contrast, 36 ($P < 0.05$) and 48 ($P < 0.01$) hours water deprivation did result in a significant increase in the turnover of brain 5-HT (unpaired t test) (Figure 3.2). To confirm this finding, the 24 and 36 hour water deprivation periods were repeated with the same conclusion (Table 3.2).

3.7 Water deprivation and Tryptophan Concentration in Discrete Brain Regions.

There was no effect of water deprivation on the concentration of tryptophan in either the cerebral cortex ($F_{(2,18)} = 1.13$; $P > 0.05$) or the hippocampus ($F_{(2,18)} = 2.46$; $P > 0.05$) (Figure 3.3). In contrast, the concentration of tryptophan in the hypothalamus was increased in response to

water deprivation ($F_{(2,18)}$ = 11.86; P < 0.01) (Figure 3.3). Specific group comparisons were made with Dunnett's t test and showed that the 24 hour water deprivation group had a concentration of tryptophan that was significantly greater than the control group, although that of the 48 hour water deprived group was not different from the control (Figure 3.3).

3.8 Water Deprivation and 5-HT Concentration in Discrete Brain Regions.

Water deprivation for 24 or 48 hours did not result in any significant changes in the concentration of 5-HT in any of the brain areas studied [cerebral cortex : $F_{(2,18)}$ = 2.55; P > 0.05; hippocampus : $F_{(2,18)}$ = 0.84; P > 0.05; hypothalamus : $F_{(2,18)}$ = 2.85; P > 0.05] (Table 3.4). There were trends towards differences in the concentration of 5-HT in the different regions, with the highest concentration present in the hypothalamus and the lowest concentration in the hippocampus (Table 3.4).

3.9 Water Deprivation and 5-HIAA Concentration in Discrete Brain Regions.

Water deprivation had a significant effect on the concentration of 5-HIAA in both the cerebral cortex ($F_{(2,18)}$ = 11.44; P < 0.01) and the hippocampus ($F_{(2,18)}$ = 6.20; P < 0.01) (Table 3.4), although this effect was not

statistically significant in the hypothalamus ($F_{(2,18)} = 2.65$; $P > 0.05$) (Table 3.4). Specific group comparisons were made with Dunnett's t test and showed that in both cerebral cortex and hippocampus only the 48 hour water deprivation group had a concentration of 5-HIAA that was significantly greater than the control group (Table 3.4).

3.10 Water deprivation and 5-HT Turnover in Discrete Brain Regions.

Water deprivation had a significant effect on the turnover of 5-HT in both the cerebral cortex ($F_{(2,18)} = 8.64$; $P < 0.01$) and the hippocampus ($F_{(2,18} = 5.24$; $P < 0.05$) (Figure 3.4),. In the hypothalamus, although the increae in turnover appeared substantial it did not reach significance ($F_{(2,18)} = 2.96$; $P > 0.05$) (Figure 3.4). Specific group comparisons were made with Dunnett's t test and showed that in both the cerebral cortex and the hippocampus only the 48 hour water deprivation period had a significantly greater 5-HIAA : 5-HT ratio than the control group (Figure 3.4).

3.11 The design of experiments on the biochemical effects of restraint stress.

In response to the rather variable results found in the control groups with the water deprivation studies, the experiment into the effect of restraint stress on plasma corticosterone concentration and central 5-HT was designed

with a single control group.

3.12 The effect of restraint on plasma corticosterone concentration.

Restraint for 15 minutes resulted in a significant elevation of plasma corticosterone concentration ($F_{(6,35)} = 3.45$; $P < 0.01$) (Figure 3.5). Post hoc analysis confirmed that the plasma corticosterone concentration remained significantly elevated for all time points at which plasma corticosterone was measured up to two hours after the end of restraint (Figure 3.5). 2 hours after the end of restraint, plasma corticosterone concentration was still elevated compared to the control group, although a trend towards the normalisation of plasma corticosterone was evident (Figure 3.5).

3.13 The effect of restraint on brain 5-HT concentration.

There was no change in the concentration of 5-HT in the brains of rats restrained for 15 minutes when measured over the 2 hour period after the end of restraint and compared to an unrestrained group ($F_{(6,33)} = 0.77$; $P > 0.05$) (Table 3.5).

3.14 The effect of restraint on brain 5-HIAA concentration.

Restraint for 15 minutes caused a significant increase in the concentration of 5-HIAA in rat brain ($F_{(6,33)} = 2.94$; $P < 0.05$) (Table 3.5). Post hoc tests confirmed that 5-HIAA concentration remained elevated compared with the control group at all time points after the end of restraint (Table 3.5).

3.15 The effect of restraint on brain 5-HT turnover.

Restraint for 15 minutes also significantly elevated the ratio of 5-HIAA : 5-HT ($F_{(6,33)} = 5.22$; $P < 0.01$) (Figure 3.6) in rat brain. Post hoc tests confirmed that this ratio remained elevated compared with the control group at all time points at which the indoles were measured after the end of restraint, which was for a period of 2 hours.

3.16 The relationship between plasma corticosterone and 5-HT turnover or 5-HT concentration.

Correlations between the ratio of 5-HT turnover and plasma corticosterone concentrations were calculated. There was a clear negative correlation between 5-HT turnover and plasma corticosterone concentration over all the control rats used in water deprivation experiments (Figure 3.7a [$r = -0.56$; $P < 0.01$]), although for each individual control group this

correlation was much more variable [r = 0.23; P > 0.05 for 12 hours control group: r = 0.08; P > 0.05 for 24 hours control group: r = 0.90; P < 0.01 for 36 hours control group and r = 0.13; P > 0.05 for 48 hours control group]. The relationship was present in rats deprived of water for 36 and 48 hours (Figure 3.7b [r = -0.73 for 36 hours and -0.86 for 48 hours, both P < 0.01]). However, in the intervening period this relationship was not apparent (Figure 3.7b [r = 0.39 for 12 hours and r = 0.32 for 24 hours, both P > 0.05]). In restrained rats, there was no indication of a correlation between 5-HT turnover and plasma corticosterone at any time point after the end of restraint (Figure 3.7d) or in the control group (Figure 3.7c).

When correlations were performed on the relationship between 5-HT concentration and plasma corticosterone concentration only the water deprived control groups showed a significant correlation (r = 0.56; P < 0.01). A significant correlation between 5-HIAA concentration and plasma corticosterone concentration was deteted after water deprivation for 36 (r = 0.73; P < 0.05) but not for 12 (r = 0.18; P > 0.05), 24 (r = 0.15; P > 0.05) or 48 hours (r = 0.50; P > 0.05).

3.17 Discussion.

Water deprivation caused a large reduction in body weight, while the body weight of non-deprived animals marginally increased, as found previously (eg Aarseth and Klug, 1972; Duncan et al., 1989; Heilig et al., 1989). It was evident from these results that the major changes in body weight occurred during the rat's active phase. The loss of body weight did not vary in a linear fashion, but was skewed with most of the weight loss occuring overnight.

For each period of water deprivation, the plasma corticosterone concentration was increased compared to its control group, confirming that water deprivation is a stressful procedure. There was no relationship between the magnitude of the increase in corticosterone concentration and duration of deprivation, suggesting that as little as 12 hours water deprivation may have had a maximal effect on the corticosterone stress response.

It may be argued that the increase in hematocrit seen in response to water deprivation (Duncan et al., 1989) was the cause of the increase in concentration of plasma corticosterone, ie that the corticosterone concentration increased stoichiometrically because of the reduction in plasma volume. However, a previous investigation reported that the increase in the hematocrit after 48 hour water deprivation was from 37.7 ± 2.4 % to 43.0 ± 2.3 % (Duncan et al., 1989), which is not sufficient to account for the observed doubling in plasma corticosterone concentration

after water deprivation.

There was some variation in the basal corticosterone concentration, but this may be due to the fact that these experiments were done in different batches of rats and carried out at different times over a four month period. A similar degree of variation in the basal corticosterone concentration has been reported (Gaillet et al., 1991).

Previous work has suggested that animals that are water deprived and then receive an electric shock have higher plasma corticosterone concentrations than those who do not receive the shock (File et al., 1988). One study also reported that water depletion, using frusemide, did not result in an increase in corticosterone concentration in intact rats. This study is difficult to interpret because rats had access to water for 7 days after injection of frusemide before the plasma corticosterone concentration was determined (Bealer and Schneider, 1985).

In contrast to the deprivation-time-independent effect of water deprivation on corticosterone concentration, there was a deprivation-time-dependent effect of water deprivation on the 5-HIAA to 5-HT ratio, with only the more prolonged periods of water deprivation producing increases in this measure. There was again some unexpected variation in the control ratios of 5-HIAA to 5-HT and in response to this, the restraint investigation was planned as a single experiment using a single control group. Effects of water deprivation on whole brain 5-HT turnover were consistent with an activation of 5-HT mechanisms in response to the

stressor. The changes observed here in response to water deprivation in whole brain 5-HT systems were similar to those found in the present study with restraint and would appear to be consistent with an activation of 5-HT mechanisms in response to the stressor.

Correlations between 5-HT turnover and reduced plasma corticosterone concentration indicated that there was an inverse relationship in the control rats from water deprivation experiments. The relationship was detected when all control groups were combined, but was less evident in the individual groups. This might have been a result of the relatively low slope of the relationship, from which it might be concluded that a greater range in plasma corticosterone concentrations would present a stronger relationship. Alternatively, the correlations may have arisen by chance because of the small size of each individual groups. An inverse relationship would be surprising when viewed in the context of agonists at both postsynaptic $5\text{-}HT_{1A}$ and $5\text{-}HT_{2/1C}$ receptors being able to increase plasma concentrations corticosterone (eg Haleem et al., 1989; King et al., 1989). In water-deprived rats, this relationship appeared to be lost during water deprivation of 12 and 24 hours, but reappeared after 36 and 48 hours water deprivation. As with the individual coontrol groups, it is possible that these correlations might have arisen by chance. However, the absence of any correlation in water-deprived groups at 10-12 hours might indicate that during the first 24 hours other systems within the brain assume

greater importance in regulating plasma corticosterone but that on longer deprivation their importance wanes.

A comparison between the correlations detected in water deprivation experiments with those of restraint would indicate that the correlations detected during water deprivation are not common to all forms of stress. However, the lack of any correlation in the control group for the restraint experiment must cast some degree of doubt on the validity of the correlation detected in the water deprivation studies. Further experiments are required to establish whether the inverse relationship between 5-HT turnover and plasma corticosterone detected here is present in a larger sample of control animals, and if present, how this relationship is influenced by water deprivation.

Previous work had investigated the effect of restraint stress on 5-HT turnover in different areas of the rat brain. Differences in the turnover of 5-HT between anatomically distinct brain regions have been observed after restraint stress, although there has been little agreement between different workers in the field as to exactly what the anatomical distribution of an increase in 5-HT turnover in response to restraint is. In particular, an increase in 5-HIAA accumulation has been seen after restraint in the cerebral cortex and hippocampus, without significant changes in the brain stem and diencephalon (Morgan et al., 1975), but Mitchell and Thomas (1988) did not observe any changes in 5-HIAA in the frontal cortex or in the hippocampus of restrained rats, noting instead

increases in amygdala and hypothalmus.

The results from the investigation into the regional effects of water deprivation suggested that there were no consistent changes in the tryptophan content of the cerebral cortex, the hippocampus or the hypothalamus. In no area was the 5-HT content altered in response to 24 or 48 hour water deprivation, which is consistent with there being no change in the precursor of 5-HT in these regions. Changes in the 5-HIAA concentration in response to water deprivation paralleled those in the ratio of 5-HIAA to 5-HT and indicated increases only after 48 hours water deprivation in the cerebral cortex and the hippocampus, but not the hypothalamus. This clearly demonstrated a fact evident in the studies with whole brain 5-HT turnover measures, namely that the increase in turnover was due to an increase in the concentration of the metabolite with constant 5-HT concentrations. This pattern has been established for other stressors such as restraint (Curzon et al., 1972) and food deprivation (Knott et al., 1973).

The results of these experiments give no indication for a possible mechanism by which water deprivation is associated with an increase in brain 5-HT turnover. One possibility which may be worth investigation is the renin-angiotensin system. Water deprivation produces increases in plasma concentrations of angiotensin II (Di Nicolantonio and Mendelsohn, 1986) and angiotensin II can also stimulate the release of 5-HT in in vivo preparations (Nahmod et al., 1978). This is only one among many possibilities.

Table 3.1 The effect of water deprivation on body weight.

Duration (hrs)	N	Initial Body Weight (g)	Final Body Weight (g)	% Change
control	6	236 ± 16	241 ± 18	+ 2.1
12 hours	6	183 ± 7	172 ± 7	- 6.0
control	6	231 ± 12	239 ± 4	+ 3.5
24 hours	6	233 ± 9	212 ± 5	- 8.2
control	6	227 ± 15	241 ± 18	+ 6.2
36 hours	6	198 ± 26	172 ± 24	- 13.1
control	6	223 ± 18	239 ± 4	+ 7.2
48 hours	6	232 ± 13	198 ± 5	- 14.6

Table 3.2 The effect of 24 and 36 hour water deprivation on brain 5-HT turnover.

Duration	N	Controls	Water Deprived
24 hours	6	1.24 ± 0.04	1.32 ± 0.05
36 hours	6	0.97 ± 0.07	1.50 ± 0.06**

Comparisons were made by Student's unpaired t test ** P < 0.01.

Table 3.3 The effect of water deprivation on brain indole concentration.

Duration	N	5-HT Concentration (ng/g brain tissue)	5 - H I A A
12 hours			
controls	6	565.1 ± 32.6	608.7 ± 46.1
stressed	6	600.2 ± 53.3	753.0 ± 38.9*
24 hours			
controls	6	260.0 ± 20.5	460.5 ± 51.2
stressed	6	317.4 ± 16.5	587.0 ± 41.5
24 hours			
controls	6	355.8 ± 19.2	438.1 ± 17.8
stressed	6	358.1 ± 24.0	471.2 ± 30.6
36 hours			
controls	6	539.5 ± 30.9	499.9 ± 41.5
stressed	6	680.6 ± 33.9*	908.6 ± 37.6**
36 hours			
controls	6	662.0 ± 64.8	624.7 ± 42.1
stressed	6	791.6 ± 63.5	1187.4 ± 95.8**
48 hours			
controls	6	346.2 ± 57.0	599.3 ± 111.6
stressed	6	374.6 ± 56.1	919.9 ± 145.7

Comparisons were made between water-deprived group and control by Student's t test * P < 0.05; ** P < 0.01.

Table 3.4. The effect of water deprivation on indole concentration in discrete brain regions (n = 7).

Region + Duration of Deprivation	5-HT concentration (ng/g brain tissue)	5-HIAA concentration (ng/g brain tissue)	Ratio 5-HIAA/5-HT (-)
cerebral cortex			
control	209.4 ± 21.8	182.4 ± 47.7	1.02 ± 0.29
24 hours	155.2 ± 32.1	394.0 ± 121.0	2.61 ± 0.35
48 hours	248.7 ± 32.9	964.7 ± 161.3*	4.09 ± 0.78*
F value	$F_{(2,18)}$=2.55	$F_{(2,18)}$=11.44	$F_{(2,18)}$=8.64
P value	P > 0.05	P < 0.01	P < 0.01
hippocampus			
control	147.6 ± 34.6	228.7 ± 59.4	1.48 ± 0.16
24 hours	137.3 ± 18.4	424.3 ± 85.2	2.98 ± 0.52
48 hours	178.5 ± 10.7	708.9 ± 132.0*	4.01 ± 0.79*
F value	$F_{(2,18)}$=0.84	$F_{(2,18)}$=6.20	$F_{(2,18)}$=5.24
P value	P > 0.05	P < 0.01	P < 0.05
hypothalamus			
control	594.5 ± 125.4	496.1 ± 177.9	0.87 ± 0.25
24 hours	336.2 ± 53.1	500.3 ± 130.2	1.52 ± 0.30
48 hours	531.9 ± 24.3	901.6 ± 112.8	1.70 ± 0.20
F value	$F_{(2,18)}$=2.85	$F_{(2,18)}$=2.65	$F_{(2,18)}$=2.96
P value	P > 0.05	P > 0.05	P > 0.05

* P < 0.05 compared to control by Dunnett's t test after a significant one way ANOVA.

Table 3.5. The effect of 15 minutes restraint stress on brain indole concentration in whole brain less cerebellum (n = 6).

Time after end of restraint. (minutes)	5-HT concentration (ng/g brain tissue)	5-HIAA concentration (ng/g brain tissue)
control	286.5 ± 64.4	450.9 ± 46.7
0	278.5 ± 57.1	742.9 ± 69.1**
15	209.8 ± 13.0	684.4 ± 19.4*
30	204.1 ± 16.2	693.5 ± 53.9**
60	200.2 ± 26.5	626.7 ± 27.4*
90	228.3 ± 25.1	657.2 ± 68.6*
120	255.9 ± 24.0	624.1 ± 50.1*
F value	$F_{(1,83)}$= 0.77	$F_{(6,83)}$= 2.94
P value	P > 0.05	P < 0.01

* P < 0.05; ** P < 0.01 compared to control by Dunnett's t test after a significant one way ANOVA.

Figure 3.1. The effect of water deprivation on plasma corticosterone concentration. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test ** P < 0.01 compared to own control.

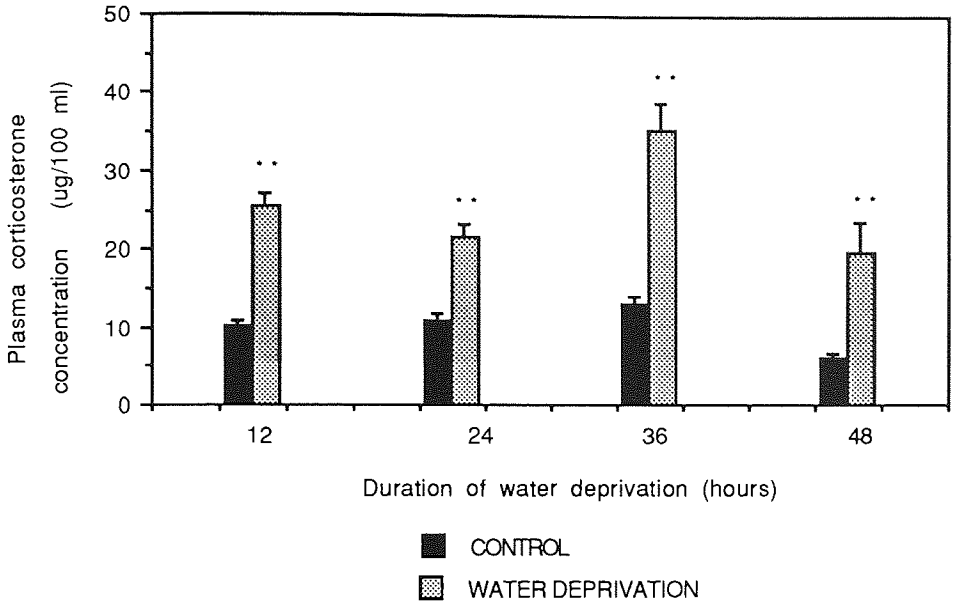


Figure 3.2. The effect of water deprivation on whole brain less cerebellum 5-HT turnover. The graph shows mean ± sem of 6 observations per group (24 h and 36 h water-deprived groups n = 5). Comparisons by unpaired t test * P < 0.05 ** P < 0.01

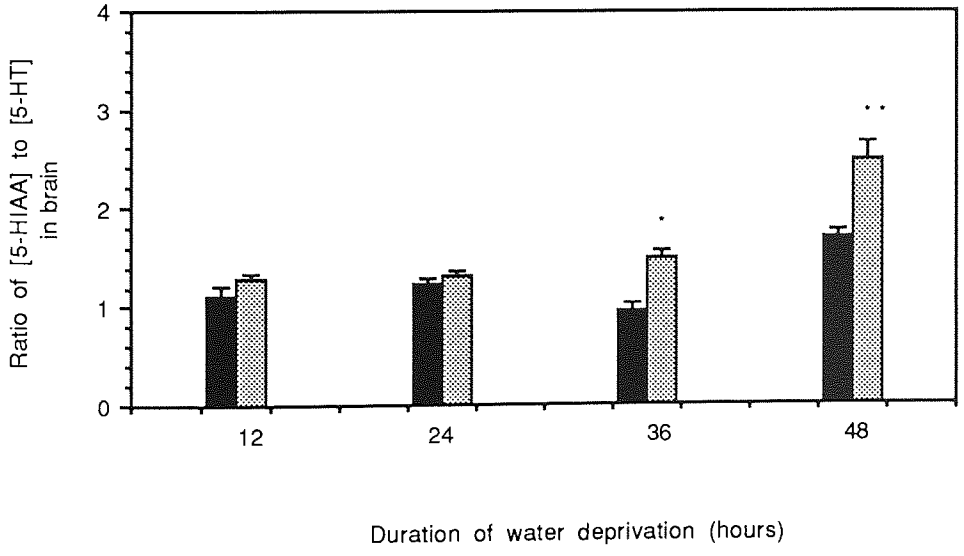
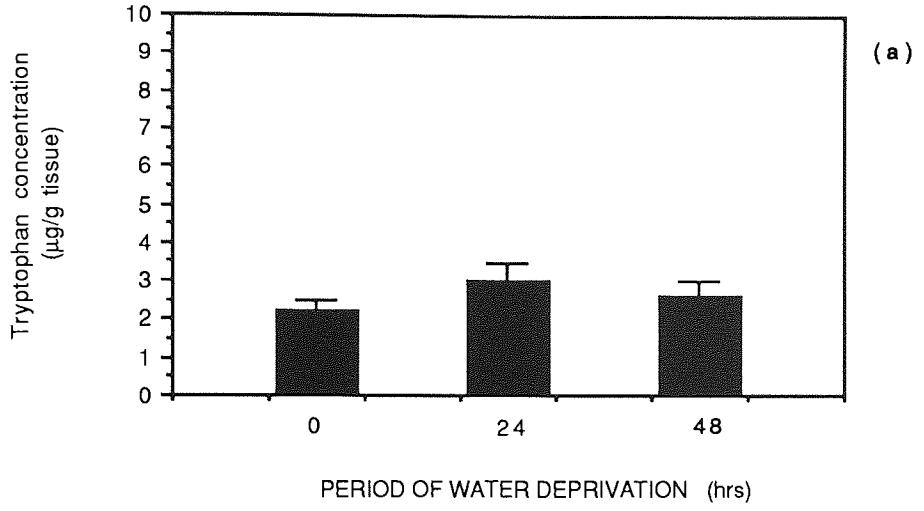
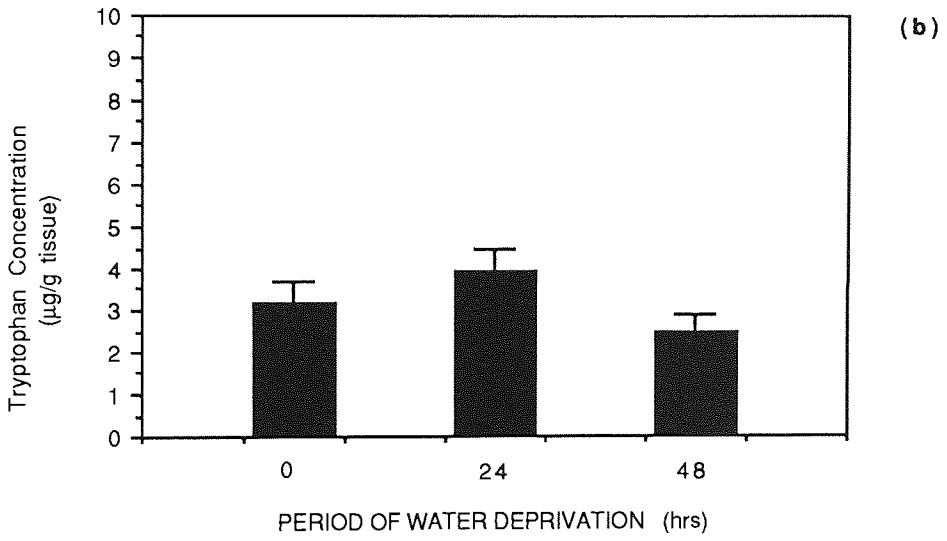


Figure 3.3. The effect of water deprivation on tryptophan concentration in (a) the cerebral cortex, (b) the hippocampus and (c) the hypothalamus. The graphs show mean ± sem of 7 observations per group. Comparisons by Dunnett's t test ** P < 0.01.





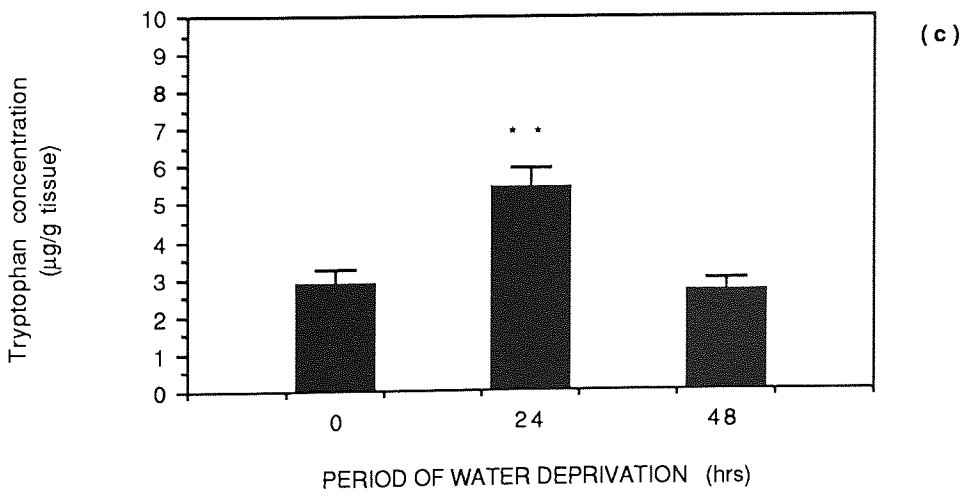
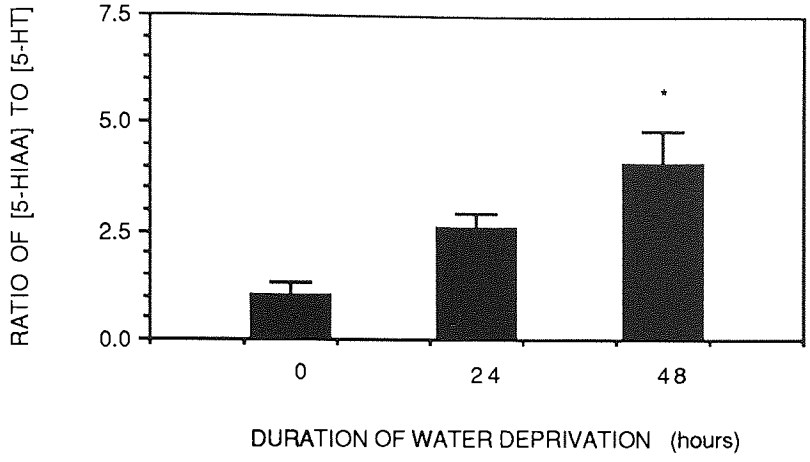
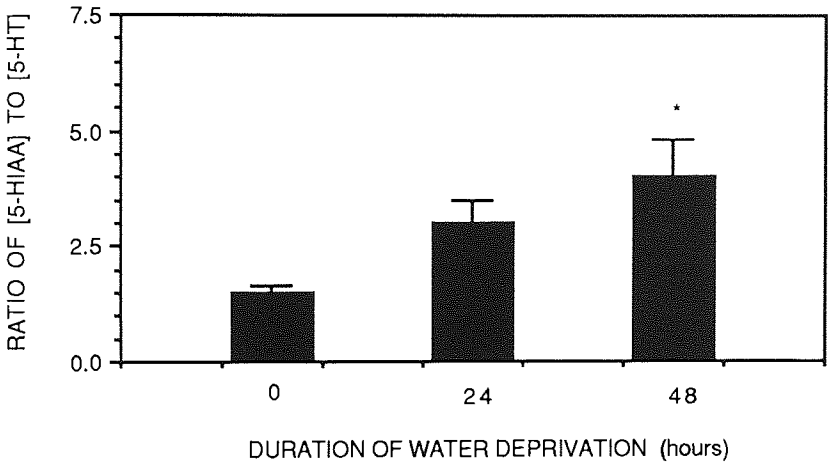


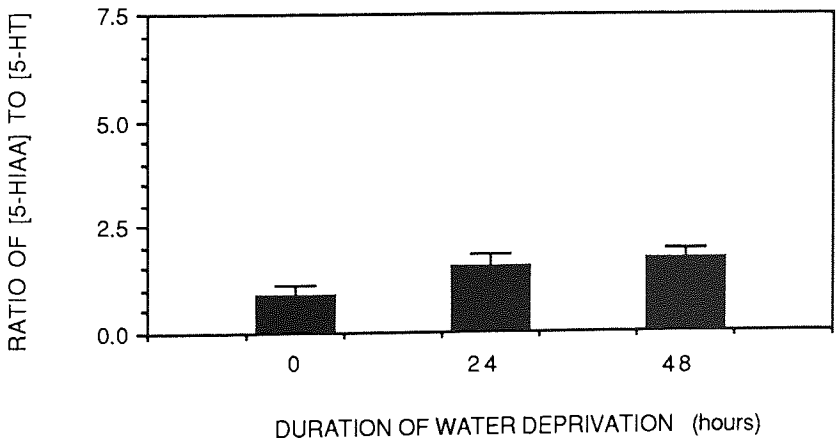
Figure 3.4. The effect of water deprivation on 5-HT turnover in (a) the cerebral cortex, (b) the hippocampus and (c) the hypothalamus. The graphs show mean ± sem of 7 observations per group. Comparisons by Dunnett's t test * P < 0.05.



(a)



(b)



(c)

Figure 3.5. The effect of restraint on plasma corticosterone concentration. The graph show mean ± sem of 6 observations per group. Comparisons by Dunnett's t test ** P < 0.01 compared to control.

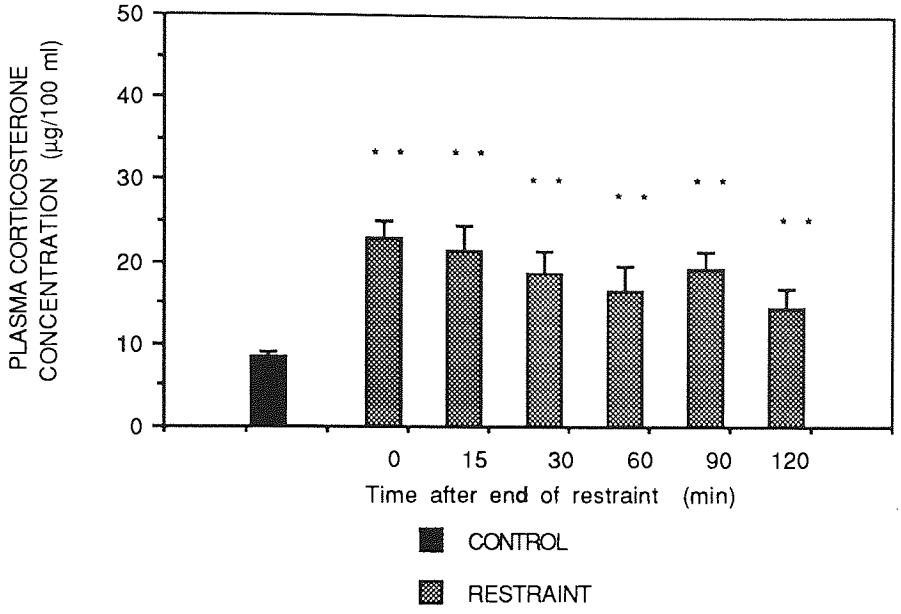


Figure 3.6. The effect of restraint on brain 5-HT turnover. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test ** P < 0.01 compared to control.

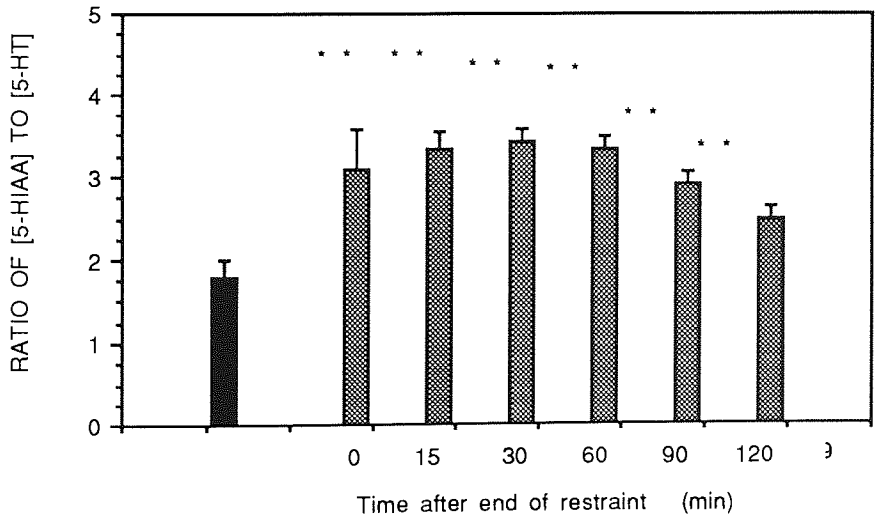


Figure 3.7a Scattergram of plasma corticosterone concentration and 5-HT turnover of control (not water-deprived) groups used in water deprivation experiments. The graph shows individual data points from the same rats within each group.

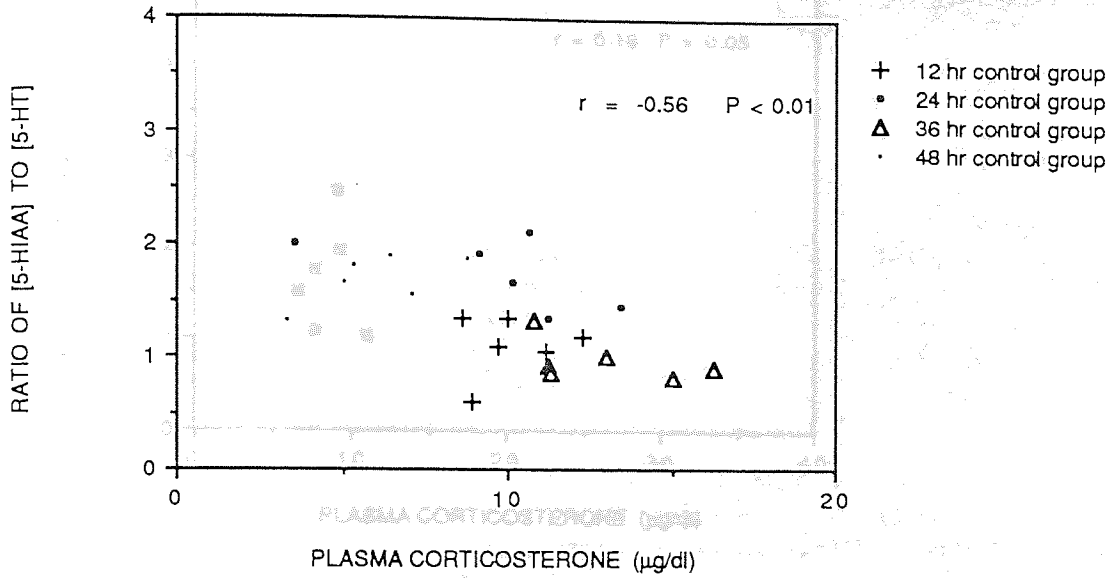


Figure 3.7b. Scattergram of plasma corticosterone and 5-HT turnover of water-deprived groups used in water deprivation experiments. The graph shows individual data points from the same rats within each group.

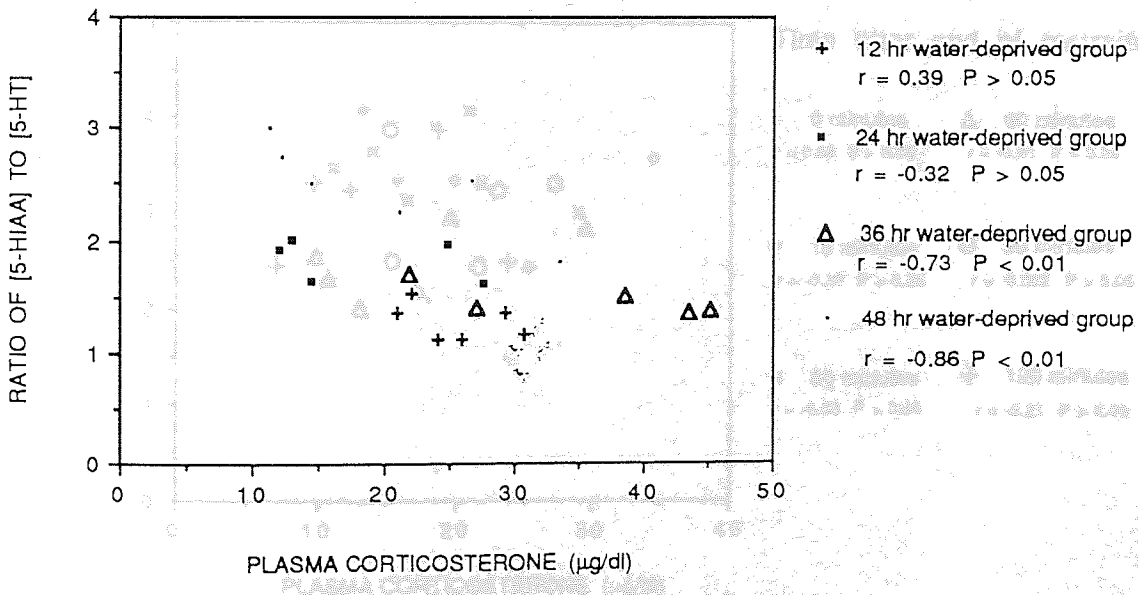


Figure 3.7c. Scattergram of plasma corticosterone concentration and 5-HT turnover of control (not restrained) rats. The graph shows individual data points from the same rats.

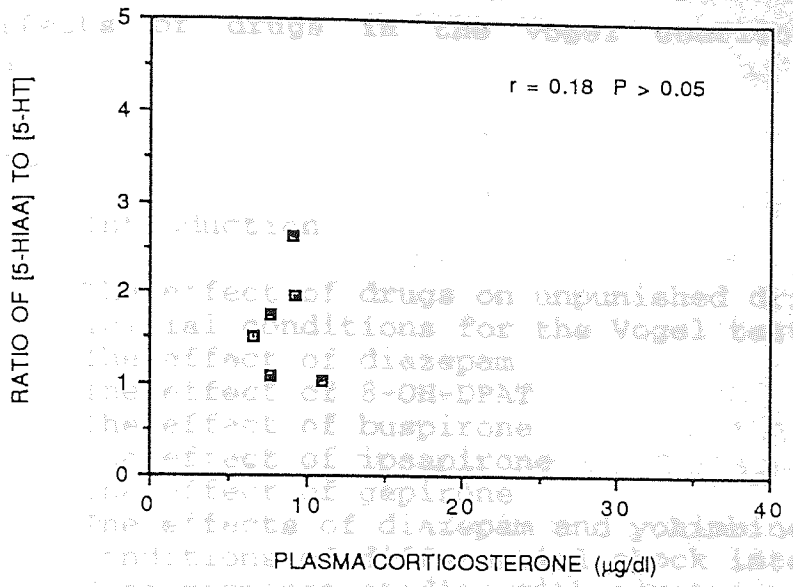
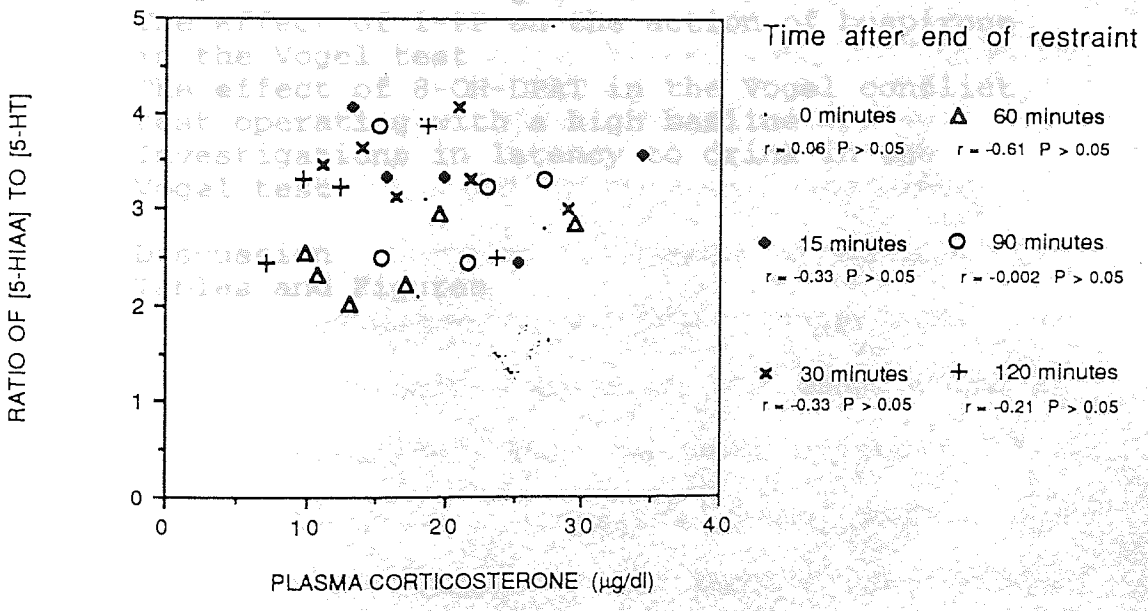


Figure 3.7d. Scattergram of plasma corticosterone and brain 5-HT turnover of restrained rats. The graph shows individual data points from the same rats within each group.



Chapter 4

The effects of drugs in the Vogel conflict model of anxiety.

Contents

4.1 Introduction.

The Vogel conflict test (or the punished drinking test) originated from the work of Leaf and Muller (1965) who reported that repeated administration of footshock suppressed licking of a water spout in rats. Vogel et al. (1971) used this information to produce a one-trial conflict test based on the suppression of drinking in water-deprived rats by the delivery of an electric shock to the floor of the cage on licking the spout of a water-bottle. For the test, Vogel et al. used rats that had been deprived of water for 48 hours prior to the experiment and were then placed in the experimental chamber with a water-bottle 30 minutes after intraperitoneal injection of the substance under test. When the spout of the water bottle was licked, an electrical circuit was completed which allowed the delivery of shock to the rat every 20th time it made contact with the spout of the water-bottle.

Vogel et al. (1971) reported that "anxiolytic" drugs in this test are able to increase the number of licks of the water spout during punishment, and that agents from other classes of psychotropic drugs, eg d-amphetamine, scopolamine and pemoline, were without effect. Other reports have subsequently confirmed the action of "anxiolytic" drugs in this test (eg Gardner and Piper, 1982; Sanger et al., 1985). Conflict engendered by the electric shock diminishes the number of licks made in this test and the ability of "anxiolytics" to increase licking

is attributed to their abiliy to reduce the conflict component of the test (Vogel et al., 1971).

A major benefit of this procedure is that it requires no training of experimental animals and is thus less time consuming than, for instance, the Geller-Seifter test. A potential disadvantage is that it requires water deprivation for a considerable period of time, which is likely to be a powerful stressor.

The Vogel test is widely held to generate data with an unusually large variance (Barrett and Gleeson, 1991), despite the initial claims that this was not so (Vogel et al., 1971). To overcome this, a modification to the test has been used in which animals are pretested several hours before the test begins. The purpose of this is to identify rats which demonstrate clear and reliable licking behaviour and to exclude from experiments those rats which do not.

An alternative favoured by some (Kilts et al., 1982; Schefke et al., 1989) is to train rats to obtain most of their daily water intake within the shock chamber. Once animals have habituated to this procedure, introduction of a signalled shock results in the association of the shock with the signal, such that rats do not drink in the presence of the signal. This procedure is called the conditioned suppression of drinking and "anxiolytic" drugs, including diazepam and buspirone, increase the number of shocks accepted in this test too (McCloskey et al., 1987; Schefke et al., 1989). This approach seems to cast aside the main benefit of the Vogel test, namely that it does not

need long-term training of animals.

It would be expected that agents which increase thirst drives would generate false positive results in the Vogel test. This issue has been discussed (Carli and Samanin, 1982) with the conclusion that, even when agents are positively dipsogenic, genuine anticonflict effects may still arise. The fact remains that agents that increase punished drinking may be doing so either because they increase drinking motivation or because they have anticonflict effects.

Many authors have replicated the findings of Vogel et al. (1971) that this test is capable of detecting "anxiolytic" properties of drugs that act through the $GABA_A$ receptor complex. The benzodiazepines chlordiazepoxide (Gardner and Piper, 1982), diazepam (ibid., Shibata et al., 1989), clobazam (ibid.) flunitrazepam (ibid.) and lorazepam (Petersen et al., 1982) all increased punished drinking, as did the barbiturate phenobarbitone (Petersen et al., 1982; Shibata et al., 1989) and the GABA transaminase inhibitor sodium valproate (Gardner and Piper, 1982).

Agents acting via systems other than the $GABA_A$ and serotonergic systems have been reported to be active in the Vogel test. An involvement of the dopamine system in anxiety has been postulated based on evidence that the dopamine agonist apomorphine (Hjörth et al., 1986) and that enantiomers of 3-(3-hydroxy-phenyl)-N-N-propylpiperidine (3-PPP), which are respectively an agonist and an antagonist at dopamine autoreceptors (Hjörth et al.,

1987a), are active in the Vogel conflict test.

A number of adrenergic α_2 antagonists have "anxiolytic" activity in the Vogel conflict procedure. Gower and Tricklebank showed that idazoxan, 1-PP, WY 26392 and yohimbine all increased punished drinking. Yohimbine has "anxiogenic" properties in humans (Holmberg and Gershon, 1961) and in animals in the elevated X-maze model of anxiety (Handley and Mithani, 1984; Pellow et al., 1985). It has a paradoxical "anxiolytic" action in the Vogel conflict test (Baldwin et al., 1989) and it was therefore of interest to investigate whether it was possible to alter the response to yohimbine in the Vogel conflict test.

A number of authors (eg Barrett and Gleeson, 1991; Handley, 1991; Lader, 1991b) have observed that, of the many animal models of anxiety, the Vogel conflict test has a good record in detecting "anxiolytic" effects of 5-HT_{1A} receptor ligands.

The initial findings with 8-OH-DPAT in the Vogel test (Engel et al., 1984) suggested that this drug was "anxiolytic" and that this effect was due to a pre-synaptic action because it was reversed by pCPA pretreament. Higgins et al. (1988) also observed an anticonflict effect of 8-OH-DPAT in the Vogel test, and as this was injected into the dorsal Raphé nucleus, it was attributed to a mechanism presynaptic with respect to the serotonergic neurones of the dorsal Raphé. However, others (e.g. Carli and Samanin, 1988; Moser et al., 1990) have not found a specific anti-conflict action of the drug in this test, although 8-OH-

DPAT was found to antagonize an "anxiolytic" effect of the 5-HT_{1A} ligand MDL 73500EF and also of buspirone (Moser et al., 1990).

The action of buspirone in the Vogel test appears to be quite reliable (eg Brocco et al., 1990; Eison, et al., 1986; Gower and Tricklebank, 1988; Moser et al. 1990; Oakley and Jones, 1983; Schefke et al., 1989; Wada and Fukuda, 1991). Although many authors have identified "anxiolytic" activity, the mechanism of this effect is not definitively established. Thus, Eison et al. (1986) concluded that it derived from a presynaptic effect on serotonergic neurones, although Moser et al. (1990) concluded it was not likely to be due to 5-HT_{1A} receptor action on the grounds that 8-OH-DPAT did not have the same effect. Gower and Tricklebank (1988) believed that the activity was due to the adrenergic α_2 antagonist metabolite 1-PP, but did not report the effect of 1-PP and buspirone in combination in the Vogel test, despite showing that buspirone did have an additive effect with other adrenergic α_2 antagonists. However, the anticonflict effect of buspirone was antagonized by clonidine, further implicating the adrenergic α_2 receptor as the site of action (Gower and Tricklebank, 1988).

Gepirone (Eison et al., 1986) and ipsapirone (Chojnacka-Wojcik et al., 1991) have also been reported to have "anxiolytic" activity in the Vogel test, the latter compound probably acting through post-synaptic 5-HT_{1A} receptors for this effect (Chojnacka-Wojcik, et al., 1991).

Less selective drugs acting on the serotonergic system have been investigated in drinking conflict tests. In a conditioned suppression of drinking test metergoline was without effect (Commissaris and Rech, 1982), although quipazine and LSD had some weak "anxiolytic" activity (Commissaris and Rech, 1982). Methysergide, cyproheptadine and cinanserin are also inactive in this conditioned suppression of drinking test (Kilts et al., 1982). It has been shown that lesioning of the sertonergic system produces an "anxiolytic" effect on the Vogel conflict test (Söderpalm and Engel, 1990). This effect of lesioning 5-HT systems has recently been shown to be abolished by adrenalectomy (Söderpalm and Engel, 1992) and furthermore, that corticosterone treatment could reinstate this effect (ibid.). The precursor to 5-HT, 5-HTP, has been reported to be both "anxiolytic" at low doses and "anxiogenic" at higher doses in a Vogel-type conflict test (Hjörth et al., 1987b).

Finally, there are no reports of the action of serotonin uptake inhibitors in the Vogel conflict test. In view of the involvement of serotonin in the action of many drugs that are active in the Vogel test, it was of interest to investigate the effect of an inhibitor of serotonin uptake in the Vogel conflict test.

Results.

4.2 The effects of drugs on unpunished drinking.

In rats that had been deprived of water for 24 hours, neither diazepam (2.5 mg/kg) nor buspirone (2 mg/kg) changed the number of licks made in an unpunished drinking test ($F_{(2,44)}$ = 0.06; P > 0.05; Table 4.1).

4.3 Initial conditions for the Vogel Test.

The standard conditions which were in operation in the laboratory at Wellcome were used at the outset of experiments in the Vogel test. These conditions consisted of 24 hours water deprivation; a 3 minute test duration; 1.0 s shock duration and 0.03 mA direct current shock intensity.

4.3.1 The effect of diazepam under initial conditions.

Diazepam, given ip at doses of 1.0 and 2.5 mg/kg 30 minutes prior to the test, resulted in an increase in the number of licks made, although this increase was only marginally significant ($F_{(2,17)}$ = 2.97; 0.05 < P < 0.1). Post hoc tests suggested that this was due to the higher dose (Table 4.2).

4.3.2 The effect of 8-OH-DPAT under initial conditions.

8-OH-DPAT, at 0.1 and 0.2 mg/kg 10 minutes prior to the

test, resulted in a trend towards an increase in the number of licks made, although this did not reach significance ($F_{(2,21)}$ = 0.66; P > 0.05) (Table 4.2).

4.3.3 The effect of buspirone under initial conditions.

Buspirone, at doses of 0.5 amd 1.0 mg/kg, given ip 30 minutes before the test did not result in any significant changes in the number of licks made ($F_{(2,22)}$ = 1.34; P > 0.05) (Table 4.2).

4.3.4 The effect of ipsapirone under initial conditions.

Ipsapirone, at doses of 0.1 and 1.0 mg/kg, given ip 30 minutes before the test did not result in any significant changes in the number of licks made ($F_{(2,20)}$ = 0.70; P > 0.05) (Table 4.2).

4.3.5 The effect of gepirone under initial conditions.

Gepirone, at doses of 0.5 amd 2.0 mg/kg, given ip 30 minutes before the test did not result in any significant changes in the number of licks made ($F_{(2,21)}$ = 1.65; P > 0.05) (Table 4.2).

4.4 The effects of diazepam and yohimbine under conditions of differential shock intensity.

Because of the lack of success with the 5-HT_{1A} receptor ligands with the initial conditions, an attempt was made to find the optimal conditions under which an "anxiolytic" effect may appear. In addition to diazepam, yohimbine, the adrenergic α_2 antagonist, was used as a standard compound. Table 4.3 depicts the results of varying the shock intensity on the action of both diazepam and yohimbine. Yohimbine (1.25 and 2.5 mg/kg, ip 30 minutes) failed to influence the number of licks made under any of the tested conditions, whereas diazepam (2.5 mg/kg, ip 30 minutes) significantly increased the number of licks, in every instance.

4.5 Dose-response studies with serotonergic agents.

Thus far, there had been little success using agents other than diazepam in the Vogel conflict test. With Home Office approval for 48 hour water deprivation, dose-response curves were conducted under the same experimental conditions, and including diazepam as an active control group, as those for which an "anxiolytic" effect of buspirone had been documented (Moser et al., 1990). These conditions were as follows: 48 hours water deprivation; 5 minutes test duration; 0.5 s shock duration; 0.4 mA shock intensity; FR 20.

4.5.1 The effect of buspirone in the Vogel conflict test.

Buspirone, over the dose range 0.25 - 8 mg/kg and given 30 minutes prior by the intraperitoneal route, produced an increase in the number of licks made in the five minute session ($F_{(5,51)} = 4.06$; $P < 0.01$) (Figure 4.1). A higher dose of 16 mg/kg produced marked flat body posture in some animals which appeared to impair responding in the Vogel test and resulted in 3 out of 7 animals failing to initiate licking in the test. Diazepam (2 mg/kg) also increased the number of licks.

4.5.2 The effect of ipsapirone in the Vogel conflict test.

Under the same conditions as buspirone and over the same dose range there was a significant main effect of drug treatment on the number of licks ($F_{(5,42)} = 5.11$; $P < 0.01$). Post hoc test revealed that ipsapirone failed to influence the number of licks made in the Vogel test (Figure 4.2) and there were no apparent signs of the 5-HT syndrome. The significant main effect was attributable completely to diazepam (2 mg/kg), which increased the number of licks (Figure 4.2).

4.5.3 The effect of 8-OH-DPAT in the Vogel conflict test.

There was a significant main effect of drug treatment on

the number of licks ($F_{(5,59)}$ = 4.81; P < 0.01). Post hoc testing confirmed that 8-OH-DPAT, given i.p. 10 minutes before testing over the dose range 0.025 - 0.2 mg/kg, failed to influence the number of licks made in the Vogel test (Figure 4.3). There were no signs of the 5-HT syndrome at any of the doses used. The significant main effect was completely attributable to diazepam (2 mg/kg), which increased the number of licks (Figure 4.3).

4.5.4 The effect of fluoxetine in the Vogel conflict test.

There was a significant effect of drug treatment on the number of licks ($F_{(5,51)}$ = 6.07; P < 0.01). Fluoxetine over the dose range 1.25 - 10 mg/kg and given i.p. 30 minutes prior to the test produced an increase in the number of licks accepted which was equivalent in magnitude to that of diazepam (2 mg/kg) at the 5 and 10 mg/kg doses (Figure 4.4). A higher dose of fluoxetine, 20 mg/kg, did not have any effect on the number of licks made in the Vogel test (Figure 4.4).

4.6 Investigations into the mechanism of the "anxiolytic" action of buspirone.

Antagonism of the "anxiolytic" effect of buspirone was attempted using the mixed 5-HT_{1A}/ß receptor antagonists pindolol and propranolol. A dose of 4 mg/kg of buspirone was chosen as the standard to produce a reliable

"anxiolytic" effect.

4.6.1 The effect of (±)-pindolol on the action of buspirone in the Vogel test.

(±)-Pindolol, given 45 minutes prior to the test at doses of 5 and 10 mg/kg, caused an increase in the number of licks made (Table 4.4; $F_{(1,28)} = 14.06$; $P < 0.01$) (Figure 4.5). Buspirone produced an increase in the number of licks made only in the experiment testing the effect of 5 mg/kg pindolol ($F_{(1,28)} = 15.70$; $P < 0.01$) (Figure 4.5). The combination of both pindolol and buspirone produced an additive effect in the number of licks accepted when buspirone was active (interaction $F_{(1,28)} = 0.65$; $P > 0.05$) (Figure 4.5) and was no different to pindolol treatment alone in the experiment where buspirone was inactive (Experiment 1, Table 4.4).

4.6.2 The effect of (±)-propranolol on the action of buspirone in the Vogel test.

(±)-Propranolol, given 45 minutes prior to the test at a dose of 10 mg/kg, caused an increase in the number of licks made ($F_{(1,28)} = 13.29$; $P < 0.01$) as did buspirone (4 mg/kg) ($F_{(1,28)} = 8.48$; $P < 0.01$) (Figure 4.6). The combination of both propranolol and buspirone produced an additive effect in the number of licks accepted (interaction $F_{(1,28)} = 0.16$; $p > 0.05$) (Figure 4.6).

A higher dose of (±)-propranolol, 40 mg/kg, caused the rats to lose posture in their home cage and resulted in only 4 out of 8 rats in the propranolol-treated group initiating the session (Experiment 2, Table 4.4).

4.6.3 The effect of 1-PP on the action of buspirone in the Vogel test.

There was a significant interaction between buspirone and 1-PP ($F_{(1,28)} = 6.14$; $P < 0.05$). Post hoc tests confirmed that 1-PP, at a dose of 4 mg/kg given i.p. 45 minutes before the Vogel test, had no effect on the number of licks made, that buspirone (4 mg/kg) significantly increased the number of licks and that 1-PP significantly antagonised the "anxiolytic" effect of buspirone (Figure 4.7).

4.7 The effect of 8-OH-DPAT in the Vogel conflict test operating with a high basline.

The intensity of the shock was reduced to 0.01 mA in order to raise the baseline of the test, although all other parameters remained the same. 8-OH-DPAT was given in doses of 0.05 - 0.2 mg/kg ip 10 minutes before the start of the test. Diazepam was included as an active control.
Under these conditions, there was no effect of either treatment with diazepam or 8-OH-DPAT ($F_{(4,29)} = 0.74$; $P > 0.05$) (Table 4.5).

4.8 Investigations in latency to drink in the Vogel Test.

In almost all of the above experiments, the latency to the first lick was recorded. There was no effect from any agent tested on the latency to drink in the Vogel test (Table 4.6).

4.9 Discussion.

At single doses, neither buspirone nor diazepam increased drinking in the absence of electric shock in a 5 minute test session in rats that had been deprived of water for 24 hours. Although this is not definitive evidence that the effect of these drugs on drinking in the presence of punishment was not due to a dipsogenic action, it does imply that the effects observed in the presence of punishment were not compromised by an increase in licking behaviour per se. These results are consistent with previous reports for both buspirone (Wada and Fukuda, 1991) and benzodiazepines (Falk and Burnidge, 1970) indicating that these drugs do not have any dipsogenic activity which could account for the observed increases in punished drinking.

It is possible that other ligands which increased punished drinking were doing so by a dipsogenic action. It could be that fluoxetine, pindolol and propranolol were active in this test for this reason rather than a true anticonflict effect. There is some evidence that adrenergic ß-blockers do in fact, increase drinking (Carli and Samanin, 1982).

Responses to diazepam under the initial conditions under which the test was conducted indicated that there was a large variance in the results. This may have resulted in only trends towards an effect being present, rather than significant effects being detected.

Results with the 5-HT_{1A} agonists under initial conditions

were disappointing. No effect was observed with 8-OH-DPAT, ipsapirone or gepirone or buspirone. Investigations with yohimbine were intended to find conditions under which this agent, which is active in the Vogel test according to published literature (Baldwin et al., 1989), was able to produce a clear and reliable "anxiolytic" effect. Doses of yohimbine were chosen on the basis of published reports of its "anxiolytic" activity (Gower and Tricklebank, 1988; Baldwin et al., 1989). The failure of yohimbine to act in this test under the conditions used may be due to the fact that only 24 hour water deprivation was used in these experiments, whereas published work used rats deprived of water for 48 hours (Gower and Tricklebank, 1988).

The conditions of the test were then altered to mimic as closely as possible one set of conditions under which buspirone had been shown to be active (Moser et al., 1990). These conditions were chosen because they were thought to be typical of reports indicating that buspirone was active in this test.

Under these conditions, buspirone had a reliable "anxiolytic" effect which was marginally significant at 1 mg/kg and maximal at 4 mg/kg. Increasing the dose further produced no greater "anxiolytic" activity (8 mg/kg) and at the highest dose tested (16 mg/kg) there were signs of the 5-HT syndrome, particularly flat body posture, which was observed to impair animals' responding.

The lack of effect of 8-OH-DPAT under these conditions is in contrast to the finding of Engel et al. (1984), but is

in agreement with Carli and Samanin (1988) and with Moser et al. (1990). The reason for this discrepancy is difficult to identify. Some differences in the detail of the procedures used here and by Moser et al (1990) are evident when compared with that of Engel et al. (1984). For instance, the latter authors used a slightly smaller shock intensity than that used here and rats were given a glucose solution instead of water to drink in the test. These factors might produce a greater incentive to drink than the conditions used here, but this would be presumed to have as great an effect on undrugged rats as on drug-treated rats. These differences appear so minor that it is difficult to believe that they alone were responsible for the observed differences in experimental outcome.

There was no effect of ipsapirone in the present studies. This finding is in direct contrast to Chojnacka-Wojcik et al. (1991) who demonstrated that ipsapirone, over a similar dose range to that used here, by the same route and in similar experimental conditions, acts at postsynaptic 5-HT_{1A} receptors to produce an increase in the number of shocks accepted in a Vogel conflict test.

The serotonin specific reuptake inhibitor, fluoxetine, produced an increase in the number of licks made in this test. There are no reports of the effect of this group of compounds in the Vogel conflict test. The bell-shaped dose-response curve is typical of the effects of many "anxiolytic" drugs.

The activity of buspirone and of fluoxetine in the present

experiments might be the result of an analgesic action of these compounds. Buspirone (Rogers and Giordano, 1990) and fluoxetine (Hynes et al., 1985) have both demonstrated analgesic activity in tests where mechanical or thermal stimuli have been used as a painful stimulus. However, buspirone has also been reported to have no intrinsic analgesic property in the mouse tail-flick test (Millan and Colpeart, 1991). The relevance of any analgesic activity in these tests to the use of electric shock in the Vogel test is unclear, but it remains a possibility that analgesic effects contribute to the increases in punished drinking observed here.

The mechanism of the "anxiolytic" effect of buspirone was not proven by the experiments conducted. The "anxiolytic" effect of both propranolol and pindolol was not unexpected, given evidence that ß-blockers increase drinking in the Vogel test (Carli and Samanin, 1982). An "anxiolytic" effect of propranolol injected into the hippocampus has been reported (Kataoka et al., 1991). It is conceivable that the observed action with both these drugs reflected partial agonist activity at the 5-HT_{1A} receptor (Hjörth and Carlsson, 1986). This may lead to the conclusion that the directly additive effect of the "anxiolytic" effect of buspirone with lower doses of the mixed 5-HT_{1A}/adrenergic ß receptor antagonists was due to the combination of partial agonist action by both buspirone and its alleged antagonists. However, this conclusion is not supported by the dose-response study with buspirone, which indicated

that the 4 mg/kg dose was at the top of the maximally effective dose range. Indeed, this dose was used because it produced the maximum "anxiolytic" effect, without producing any clear signs of the 5-HT syndrome.

That the effects of pindolol and propranolol were very similar in these experiments is tentative evidence in favour of a postsynaptic 5-HT_{1A} receptor being involved in this effect of buspirone. There is evidence from microdialysis studies that propranolol is a poor antagonist at presynaptic 5-HT_{1A} receptors (Sharp and Hjörth, 1990), but it is a good postsynaptic antagonist according to electophysiological experiments (Sprouse and Aghajanian, 1986). Pindolol does not appear to possess this discriminatory profile (Sharp and Hjörth, 1990).

It is difficult to argue that buspirone was acting through 5-HT_{1A} receptors, as an agonist, to produce an "anxiolytic" effect firstly because pindolol and propranolol did not antagonize this effect and secondly because ipsapirone and, crucially, 8-OH-DPAT, did not produce the same effect. The possibility that the metabolite of buspirone, 1-PP was responsible was not supported by experimental evidence with the combination of 1-PP and buspirone. At best, because buspirone's effect was maximal at the dose used, 1-PP should have had an equivalent "anxiolytic" effect with buspirone, if it is assumed that 1-PP is the cause of the "anxiolytic" effect of buspirone, but the combination should be no better than either compound alone. Instead, 1-PP was found to significantly antagonize the effect of

buspirone without itself having any effect.

After a 10 mg/kg oral dose of buspirone, the brain 1-PP concentration was found to be 7.8 nmol/g brain tissue, whereas that of buspirone was just 0.6 nmol/g (Caccia et al., 1986). It is possible that 1-PP was interacting with 5-HT_{1A} receptors to prevent the action of buspirone and so reduce the "anxiolytic" effect of buspirone. 1-PP has some 5-HT_1 affinity (IC_{50} = 1.6 µM; Caccia et al., 1986) although this is negligible compared with its affinity for α_2 adrenoceptors (IC_{50} = 25 nM; Caccia et al., 1986). A similar effect of 1-PP and other α_2 adrenoceptor antagonists on a 5-HT_{1A} mediated effect has been reported (Handley and Dursun, 1992). It is established that 5-HT_{1A} receptor agonists antagonize the occurence of head shakes in the mouse after treatment with the 5-HT_2 agonist DOI (Handley and Dursun, 1991). 1-PP significantly antagonizes the effect of buspirone at doses which do not themselves have any effect. This lends support to the view that not all behavioural effects of buspirone are attributable to action of its metabolite on α_2 adrenoceptors.

From this evidence it is impossible to make a logical statement about the "anxiolytic" action of buspirone in the Vogel conflict test. The latency to the first lick was measured in most experiments. It was thought that this would provide information regarding the ability of the animal to withhold a response. Soubrié has eloquently argued that many, if not all, "anxiolytic" effects of the benzodiazepines and serotonergic drugs may be attributable

not to a specific "anxiolytic" activity, but to an inability to wait before responding. Thus, in the Geller-Seifter test, apparent "anxiolytic"-like increases in the number of bar presses would in fact not be related to "anxiety", but rather reflect the fact that the animal cannot withhold the learned response. Clinical studies too have suggested that serotonin is involved in the control of impulsivity, with individuals who have low 5-HT tone being more likely to commit suicide and show aggressive behaviour (López-Ibor, 1988). Results from the present experiments indicated that the "anxiolytic" drug effects observed were never related to a reduction in the latency to the first lick, which might suggest that the observed increases in drinking represented, if not an "anxiolytic" action, then an action independent from a reduction in impulse control. Wada and Fukuda (1991) reported that buspirone increased the time elapsed before the first shock was accepted, despite there being an "anxiolytic"-like effect. The dose used also decreased the number of arm entries in an elevated X-maze test (Wada and Fukuda, 1991), indicating that it may have produced some behavioural impairment which caused the increase in latency. One conclusion might be that the "anxiolytic" effect of buspirone is not caused by an increase in impulsivity in buspirone-treated rats.

Table 4.1 The effects of drugs on unpunished drinking.

DRUG	Saline	Diazepam	Buspirone
DOSE		2.5 mg/kg	2.0 mg/kg
N	28	12	7
LICKS	920 ± 37	1030 ± 89	848 ± 87

Statistical analyses were made by one way ANOVA, which was not significant.

Table 4.2. The effect of drugs in the Vogel conflict test. For conditions see section 4.3.

DRUG	N	DOSE (mg/kg)	NO. OF LICKS
Diazepam	6	0.0	74.2 ± 29.1
	6	1.25	88.1 ± 8.9
	6	2.5	149.9 ± 28.3*
8-OH-DPAT	8	0.0	103.4 ± 10.7
	7	0.1	125.1 ± 9.6
	9	0.2	148.1 ± 41.7
Buspirone	8	0.0	147.9 ± 34.1
	8	0.5	96.9 ± 14.6
	9	1.0	174.8 ± 43.8
Ipsapirone	7	0.0	142.4 ± 26.9
	7	0.1	141.7 ± 28.5
	8	1.0	115.1 ± 17.9
Gepirone	8	0.0	141.1 ± 32.2
	8	0.5	131.5 ± 14.6
	8	2.0	275.5 ± 102.8

Statistical analyses were made by one way ANOVA followed by Dunnett's t test * $P < 0.05$.

Table 4.3. The effect of diazepam and yohimbine in the Vogel conflict test under conditions of differential shock intensity.

DRUG	SHOCK INTENSITY (mA)	N	DOSE (mg/kg)	NO. OF LICKS
Diazepam	0.03	6	0.0	74.2 ± 29.1
		6	1.0	88.1 ± 8.9
		6	2.5	149.9 ± 28.3*
Diazepam	0.15	10	0.0	82.0 ± 13.8
		9	1.0	79.3 ± 11.0
		9	2.5	120.8 ± 15.3*
Diazepam	0.40	7	0.0	54.0 ± 8.9
		8	1.0	85.0 ± 10.1
		8	2.5	82.9 ± 8.9*
Yohimbine	0.03	7	0.0	123.4 ± 26.8
		7	1.25	116.9 ± 19.6
		6	2.50	77.7 ± 11.7
Yohimbine	0.15	9	0.0	96.2 ± 16.5
		9	1.25	70.8 ± 10.2
		8	2.5	95.0 ± 19.2
Yohimbine	0.40	7	0.0	53.4 ± 6.3
		6	1.25	46.8 ± 6.7
		7	2.5	58.3 ± 6.5

Statistical analyses were made by one way ANOVA which, if significant, was followed by Dunnett's t test * $P < 0.05$.

Table 4.4. The effect of pindolol and propranolol on the action of buspirone in the Vogel test.

Treatment	DOSE	N	NO. OF LICKS
Experiment 1			
Saline	0.0	6	167.8 ± 25.5
Buspirone	4.0	5	151.8 ± 31.5
Pindolol	10	7	617.9 ± 178.4**
Buspirone + Pindolol	4.0 10	4	593.2 ± 184.0
Experiment 2			
Saline	0.0	8	121.9 ± 15.9
Buspirone	4.0	7	398.7 ± 69.2
Propranolol	40	4	442.5 ± 134.2**
Buspirone + Propranolol	4.0 40	2	501.0 ± 139.0

Two way ANOVA was not performed on these data because of the number of animals failing to initiate the session. ** P < 0.01 compared to saline by Student's unpaired t test.

Table 4.5. The effect of 8-OH-DPAT and diazepam in the Vogel conflict test operating with a high baseline.

DRUG	N	DOSE (mg/kg)	NO. OF LICKS
Saline	7	0.0	1079.3 ± 65.3
8-OH-DPAT	7	0.05	727.7 ± 196.7
	7	0.10	811.1 ± 172.4
	7	0.20	940.6 ± 184.2
Diazepam	6	2.0	998.9 ± 181.1

Statistical comparison was made by one way ANOVA, which was not significant.

Table 4.6. The effect of 5-HT_{1A} ligands on latency to drink in the Vogel conflict test.

DRUG	DOSE (mg/kg)	N	LATENCY (s)
8-OH-DPAT	0.0	11	34.7 ± 16.9
	0.025	11	14.5 ± 3.3
	0.050	11	27.8 ± 8.0
	0.10	11	20.8 ± 3.6
	0.20	10	9.0 ± 2.3
Diazepam	2.0	11	27.4 ± 7.4
F value			
Buspirone	0.0	10	28.5 ± 11.3
	0.25	7	34.6 ± 7.1
	1.0	7	29.9 ± 12.6
	4.0	11	15.8 ± 5.4
	8.0	9	35.3 ± 13.5
Diazepam	2.0	10	31.4 ± 9.7
F value			
Ipsapirone	0.0	8	12.1 ± 4.0
	0.25	8	25.6 ± 4.6
	1.0	8	53.8 ± 35.0
	4.0	8	24.0 ± 7.4
	16.0	8	21.5 ± 4.0
Diazepam	2.0	8	25.8 ± 5.7
F value			
Gepirone	0.0	8	24.6 ± 6.2
	0.5	8	49.0 ± 16.7
	2.0	8	29.9 ± 10.2
F value			
Fluoxetine	0	10	24.2 ± 8.4
	10	10	31.8 ± 7.2
	20	9	68.0 ± 25.3
Diazepam	2	10	33.9 ± 9.0
F value			

Results were analysed by one way ANOVA, although no experiment gave a significant main effect.

Figure 4.1. The effect of buspirone in the Vogel conflict test. The graph shows mean ± sem of 8-10 observations per group. Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control.

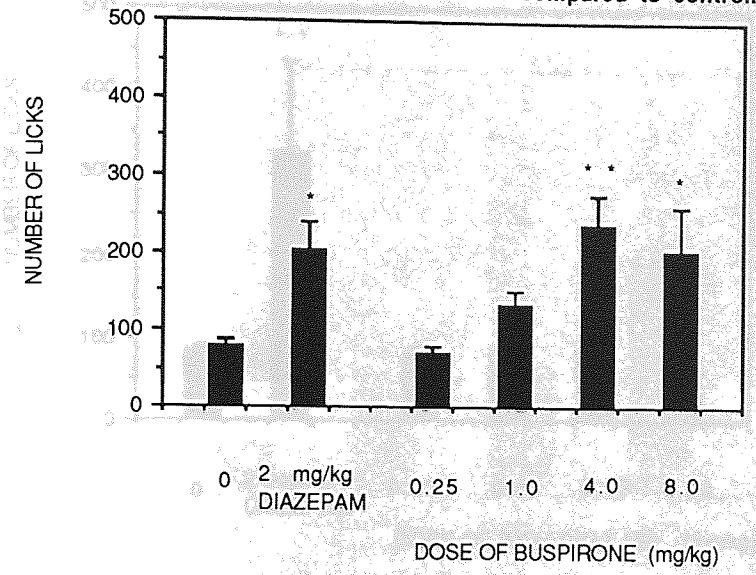


Figure 4.2. The effect of ipsapirone in the Vogel conflict test. The graph shows mean ± sem of 8 observations per group. Comparisons by Dunnett's t test ** P < 0.01 compared to control.

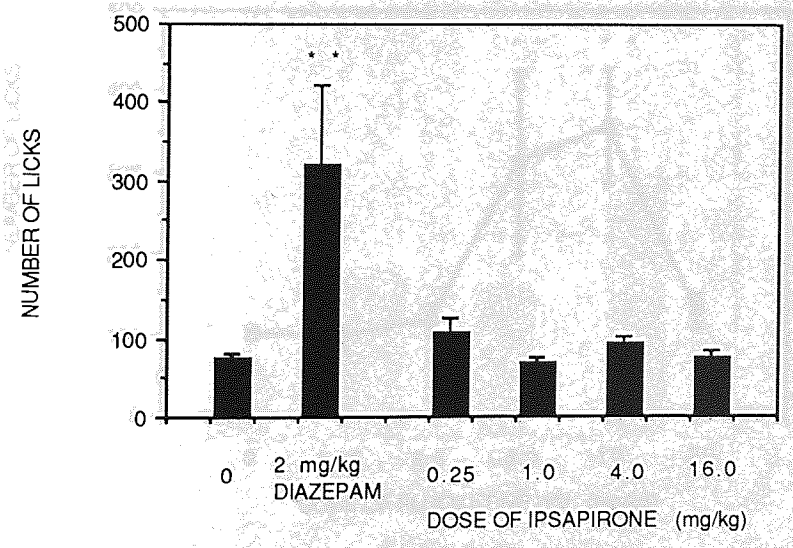


Figure 4.3. The effect of 8-OH-DPAT in the Vogel conflict test. The graph shows mean ± sem of 10 or 11 observations per group. Comparisons by Dunnett's t test ** P < 0.01 compared to control.

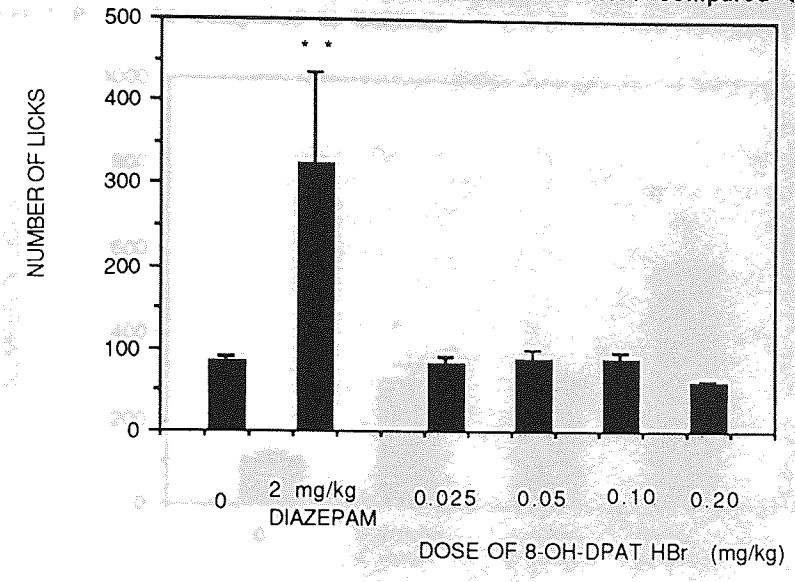


Figure 4.4. The effect of fluoxetine in the Vogel conflict test. The graph shows mean ± sem of 7-10 observations per group (control group n = 17). Comparisons by Dunnett's t test. ** P < 0.01 compared to control.

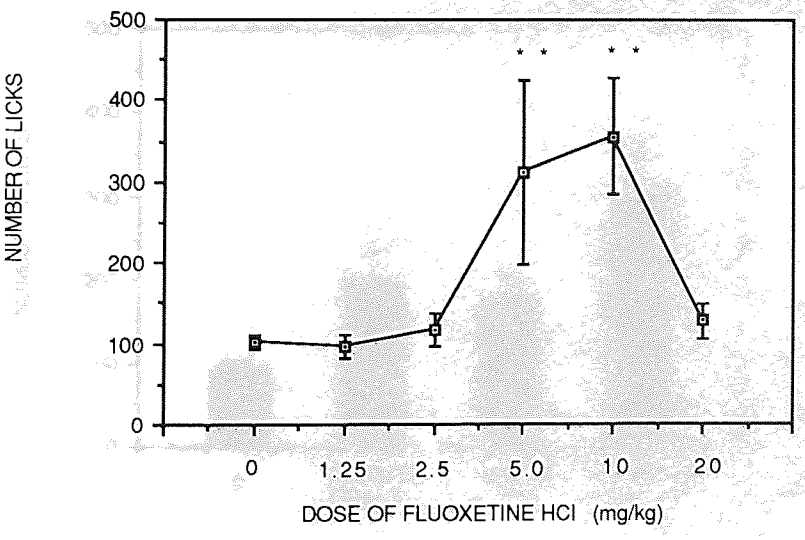


Figure 4.5. The effect of pindolol on the action of buspirone in the Vogel conflict test. The graph shows mean ± sem of 8 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control; ∞∞ P < 0.01 compared to buspirone group.

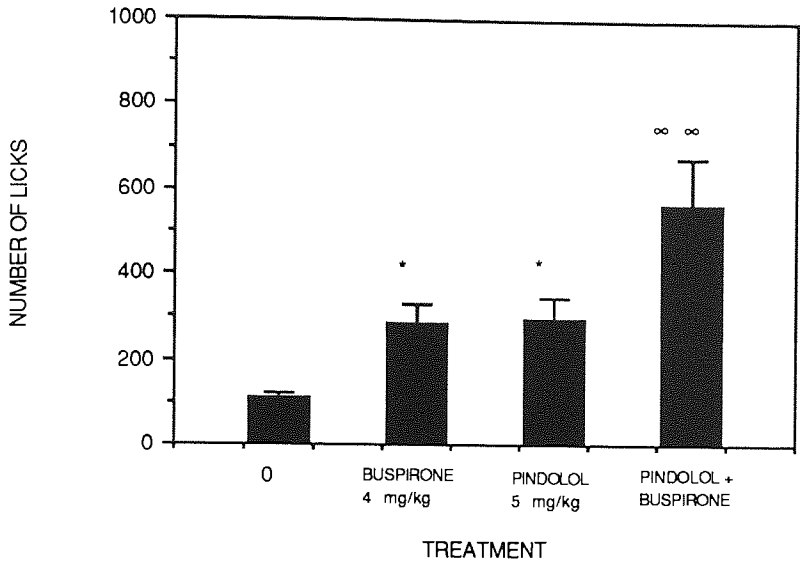


Figure 4.6. The effect of (±) propranolol on the action of buspirone in the Vogel conflict test. The graph shows mean ± sem of 8 animals per group. Comparisons by Tukey's test * P < 0.05 compared to control; ∞ P < 0.05 compared to buspirone group.

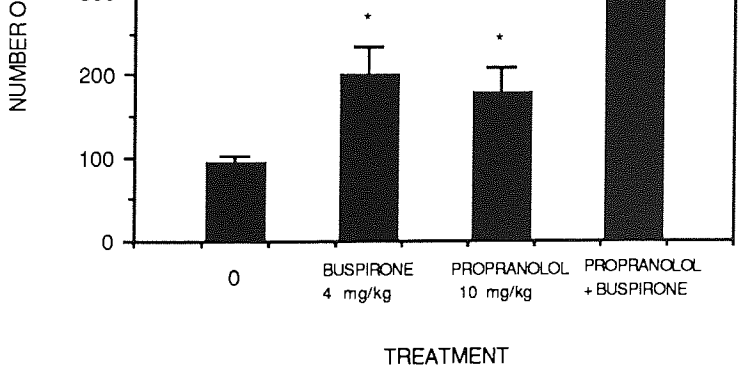
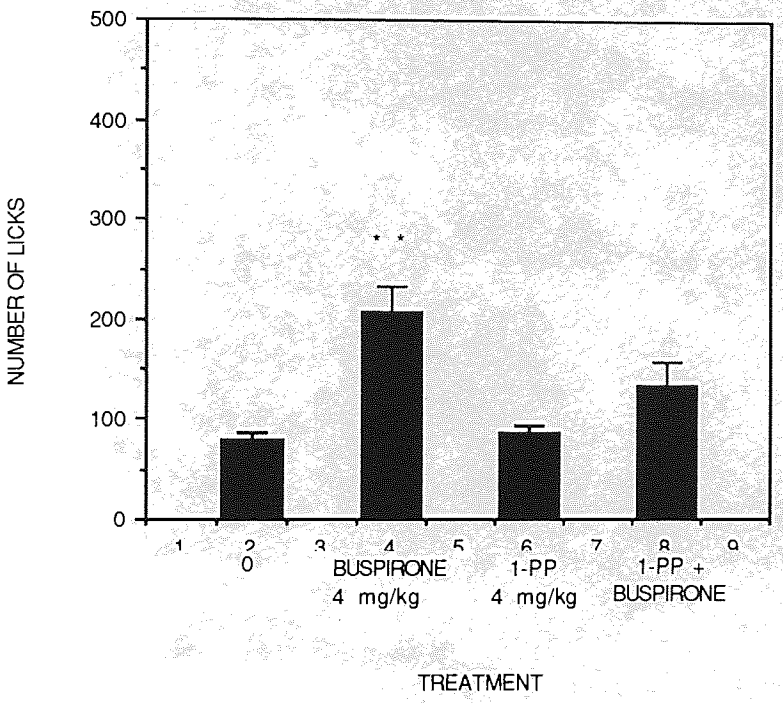


Figure 4.7. The effect of 1-PP on the action of buspirone in the Vogel conflict test. The graph shows mean ± sem of 8 observations per group. Comparisons by Tukey's test ** P < 0.01 compared to control.



Chapter 5

The effects of drugs in elevated X-maze model of anxiety.

Contents

5.1 Introduction.

The concept of the elevated X-maze arose from work carried out by Montgomery (1955) in which the behaviour of rats in Y-shaped maze was assessed with particular regard to exploration of arms that were either enclosed or open. These experiments lead to the conclusion that open and elevated spaces created greater aversion in rodents than enclosed spaces. The elevated X-maze was first used to investigate drug effects on "anxiety" by Handley and Mithani (1984), who adapted Montgomery's Y-maze into an X-maze to make the test symmetrical and thus allow direct comparisons between the two types.

The basis of the test is that, because rats find the open arms of the maze more aversive than the enclosed arms, there is less exploration of the open arms. "Anxiolytic" drugs are believed to reduce the unwillingness of rats to enter the open arms and so increase the number of open arm entries, whereas "anxiogenic" drugs magnify the unwillingness of rats to enter the open arms and so reduce the number of open arm entries. As it may be thought that drugs which increase locomotor activity would increase the number of open arm entries, the measure used to quantify anxiety is the ratio of open : total arm entries, rather than the absolute number of open arm entries.

Handley and Mithani confirmed that the test was able to detect an "anxiolytic" effect, that is an increase in the open : total entry ratio, of diazepam and of the adrenergic

α_2 agonists guanebenz and azepexole; an "anxiogenic" action, that is a reduction in the open : total entry ratio was seen in response to a number of drugs acting by different mechanisms. For instance, the α_2-adrenoceptor antagonists, idazoxan and yohimbine, caused a reduction in the open : total ratio as did the $GABA_A$ antagonist picrotoxin and the "anxiogenic" agent ACTH (Handley and Mithani, 1984).

Subsequently, Pellow et al. (1985) reported that the test was also able to detect the "anxiolytic" action of phenobarbitone and of chlordiazepoxide and diazepam, but did not detect responses to psychotropic drugs with no known anxiolytic properties. For instance, the major tranquillizer haloperidol and the antidepressant imipramine both reduced total activity without influencing either the open : total entry ratio or the time spent on the open arms (Pellow et al., 1985). In addition, it was demonstrated that confinement of rats to the open arms for 20 minutes resulted in a higher plasma corticosterone concentration than confinement in the enclosed arms for the same length of time (Pellow et al., 1985), which suggested that the open arms were more aversive than the enclosed arms.

The elevated X-maze has often been used to investigate "anxiety"-related effects of ligands for $5\text{-}HT_{1A}$ receptors. The effect of $5\text{-}HT_{1A}$ ligands after acute administration differs between laboratories, but appears to be quite consistent within the same laboratory. There is a wide disparity in the published results of agents acting at $5\text{-}HT_{1A}$ receptors in the elevated X-maze. In many cases it is

possible to cite "anxiolytic", inactive and "anxiogenic" responses to the same compound. For instance, in this laboratory, 8-OH-DPAT, a selective 5-HT_{1A} agonist (Van Wijngaarden et al., 1990), is consistently "anxiogenic" (Critchley and Handley, 1987; Critchley et al., 1992). Other authors have reported that 8-OH-DPAT is "anxiogenic" (eg Klint, 1991; Kshama et al., 1990; Moser et al., 1990), although "anxiolytic" effects for the same compound have also been found (eg Dunn et al., 1989; Luscombe et al., 1992; Söderpalm et al., 1989). Additionally, there is a report that 8-OH-DPAT does not influence behaviour in the elevated X-maze (Pellow et al., 1987). The effect of buspirone in the elevated X-maze is also not clearly established. The majority of reports are that this drug has an "anxiogenic" profile in the elevated X-maze (Critchley et al., 1992; Klint, 1991; Moser, 1989; Moser et al., 1990; Pellow and File, 1986; Pellow et al., 1987; Wada and Fukuda, 1991) but there are also reports of opposite, "anxiolytic", effects (Dunn et al., 1989, Söderpalm et al., 1989). When injected into the dentate gyrus, buspirone had an "anxiolytic" profile in an elevated X-maze (Kostowski et al., 1989).

There are fewer reports with other 5-HT_{1A} ligands in the X-maze. Ipsapirone produces an "anxiolytic" profile in some hands (Critchley et al., 1992; Luscombe et al., 1992; Pellow et al., 1987) but in others it has the opposite effect (eg Moser, 1989; Soderpalm et al., 1989; Wright et al., 1992). Gepirone can be inactive (Critchley et al.,

1992; Motta et al., 1992), "anxiolytic" (Dunn et al., 1989; Luscombe et al., 1992) or alternately "anxiolytic" at low doses and "anxiogenic" at higher doses (Söderpalm et al., 1989).

As is evident from this literature, it is invidious to make conclusions based on results from a single experiment or indeed a single group working within the field.

The variation in response to agents thought to be acting at 5-HT_{1A} receptors has been variously attributed to dose and route of drug, strain or species of animal, pre- or post-synaptic action of the drug, control baseline of the response and the type of model being used, although no systematic variation of these parameters has been shown to be sufficient to explain the degree of variability in response to these agents. Factors which govern the response to 5-HT_{1A} ligands are assessed in the next chapter; the experiments reported in this chapter endeavoured to ascertain what effects were obtained with 5-HT_{1A} ligands under what might be termed basal operating conditions.

The elevated X-maze has been used to investigate potential "anxiolytic" or "anxiogenic" effects of a wide range of compounds acting through different mechanisms. The mixed 5-$HT_{1A/1B}$ agonist RU 24969 is reported to be "anxiogenic" (Njung'e, 1989; Critchley et al., 1987) although no effect of the 5-HT_{1B} agonist CGS 12066B was found in a maze adapted for use in the mouse (Benjamin et al., 1990).

The effect of 5-HT_2 receptor antagonists is not clear-cut. Ritanserin is apparently endowed with both "anxiolytic"

(Critchley and Handley, 1987) and "anxiogenic" (File et al., 1987) effects after acute treatment, although chronic treatment produces an "anxiolytic" effect (Wright et al., 1992). Some groups have been able to detect "anxiolytic" activity of 5-HT_3 antagonists (Costall et al., 1989; Upton and Blackburn, 1990), although there is once again some discrepancy between different laboratories (File and Johnston, 1989). Chronic treatment with the 5-HT uptake inhibitor paroxetine produced an "anxiolytic" effect on the X-maze (Cadogan et al., 1992), although the acute effects of these compounds has not been previously reported.

Naturally, potential "anxiolytic" agents that act through non-serotonergic means have also been tried in the elevated X-maze. A detailed assessment of effects of such compounds is beyond the scope of this thesis, but it is nonetheless important to be aware that effects may arise out of these mechanisms, particularly so when assessing the effects of agents whose pharmacology is unknown. The NMDA receptor antagonists AP-5 and MK-801 were "anxiolytic" (Dunn et al., 1989) and the agonist NMDA had the opposite effect (ibid.). Antagonists at receptors for the peptide cholecystokinin may also influence "anxiety" as assessed in the elevated X-maze (Singh et al., 1991).

The purpose of experiments described in this chapter was to demonstrate that it was possible to obtain "anxiolytic" and "anxiogenic" effects from standard compounds with these effects in the X-maze and to assess effects of 5-HT_{1A} receptor ligands.

Results.

5.2 The effect of non-serotonergic agents on behaviour in the elevated X-maze.

5.2.1 The effect of picrotoxin.

Picrotoxin administered i.p. 30 minutes prior to maze exposure at a single dose of 3 mg/kg, which had been previously shown to have "anxiogenic" effects in this laboratory (Mithani, 1984), produced a marked fall in the open : total entry ratio (unpaired t test, $P < 0.05$) (Figure 5.1a) and in the time spent on the open arms (unpaired t test, $P < 0.05$) (Figure 5.1b), although a significant reduction in total entries was also observed (unpaired t test, $P < 0.05$) (Table 5.1).

5.2.2 The effect of corticosterone.

There was a main effect of treatment with corticosterone, administered i.p. 30 minutes beforehand, at doses of 5 and 10 mg/kg, on both the open : total entry ratio ($F_{(2,27)} = 4.75$; $P < 0.05$) (Figure 5.2a) and the time spent on the open arms ($F_{(2,27)} = 4.44$; $P < 0.05$) (Figure 5.2b), although after post hoc comparisons only the higher dose produced significant differences. Corticosterone treatment also increased the number of total entries made ($F_{(2,27)} = 4.64$; $P < 0.05$) (Table 5.2).

5.2.3 The effect of diazepam and flumazenil.

There was a significant main effect of drug treatment on the open : total entry ratio ($F_{(3,20)} = 12.49$; $P < 0.01$), but not on the time spent on the open arms ($F_{(3,20)} = 2.00$; $P > 0.05$) or on the total entries ($F_{(3,20)} = 2.48$; $P > 0.05$) (Table 5.3). Post hoc comarisons indicated that flumazenil, given i.p. 45 minutes prior to maze exposure, significantly reduced the open : total entry ratio (Figure 5.3a) and the time spent on the open arms (Figure 5.3b) and that the differences were due solely to the 8 mg/kg dose. Diazepam, incorporated as an active control agent, significantly raised both the open : total entry ratio (Figure 5.3a) and the time spent on the open arms (Figure 5.3b).

5.2.4 The effect of flumazenil on the action of diazepam.

Diazepam at a dose of 1.5 mg/kg 30 minutes i.p. before exposure to the maze significantly increased the open : total entry ratio ($F_{(1,20)} = 7.80$; $P < 0.05$) (Figure 5.4a) and the time spent on the open arms ($F_{(1,20)} = 5.13$; $P < 0.05$) (Figure 5.4b). Flumazenil on its own at the 4 mg/kg dose i.p. 45 minutes before exposure to the maze did not influence the open : total entry ratio ($F_{(1,20)} = 1.28$; $P > 0.05$) (Figure 5.4a) or the time spent on the open arms ($F_{(1,20)} = 1.12$; $P > 0.05$) (Figure 5.4b). The combination resulted in the abolition of the "anxiolytic" effect of

diazepam on the open : total entry ratio, such that it was below that of the saline-treated animals (Figure 5.4a), although the interaction term was not significant ($F_{(1,20)}$ = 0.60; P > 0.05). The combination also produced a reduction of the time spent on the open arms also below that of saline treated rats (Figure 5.4b), but again the interaction term was not significant ($F_{(1,20)}$ = 0.44; P > 0.05). Total entries were not changed by either diazepam ($F_{(1,20)}$ = 2.90; P > 0.05) or flumazenil ($F_{1,20)}$ = 0.37; P > 0.05) (Table 5.4).

5.2.5 The effect of flumazenil on the action of corticosterone.

There was a main effect of corticosterone treatment, at a dose of 10 mg/kg i.p. given 30 minutes before exposure to the maze, on both the open : total entry ratio ($F_{(1,20)}$ = 18.51; P < 0.01) (Figure 5.5a) and the time spent on the open arms ($F_{(1,20)}$ = (Figure 5.5b). Post hoc comparisons indicated that corticosterone had an "anxiolytic" effect on both measures of anxiety. Flumazenil at a dose of 4 mg/kg i.p. given 45 minutes before exposure did not influence either the ratio ($F_{(1,20)}$ = 0.24; P > 0.05) (Figure 5.5a) or the time spent on the open arms ($F_{(1,20)}$ = 0.17; P > 0.05) (Figure 5.5b). The combination of these treatments demonstrated that flumazenil was not able to antagonize the "anxiolytic" effect of corticosterone on either the open : total entry ratio criterion (interaction term $F_{(1,20)}$ = 1.73;

$P > 0.05$) or the % time criterion (interaction $F_{(1,20)} = 0.74$; $P > 0.05$) (Figure 5.5a and b). On this occasion, corticosterone did not influence the total number of arm entries ($F_{(1,20)} = 1.70$; $P > 0.05$) (Table 5.5). Flumazenil did not alter the total number of entries ($F_{(1,20)} = 0.87$; $P > 0.05$) (Table 5.5).

5.3 The effect of serotonergic drugs on behaviour on the elevated X-maze.

5.3.1 The effect of 8-OH-DPAT.

For a considerable period of time at the start of the behavioural experiments, 8-OH-DPAT appeared to be inactive in the elevated X-maze test. In Wistar rats, a dose response curve to 8-OH-DPAT failed to show any effects of the drug (Table 5.6). The light intensity was reduced (see chapter 7) and 8-OH-DPAT was tested at a single dose of 0.2 mg/kg in rats of the Hooded Lister strain. 8-OH-DPAT produced a significant fall in both the open : total entry ratio (unpaired t test, $P < 0.01$) (Figure 5.6a) and the time spent on the open arm (unpaired t test, $P < 0.01$) (Table 5.7), although the total entries were also depressed (unpaired t test, $P < 0.01$) (Table 5.7). This experiment was repeated in Wistar rats with essentially the same result (Figure 5.6b; Table 5.8).

5.3.2 The effect of buspirone.

Buspirone, over a wide dose range (0.003 - 3.14 mg/kg), and given i.p. 30 minutes prior to exposure to the maze did not significantly influence the open : total ratio ($F_{(7,40)}$ = 1.79; P > 0.05) (Figure 5.7), nor the time spent on the open arms ($F_{(7,40)}$ = 1.69; P > 0.05) (Table 5.9). Inspection of the data suggested that the dose 0.192 mg/kg might have had "anxiolytic" effects and the dose 3.144 mg/kg might have had an "anxiogenic" effect; however neither of these were confirmed by statistical analysis. There was no effect of buspirone on total entries ($F_{(7,40)}$ = 0.56; P > 0.05) (Table 5.9) although once again, inspection of the data suggested that this was depressed, in this case at the highest dose used.

5.3.3 The effect of ipsapirone.

Ipsapirone, over a wide dose range (0.003 - 3.14 mg/kg) and given i.p. 30 minutes prior to exposure to the maze produced a significant main effect on both the open : total ratio ($F_{(6,35)}$ = 9.23; P < 0.01) (Figure 5.8) and the time spent on the open arms ($F_{(6,35)}$ = 5.90; P , 0.01) (Table 5.10). Post hoc comparisons revealed that only the dose 0.012 mg/kg significantly raised the ratio, and the time spent on the open arms. One way analysis of variance suggested that there was a main effect of ipsapirone on total entries ($F_{(6,35)}$ = 2.54; P < 0.05), but post hoc

comparisons suggested that no group was significantly different from the control group in this respect (Table 5.10).

5.3.4 The effect of RU 24969.

RU 24969 administered i.p. 30 minutes prior to exposure to the maze at a dose of 1 mg/kg, previously shown in this laboratory to have "anxiogenic" properties (Njung'e, 1989), resulted in a reduction of the open : total entry ratio (unpaired t test, $P < 0.1$) (Figure 5.9a) and of the time spent on the open arms (unpaired t test, $P < 0.1$) (Figure 5.9b). A single animal did not respond to treatment with RU 24969 in this fashion and caused the reduction in both the ratio and open arm time data to lose significance at the 0.05 level. RU 24969 did not influence the number of arm entries made (Table 5.11). Rats that had received RU 24969 were markedly more difficult to handle than the controls, with behaviour indicative of bad temper such as screeching when picked up, wriggling and struggling during handling, being displayed.

5.3.5 The effect of CGS 12066B.

CGS 12066B administered i.p. 30 minutes prior to maze exposure in the dose range 0.2 - 5.0 mg/kg produced a significant main effect on the open : total entry ratio ($F_{(3,20)} = 4.93$; $P < 0.05$) (Figure 5.10a) and in the time

spent on the open arms ($F_{(3,20)} = 2.53$; $P < 0.05$) (Figure 5.10b). Specific comparisons showed that the 1 mg/kg ($P < 0.05$) and 5 mg/kg ($P < 0.01$) doses significantly decreased the open : total ratio, but only the 5 mg/kg dose significantly ($P < 0.05$) reduced the time spent on the open arms. The total entries remained unchanged ($F_{(3,20)} = 0.35$; $P > 0.05$) (Table 5.12).

5.3.6 The effect of fluoxetine.

Fluoxetine over the dose range 1.25 - 10 mg/kg and given i.p. 30 minutes prior to the test produced a dose-related fall in the open : total entry ratio ($F_{(5,39)} = 7.09$; $P < 0.01$) and in the time spent on the open arms ($F_{(5,39)} = 5.54$; $P < 0.01$) (Figure 5.11a; Figure 5.11b). The number of total arm entries made was reduced ($F_{(5,39)} = 9.52$; $p < 0.01$), but only by the 10 mg/kg dose. A higher dose of fluoxetine (20 mg/kg) failed to have any effect on "anxiety"-related behaviour (Figure 5.11a; Figure 5.11b), but did reduce the total number of arm entries (Table 5.13).

5.3.7 The effect of pindolol on the action of 8-OH-DPAT.

Previous work from this laboratory had suggested that ß adrenoceptor antagonists could block the "anxiogenic" effect of 8-OH-DPAT in the elevated X-maze without themselves having any effect (Njung'e et al., 1993). One exception to this was pindolol, which did antagonize the

"anxiogenic" effect of 8-OH-DPAT, but the dose chosen also caused a significant "anxiolytic" effect in its own right. This experiment was conducted to test whether a lower dose of pindolol, without any "anxiolytic" effect on its own, could still antagonize the action of 8-OH-DPAT.

In the initial 6 animals used in each group, 8-OH-DPAT, at a dose of 0.1 mg/kg given i.p. 10 minutes before exposure to the maze caused a significant fall in the open : total entry ratio ($F_{(1,20)} = 6.30$; $P < 0.05$) .The fall in the time spent on the open arms did not reach significance ($F_{(1,20)} = 4.07$; $P > 0.05$) (Figure 5.12 b). Pindolol, at a dose of 0.5 mg/kg i.p. and given 45 minutes before exposure, did not alter either parameter (OTR $F_{(1,20)} = 1.68$; $P > 0.05$: % time $F_{(1,20)} = 0.62$; $P > 0.05$). The combination of the two treatments demonstrated that pindolol did not significantly antagonize the "anxiogenic" effects of 8-OH-DPAT, although a trend in this direction was suggested (OTR $F_{(1,20)} = 2.22$; $P > 0.05$: % time $F_{(1,20)} = 1.10$; $P > 0.05$). There was no effect of either 8-OH-DPAT ($F_{(1,20)} = 0.58$; $P > 0.05$) or of pindolol ($F_{(1,20)} = 0.07$; $P > 0.05$) on the number of total entries made (Table 5.14).

Since this experiment was conducted a further three animals per group have been added, although there are no additional data on the time spent on the open arms. When results are combined, there was a significant antagonism of the "anxiogenic" effect of 8-OH-DPAT by pindolol at a dose of pindolol which does not influence behaviour for the open : total entry ratio (Figure 5.12a).

5.4 Discussion.

The most common measures used to assess "anxiety" in the elevated X-maze are the open : total entry ratio (Handley and Mithani, 1984) and the time spent on the open arms (Pellow et al., 1985). Both were used to quantify changes in anxiety related behaviour in the present set of experiments. The total number of arm entries made is a useful measure to indicate changes in the state of the animal which could influence the open : total entry ratio. Moser (1989) has argued that a reduction in the total number of arm entries is consistent with an "anxiogenic" effect and it is not difficult to believe that a drug which has strong "anxiogenic" properties will inhibit locomotor activity in this test. "Anxiogenic" drugs do decrease the open : total entry ratio if the dose is increased beyond that which produces a selective reduction in the open : total entry ratio. The number of total entries is not, however, a reliable measure of anxiety as it is also likely to result from a sedative drug effect or from an action of a drug that produces a feeling of general malaise. The presence of reduced total entries at the same time as an "anxiolytic" effect is less difficult to interpret. In this case, it is difficult to attribute an increase in the preference of the open arms to a general reduction in activity.

The results with picrotoxin confirmed previous results with the elevated X-maze that this compound produces an

"anxiogenic"-like fall in both the open : total entry ratio and time spent on the open arms (Handley and Mithani, 1984; Pellow et al., 1985). The reduction in total arm entries which occured with both treatments may be an indication of a powerful "anxiogenic" effect, as discussed above.

Corticosterone had an "anxiolytic" effect in the elevated X-maze at doses which increased the total number of arm entries. Doses were chosen on the basis of published work indicating that plasma concentrations resulting from administration of similar amounts by the intraperitoneal route were similar to those after restraint and were active in another animal model of anxiety, the social interaction test (File et al., 1979b) Steroids are known to interact with the $GABA_A$ receptor in a similar fashion to the barbiturates (Lambert et al., 1989) and "anxiolytic" effects of steroids have been reported in the Vogel test (Crawley et al., 1986; Söderpalm and Engel, 1992) and in a two chambered exploration test in the mouse (Crawley et al., 1986). This evidence suggested that it was possible that the "anxiolytic" effect observed with corticosterone was due to modulation of the $GABA_A$ receptor. 4 mg/kg flumazenil, the benzodiazepine receptor antagonist (Hunkeler et al., 1981) was sufficient to antagonize the "anxiolytic" action of diazepam in the elevated X-maze without producing any effects of its own, whereas the higher dose was marginally "anxiogenic". The lower dose was tested against corticosterone. Flumazenil did not antagonize the increase in either the open : total entry

ratio or the time spent on the open arms brought about by corticosterone and did not itself cause any significant changes in behaviour.

A tentative conclusion might therefore be that corticosterone does not act through the benzodiazepine receptor to produce its "anxiolytic" effect; however, as this was only a single experiment, this possibility is not definitively resolved. The conclusion is supported by electrophysiological evidence which demonstrated that corticosterone did not influence the function of the chloride channel associated with the $GABA_A$ receptor, although a number of other steroids did (Gee et al., 1987).

Results to this point had demonstrated that it was possible to obtain both "anxiogenic" and "anxiolytic" effects of drugs using the elevated X-maze. Testing of 8-OH-DPAT initially indicated that no response could be obtained with this compound. After spot doses were tested, a dose-response curve with 8-OH-DPAT failed to show any effect of this drug. A previous student in this laboratory had also noted that the effect of 8-OH-DPAT was initially difficult to obtain. With RU 24969, despite the lack of a significant effect, the magnitude of response in 5 out of 6 animals was sufficient to indicate that "anxiogenic" effects could result from serotonergic manipulations under the present conditions.

Persevering with 8-OH-DPAT in the elevated X-maze, conditions were eventually discovered under which it produced an "anxiogenic" effect. The reasons for the

variability in response to 8-OH-DPAT forms the basis of a subsequent chapter and therefore the details are not presented here. Once an "anxiogenic" effect of 8-OH-DPAT was found, it was tested in both hooded Lister and Wistar rats and tested positively "anxiogenic" both times. This was the only occasion on which Hooded Lister rats were used in experiments for this thesis.

Previous work form this laboratory has suggested that ß-adrenoceptor antagonists are able to prevent the "anxiogenic" action of 8-OH-DPAT at doses which do not influence "anxiety" (Njung'e et al., 1992). However, one exception to this was pindolol, which could block the "anxiogenic" effects of 8-OH-DPAT, but only at a dose where pindolol itself was "anxiolytic" (Njung'e et al., 1992). The purpose of this experiment was to use a lower dose of pindolol to block the effect of 8-OH-DPAT which would not itself influence X-maze behaviour. The results suggested that pindolol could antagonize an "anxiogenic" action of 8-OH-DPAT, but this antagonism was not significant. A further three animals per group have now been tested an the amalgamated results indicate that pindolol, like many other adrenergic ß blockers can antagonize 8-OH-DPAT's "anxiogenic" effect.

At this stage, with a reliable "anxiogenic" effect of 8-OH-DPAT established, the effects of other serotonergic ligands were assessed. The failure of buspirone to modulate "anxiety" was disappointing. Over the same dose range a biphasic effect of buspirone was shown by Söderpalm et al.

(1989). It does not seem possible to explain why the present study failed to detect either of these effects. An "anxiogenic" effect at the highest dose, 3.14 mg/kg, was indicated, but was not significant. This dose also appeared to reduce total entries, but again this effect was not significant. Results with ipsapirone were very similar. However, at a low dose it proved to be "anxiolytic".

In summary, the experiments with the 5-HT_{1A} receptor ligands buspirone and ipsapirone did not suggest any clear "anxiety"-related effects, even under conditions where the "anxiogenic" effect of 8-OH-DPAT was very reliable. This was disappointing, but is not inconsistent with published findings, although it is perhaps worth noting that there is such diversity in responses to 5-HT_{1A} agonists, it would be difficult to get a result that was not supported by some published evidence (see Introduction for references).

RU 24969 produced a strong "anxiogenic" effect in 5 out of 6 animals. CGS 12066B, a selective 5-HT_{1B} receptor agonist (Neale et al., 1987), produced a selective "anxiogenic" profile in the elevated X-maze in the present investigations. These results were in contrast to those of Benjamin et al. (1990) who found no effect of CGS 12066B over a similar dose range in a mouse elevated X-maze. CGS 12066B increases ultrasonic vocalizations in the rat pup isolation call test, consistent with an "anxiogenic" action. The 5-HT_{1B} receptor is probably only found in the rat and mouse (Hoyer and Middlemiss, 1989), although recent evidence from molecular biological studies have suggested

that this may not be the case (Peroutka, 1992). In humans, ducks, cows and guinea-pigs the terminal autoreceptor is of the 5-HT_{1D} subtype. Results with RU 24969 and CGS 12066B might suggest that agonist action at the terminal autoreceptor is "anxiogenic" and therefore antagonists for these receptors might have "anxiolytic" properties. The possibility that agents acting at 5-HT_{1D} receptors may influence "anxiety" in animal models has been mentioned in the literature (Rex et al., 1991) but, paradoxically in the present context, this was mentioned in the context of agonists producing "anxiolytic" effects. However, a reduction in 5-HT release subsequent to 5-HT_{1D} receptor activation is consistent with the view that reducing 5-HT tone produces anxiolysis (Iversen, 1984).

An interesting experiment which may help to clarify the role of presynaptic 5-HT_{1B} receptors in the effects of CGS 12066B and RU 24969 would be to test the effect of these compounds in rats treated with pCPA to deplete 5-HT. One radioligand binding study has suggested that there are postsynaptic 5-HT_{1B} receptors (Weissman et al., 1986) and at present it is not possible to confirm whether the effects seen with 5-HT_{1B} agonists are due to reduced release of 5-HT or to a postsynaptic agonist effect. Behavioural evidence exists that there are functionally relevant postsynaptic 5-HT_{1B} receptors (Kennett et al., 1987b).

The dose-response curve to fluoxetine indicated an increasingly powerful "anxiogenic" effects were seen as the dose was increased from 1.25 through to 10 mg/kg. At this

dose, very strong "anxiogenic" effects were seen, with only a single animal making one entry into the open arms. Yet at the 20 mg/kg dose, there was no "anxiety"-related effects, despite a reduction in the total entries. The effect of the 5-HT uptake inhibitor, fluoxetine, in the X-maze was opposite to that found using paroxetine in a chronic dosing regime on the X-maze (Cadogan et al., 1992). This temporal variation in response to 5-HT uptake inhibitors in the elevated X-maze has striking similarities to the clinical effects of the 5-HT uptake inhibitors in the clinic, where there is often a worsening of anxiety symptoms initially, but the therapeutic effect develops in continual use (Nutt and Glue, 1989).

The potency of the effect on open : total entry ratio seen at 10 mg/kg were similar to those seen with RU 24969 in those treated animals which did not enter the open arms at all. There has been a claim that RU 24969 acts to inhibit the uptake of 5-HT at concentrations that are likely to be achieved after systemic administration (1 - 10 mg/kg, Wolf and Kuhn, 1991). Given the similarity between the behavioural efects of fluoxetine and RU 24969 found here, it may be that the "anxiogenic" profile of RU 24969 in fact resides in its uptake inhibition properties rather than action at 5-HT_{1B} receptors.

Figure 5.1a. The effect of picrotoxin on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparison by unpaired t test * P < 0.05.

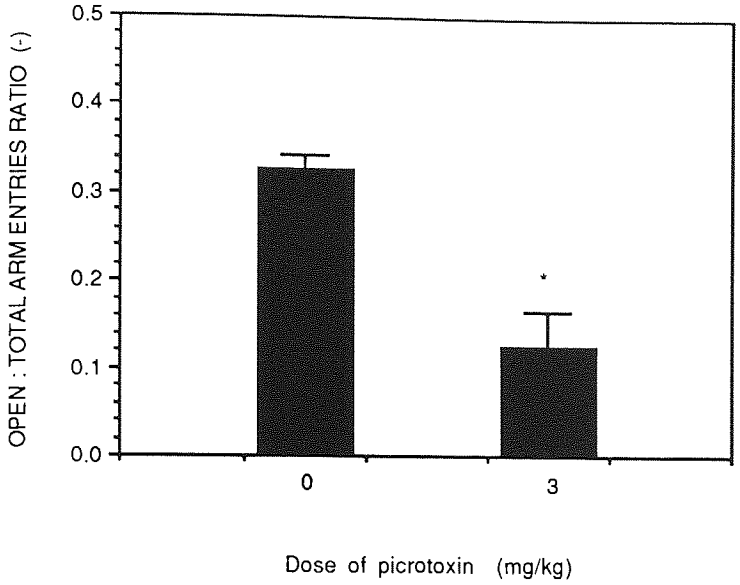


Figure 5.1b. The effect of picrotoxin on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparison by unpaired t test * P < 0.05.

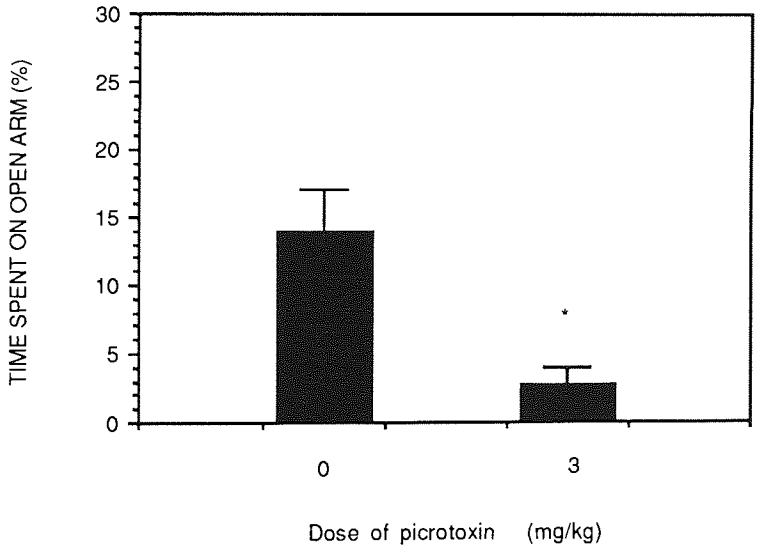


Table 5.1. The effect of picrotoxin on total entries.

DRUG	DOSE (mg/kg)	N	Total Entries
Picrotoxin	0.0	6	19.7 ± 4.2
	3.0	6	8.8 ± 2.5*

* P < 0.05 by Student's unpaired t test.

Figure 5.2a. The effect of corticosterone on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 12 observations (5mg/kg dose n = 6). Comparisons by Dunnett's t test ** P < 0.01 compared to control.

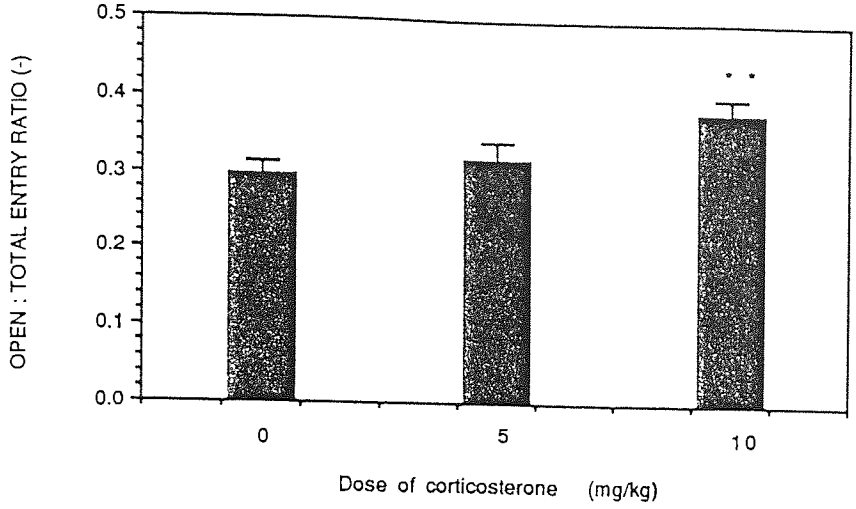


Figure 5.2b. The effect of corticosterone on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 12 observations per group (n = 6 for 5 mg/kg dose). Comparisons by Dunnett's t test * P < 0.05.

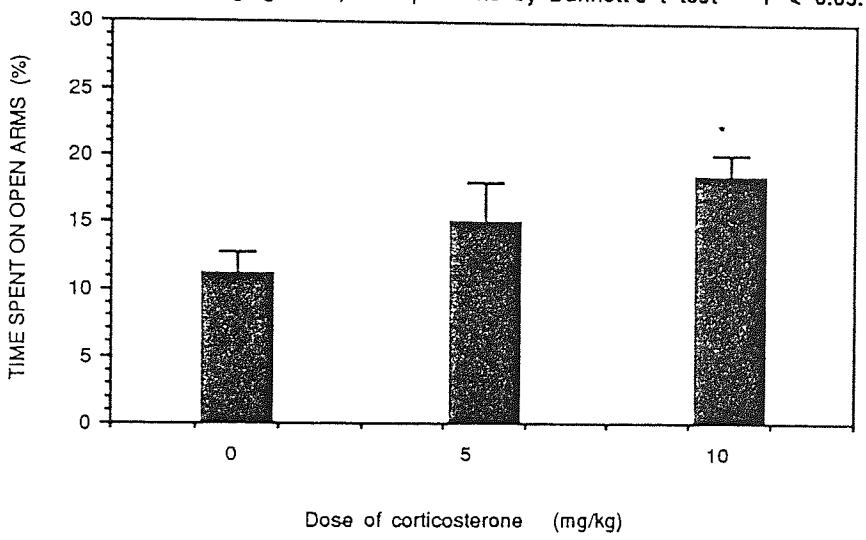


Table 5.2. The effect of corticosterone on total entries.

DRUG	DOSE (mg/kg)	N	Total Entries
Corticosterone	0.0	12	15.8 ± 1.1
	5.0	6	20.7 ± 2.4*
	10	12	21.5 ± 1.5**

Statistical comparisons were made by 1-way ANOVA and Dunnett's t test. * P < 0.05; ** P < 0.01.

Figure 5.3a. The effect of flumazenil and diazepam on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test ** P < 0.01 compared to control.

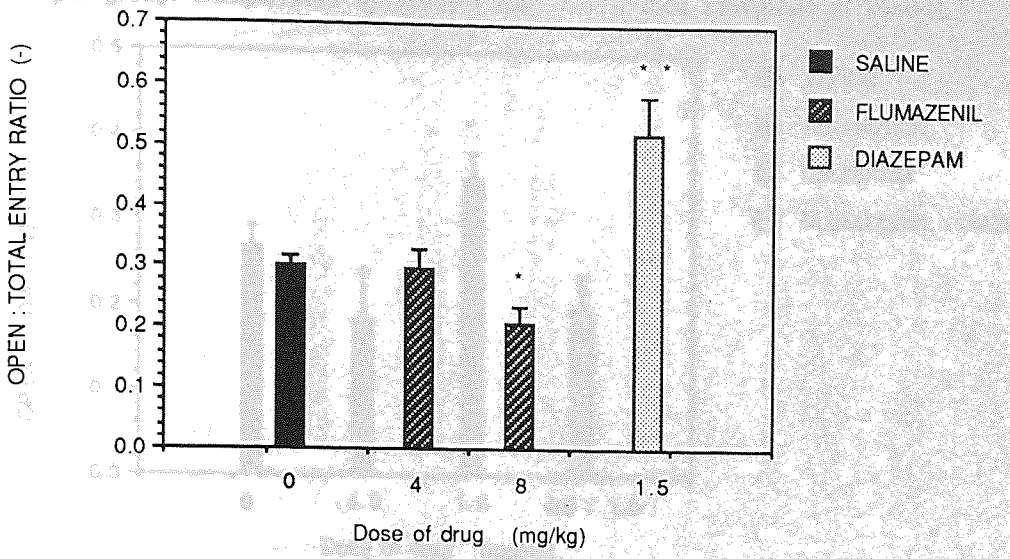


Figure 5.3b. The effect of flumazenil and diazepam on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test * P < 0.0 5 compared to control.

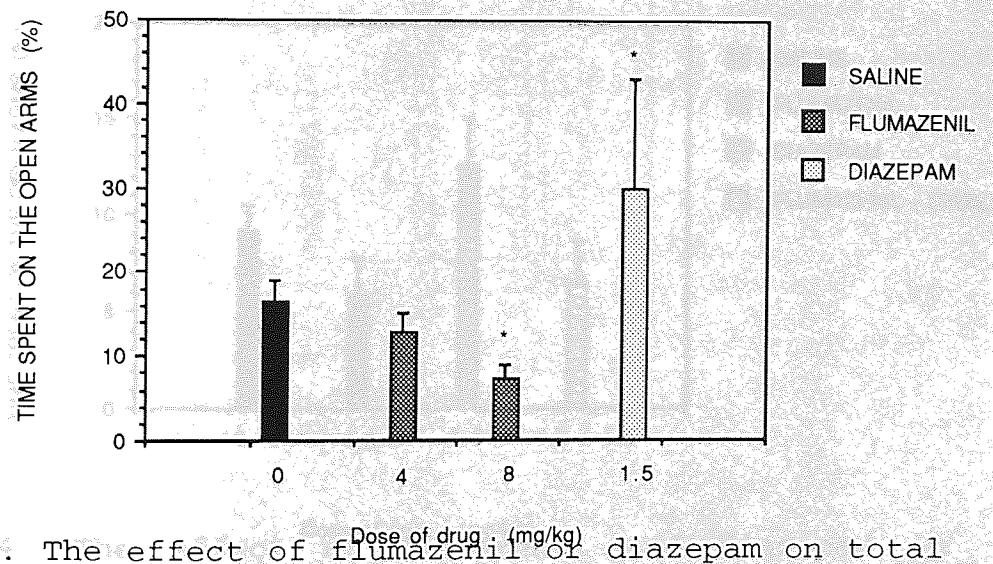


Table 5.3. The effect of flumazenil or diazepam on total entries.

DRUG	DOSE (mg/kg)	N	Total Entries
Flumazenil	0.0	6	25.2 ± 2.0
	4.0	6	23.0 ± 1.9
	8.0	6	18.3 ± 3.2
Diazepam	2.0	6	17.2 ± 2.0

Statistical analysis was by one way ANOVA - no main effect.

Figure 5.4a. The effect of flumazenil on the action of diazepam on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control.

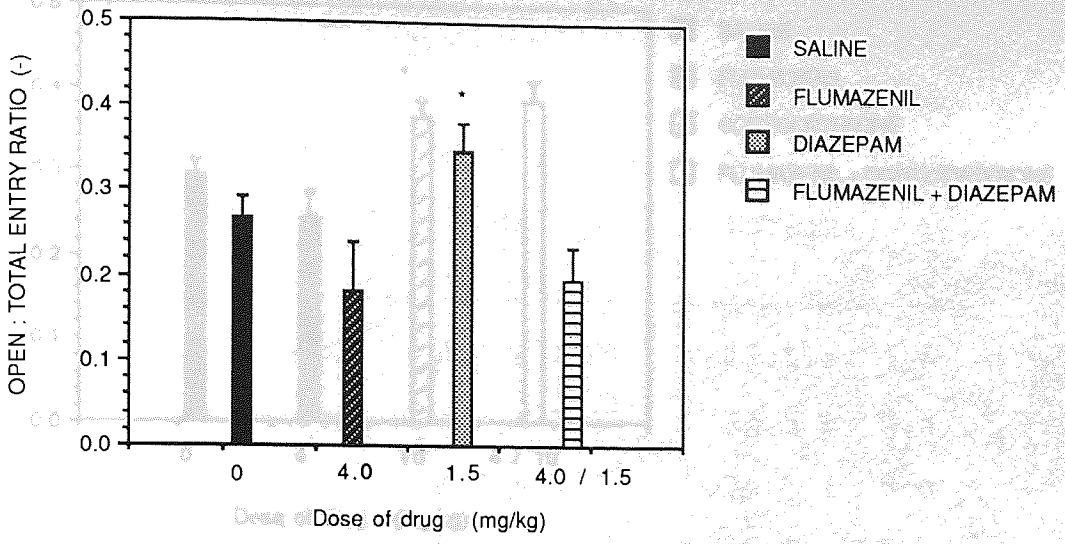


Figure 5.4b. The effect of flumazenil on the action of diazepam on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test.

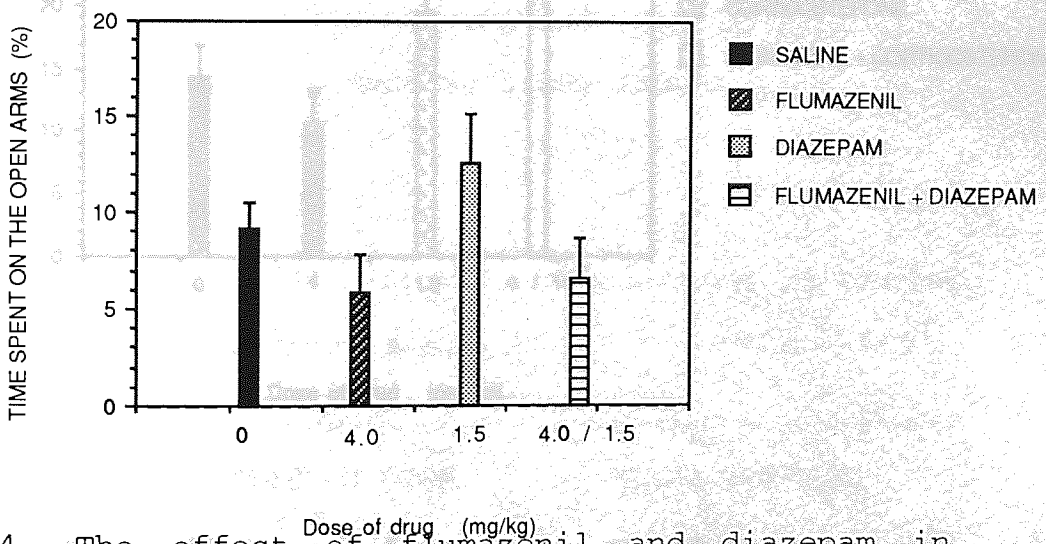


Table 5.4. The effect of flumazenil and diazepam in combination on total entries.

DRUG	DOSE (mg/kg)	N	Total Entries
Saline	0.0	6	16.2 ± 2.3
Flumazenil	4.0	6	12.5 ± 2.0
Diazepam	1.5	6	18.5 ± 2.3
Flumazenil + Diazepam	4.0 / 1.5	6	13.3 ± 3.3

Statistical comparisons were made by two way ANOVA.

Figure 5.5a. The effect of flumazenil on the action of corticosterone on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control; ∞ P < 0.05 compared to flumazenil group.

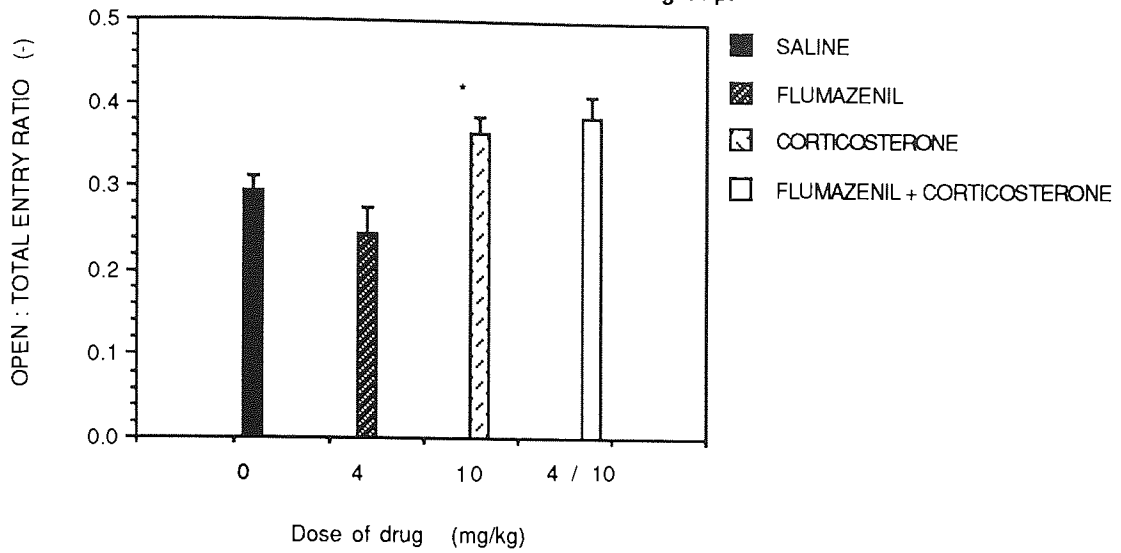


Figure 5.5b. The effect of flumazenil on the action of corticosterone on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test.

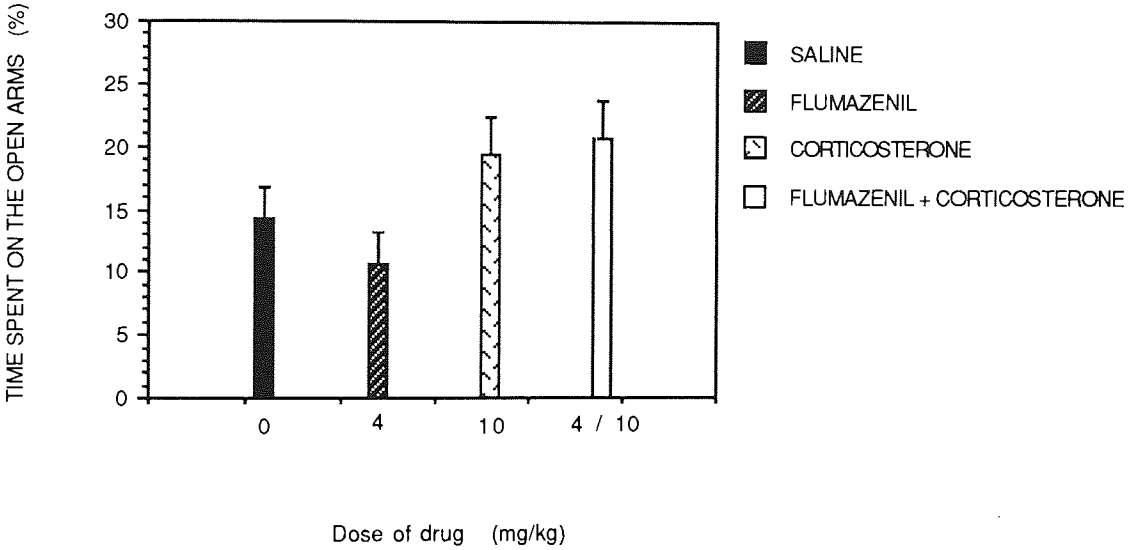


Table 5.5. The effect of flumazenil on the action of corticosterone on the total entries in the X-maze.

DRUG	DOSE (mg/kg)	N	Total Entries
Saline	0.0	6	22.7 ± 2.3
Flumazenil	4.0	6	21.8 ± 3.2
Corticosterone	10	6	27.8 ± 1.9*
Flumazenil + Corticosterone	4.0 10	6	23.7 ± 2.9

Statistical comparisons were made by Tukey's test after a 2-way ANOVA.

Table 5.6. The effect of 8-OH-DPAT on behaviour of Wistar rats on the elevated X-maze.

DOSE	N	OPEN:TOTAL RATIO (-)	TIME ON OPEN ARMS (%)	TOTAL ENTRIES
0.0	6	0.14 ± 0.05	3.3 ± 1.5	13.2 ± 1.6
0.2	6	0.22 ± 0.04	6.6 ± 1.6	24.5 ± 2.4
0.4	6	0.24 ± 0.03	8.7 ± 2.1	25.7 ± 5.0
0.8	6	0.18 ± 0.08	5.3 ± 2.2	29.3 ± 11.2
F value		$F_{(3.20)}=0.74$	$F_{(3,20)}=1.43$	$F_{(3,20)}=1.23$
P value		P > 0.05	P > 0.05	P > 0.05

Statistical analyses were made by 1-way ANOVA.

Table 5.7. The effect of 8-OH-DPAT on behaviour of Hooded Lister rats on the elevated X-maze.

DOSE	N	TIME ON OPEN	TOTAL ENTRIES
0.0	6	15.6 ± 3.9	22.3 ± 3.4
0.2	6	3.3 ± 3.3**	10.0 ± 1.8**

Comparisons were made by Student's unpaired t test. ** P < 0.01.

Figure 5.6a. The effect of 8-OH-DPAT on the open : total entry ratio in Hooded Lister rats. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test ** P < 0.01.

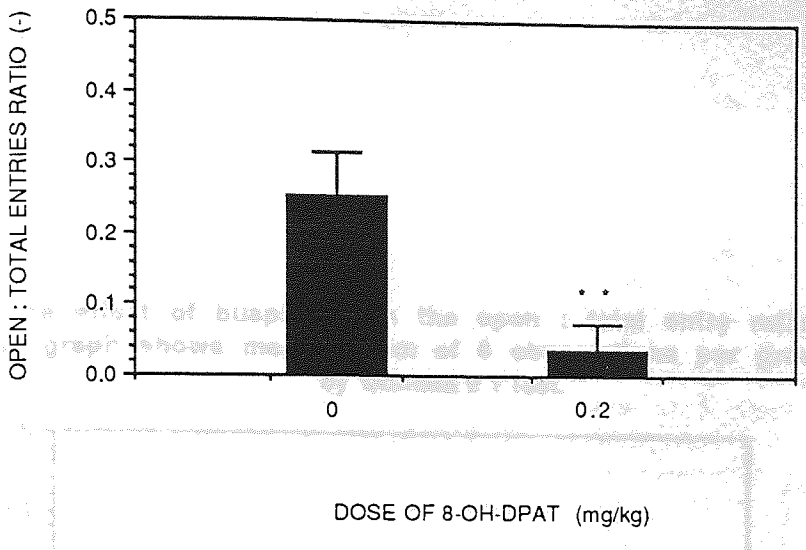


Figure 5.6b. The effect of 8-OH-DPAT on the open : total entry ratio in Wistar rats. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test ** P < 0.01.

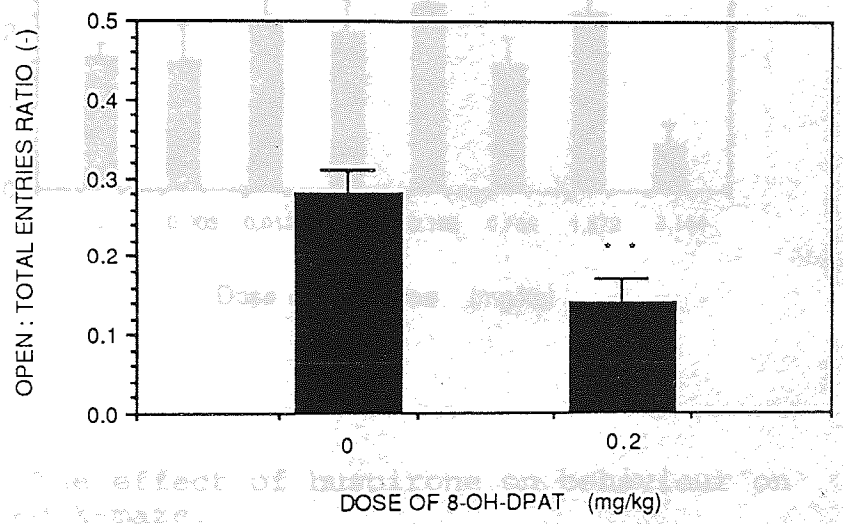


Table 5.8. The effect of 8-OH-DPAT on behaviour of Wistar rats on the elevated X-maze.

DOSE (mg/kg)	N	TIME ON OPEN ARMS (%)	TOTAL ENTRIES
0.0	6	5.8 ± 0.6	17.6 ± 3.3
0.2	6	2.5 ± 1.2*	14.0 ± 2.0

Comparisons were made by Student's unpaired t test * P < 0.05.

Figure 5.7. The effect of buspirone on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test.

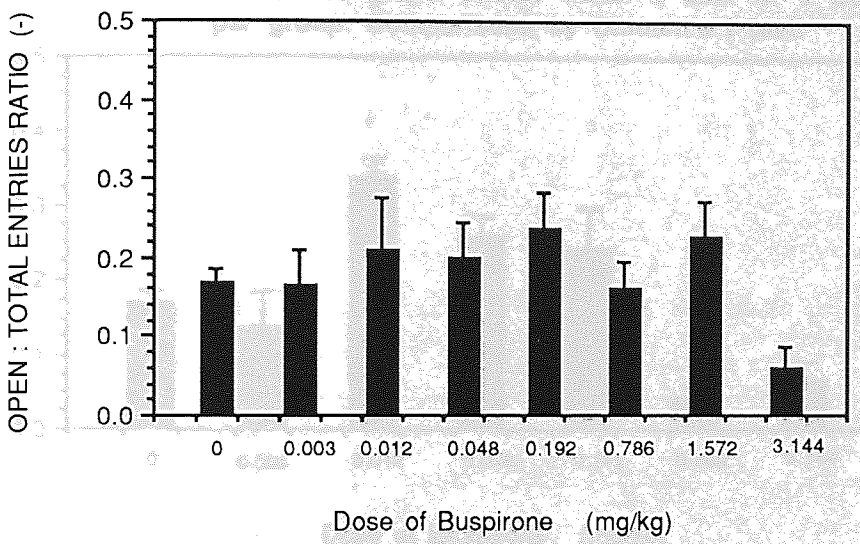


Table 5.9. The effect of buspirone on behaviour on the elevated X-maze.

DRUG	DOSE (mg/kg)	N	Time on Open Arms (%)	Total Entries
Buspirone	0.0	6	7.5 ± 1.3	22.3 ± 1.8
	0.003	6	10.1 ± 3.7	25.3 ± 4.8
	0.012	6	13.5 ± 5.1	21.5 ± 2.4
	0.048	6	9.2 ± 3.0	23.5 ± 4.2
	0.19	6	11.6 ± 3.5	22.0 ± 0.4
	0.79	6	8.7 ± 2.3	22.7 ± 1.3
	1.57	6	14.2 ± 4.4	21.8 ± 3.8
	3.14	6	1.4 ± 0.5	17.2 ± 1.9

Analysis by 1-way ANOVA did not reveal any main effect.

Figure 5.8. The effect of Ipsapirone on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test.

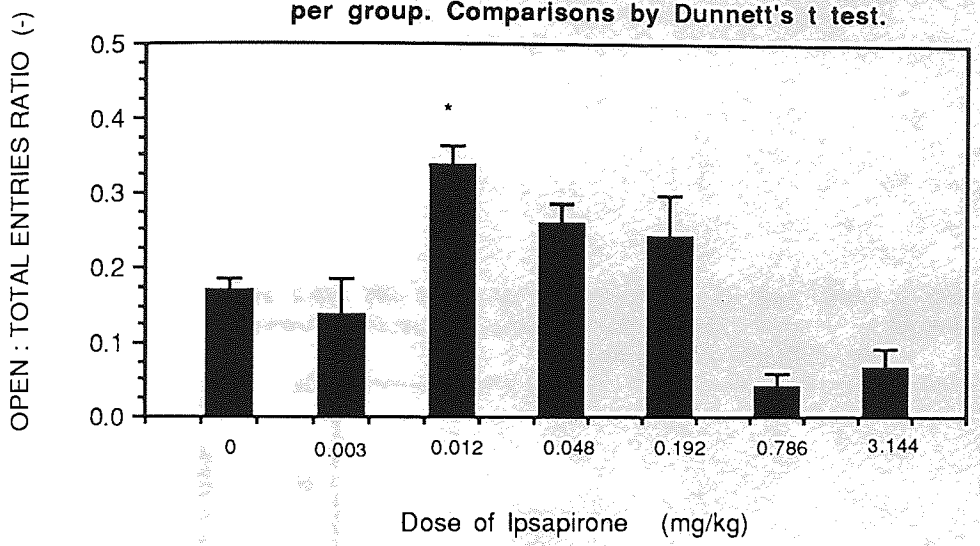


Table 5.10. The effect of ipsapirone on behaviour on the X-maze.

DRUG	DOSE (mg/kg)	N	Time on Open Arms (%)	Total Entries
Ipsapirone	0.0	6	8.2 ± 1.3	23.8 ± 2.3
	0.003	6	8.6 ± 2.7	18.5 ± 2.7
	0.012	6	19.2 ± 3.4**	28.2 ± 3.3
	0.048	6	14.2 ± 2.2	20.7 ± 1.9
	0.19	6	14.8 ± 4.2	28.3 ± 2.5
	0.79	6	1.6 ± 0.8	18.2 ± 3.4
	3.14	6	4.0 ± 1.8	20.2 ± 2.4

Comparisons were made using Dunnett's t test after a 1-way ANOVA. ** $P < 0.01$.

Figure 5.9a. The effect of RU 24969 on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test.

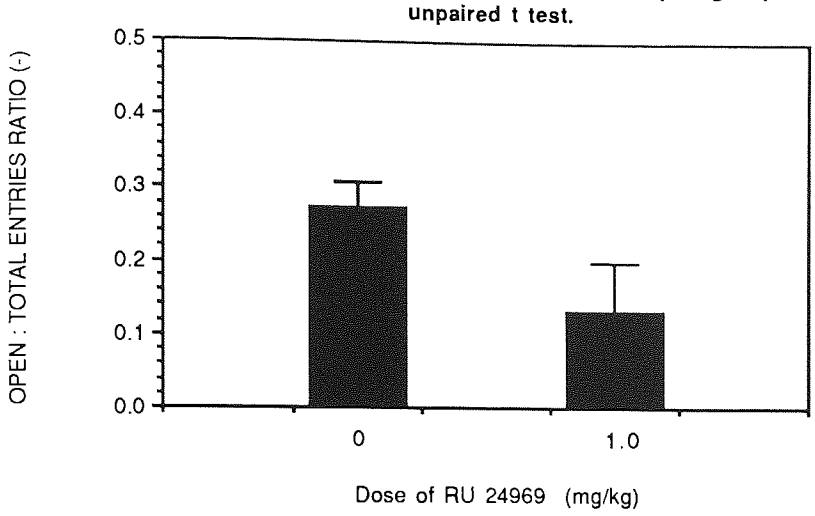


Figure 5.9b. The effect of RU 24969 on the time spent of the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test.

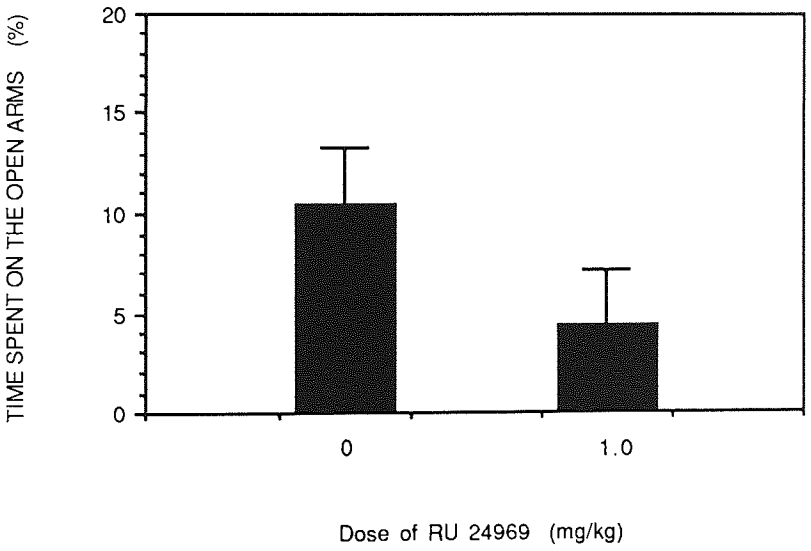


Table 5.11. The effect of RU 24969 on the total of entries made in the X-maze.

DRUG	DOSE (mg/kg)	N	Total Entries
RU 24969	0.0	6	14.8 ± 2.6
	1.0	6	12.3 ± 2.8

Statistical comparisons were made with Student's unpaired t test.

Figure 5.10a. The effect of CGS 12066B on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control.

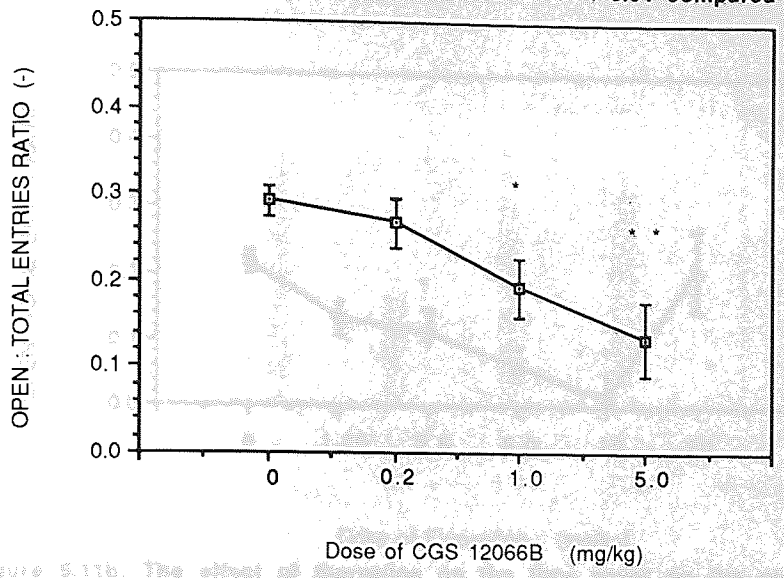


Figure 5.10b. The effect of CGS 12066B on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Dunnett's t test * P < 0.05 compared to control.

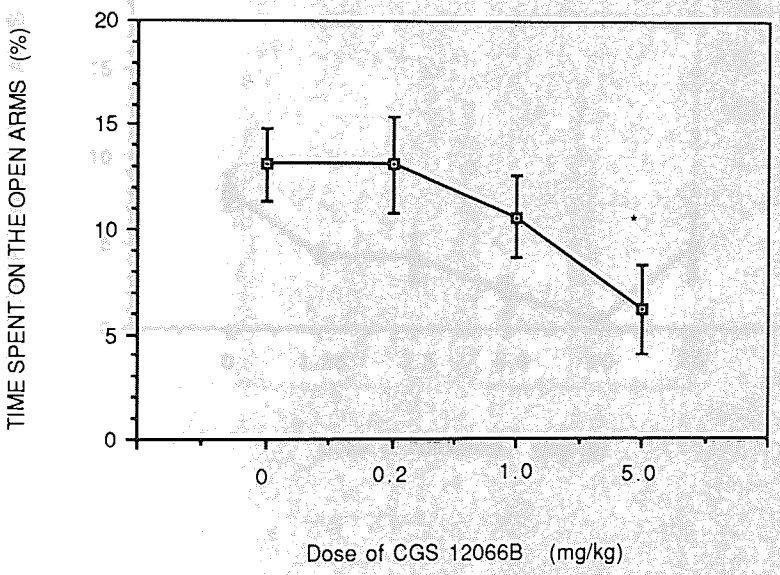


Table 5.12. The effect of CGS 12066 B on the total of entries made in the X-maze.

DRUG	DOSE (mg/kg)	N	Total Entries
CGS 12066 B	0.0	6	18.8 ± 1.6
	0.2	6	19.0 ± 2.2
	1.0	6	19.2 ± 2.7
	5.0	6	16.0 ± 3.6

Statistical comparisons were made by 1-way ANOVA followed by Dunnett's t test.

Figure 5.11a. The effect of fluoxetine on the open : total entry ratio in the elevated X-maze. The graph shows mean ± sem of 6 or 7 observations per group (control n = 13). Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control.

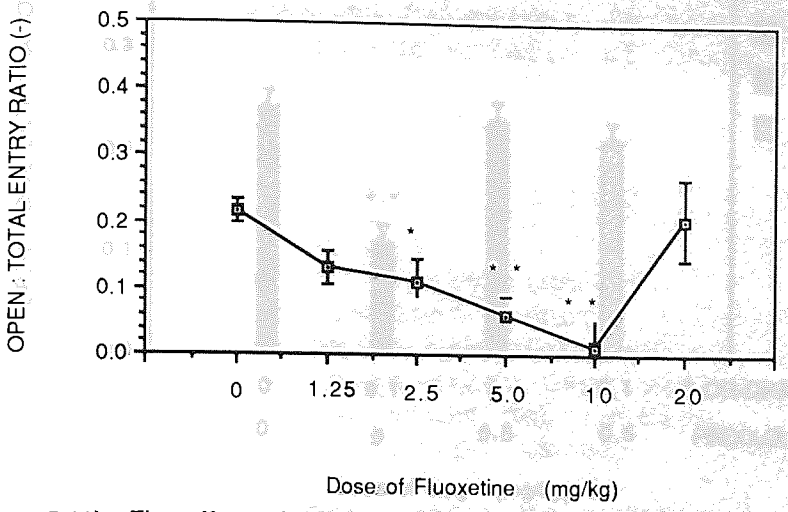


Figure 5.11b. The effect of fluoxetine on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 or 7 observation per group (control n = 13). Comparisons by Dunnett's t test ** P < 0.01 compared to control.

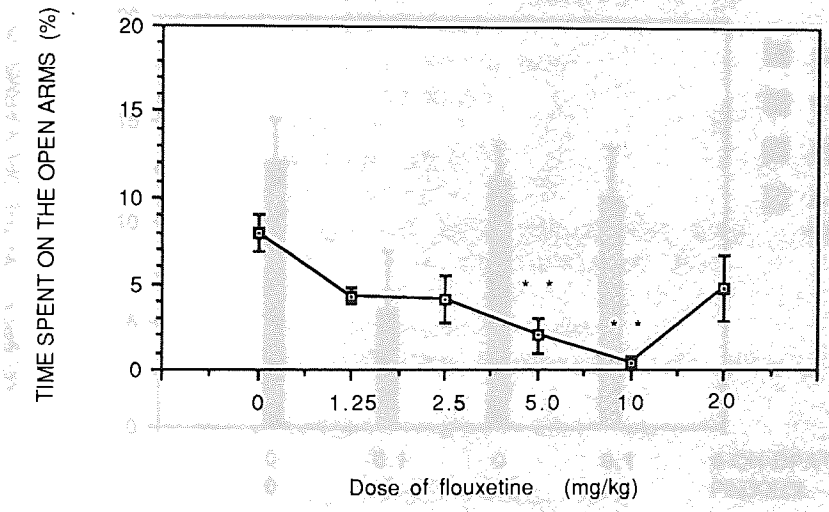


Table 5.13. The effect of fluoxetine on the total of entries made in the X-maze.

DRUG	DOSE (mg/kg)	N	Total Entries
Fluoxetine	0.0	13	21.8 ± 1.9
	1.25	6	19.5 ± 1.5
	2.5	6	19.5 ± 1.3
	5.0	6	20.5 ± 3.1
	10	7	7.6 ± 2.7**
	20	7	7.3 ± 1.9**

Statistical comparisons were made by 1-way ANOVA followed by Dunnett's t test. ** P < 0.01.

Figure 5.12a. The effect of pindolol on the "anxiogenic" action of 8-OH-DPAT on the elevated X-maze. The graph shows mean ± sem. Comparisons by Tukey's test ** P < 0.01.

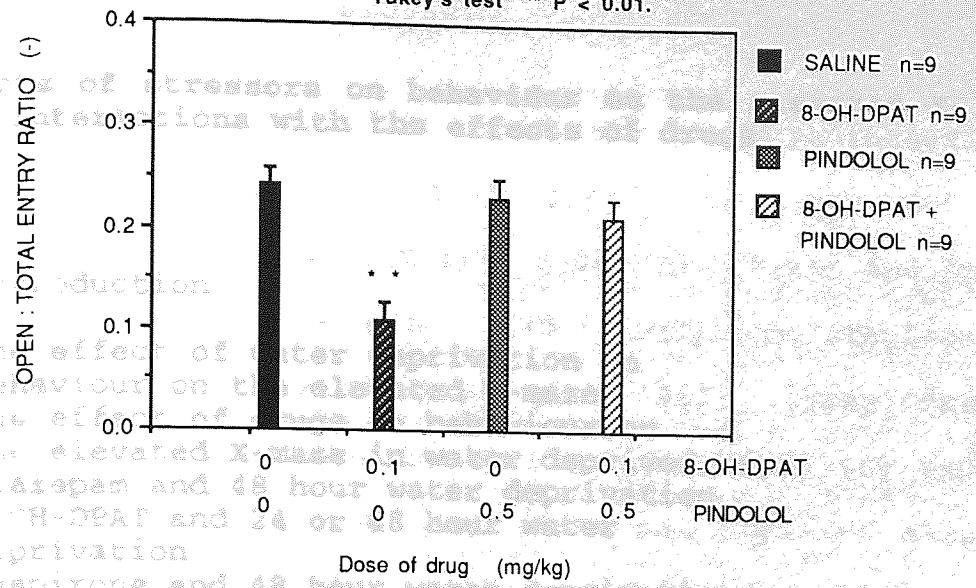


Figure 5.12b. The effect of pindolol on the "anxiogenic" action of 8-OH-DPAT on the elevated X-maze. The graph shows mean ± sem. Comparisons by Tukey's test. (b) time spent on the open arms.

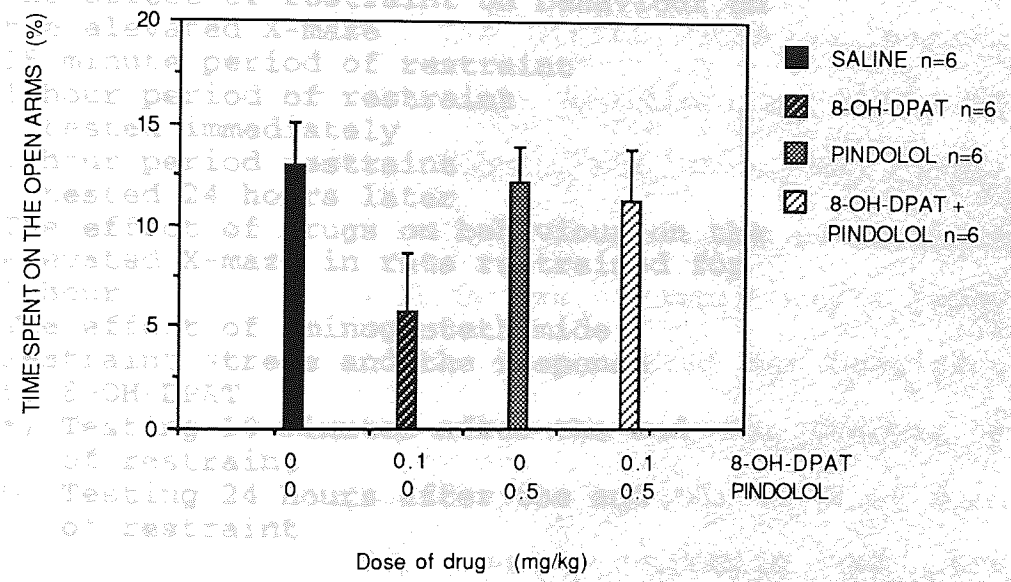


Table 5.14. The effect of 8-OH-DPAT and pindolol on the total entries made in the elevated X-maze.

DRUG	DOSE (mg/kg)	N	Total Entries
Saline	0.0	9	23.3 ± 2.0
8-OH-DPAT	0.1	9	22.0 ± 1.2
Pindolol	0.5	9	20.0 ± 3.3
8-OH-DPAT + Pindolol	0.1 0.5	9	23.1 ± 2.0

Statistical comparisons were made by 2-way ANOVA.

Chapter 6

The effects of stressors on behaviour on the elevated X-maze and interactions with the effects of drugs.

Contents

6.1 Introduction.

Studies using biochemical and electrophysiological techniques have demonstrated that ligands for presynaptic 5-HT_{1A} receptors are full agonists. 8-OH-DPAT (Blier and De Montigny, 1990; Wilkinson et al., 1991), buspirone (Trulson and Araseth, 1986; Wilkinson et al., 1987), ipsapirone (Dourish et al., 1986; Sprouse and Aghajanian, 1986) and gepirone (Blier and De Montigny, 1990; see Eison et al., 1986) have all been shown to produce a large reduction in the rate of firing of serotonergic dorsal Raphé neurones when applied locally into the dorsal Raphé nucleus or following systemic administration. Additionally, 8-OH-DPAT (Arvidsson et al., 1981; Hjörth and Magnusson, 1988) inhibits the synthesis of 5-HT by an action on presynaptic 5-HT_{1A} receptors on the cell bodies of serotonergic Raphé neurones. This has also been demonstrated for buspirone (Hjörth and Carlsson, 1982) and for ipsapirone (Goodwin et al., 1986) and is interpreted as being indicative of full agonists properties of these compounds at the somatodendritic autoreceptor (Dourish et al., 1986; Meller et al., 1990). This is in marked contrast to their effect at postsynaptic 5-HT_{1A} receptors in, for example, the hippocampus.

Here, buspirone, ipsapirone and gepirone have all been shown to act as partial agonists in electrophysiological experiments being able to produce only a fraction of the hyperpolarization response seen in these neurones with 5-

HT, and in biochemical experiments being able to produce a fraction of the inhibition of forskolin-stimulated cAMP production possessed by 5-HT.

With regard to 8-OH-DPAT there is some controversy over whether this ligand acts as a partial agonist or as a full agonist at postsynaptic receptors. In biochemical studies evidence exists to suggest that agonist action at 5-HT_{1A} receptors can both stimulate cAMP production per se (Hamon et al., 1984) and inhibit cAMP production if it is initially and artificially stimulated with either forskolin or vasoactive intestinal peptide (VIP) (De Vivo and Maayani, 1985; Bockeart et al., 1987). These effects of 8-OH-DPAT are reported to be concentration-dependent over the range 1-1000nM (eg De Vivo and Maayani, 1985, Dumuis et al., 1988). In hippocampal tissue, 8-OH-DPAT produces as full an inhibition of forskolin-stimulated cAMP production as 5-HT (De Vivo and Maayani, 1985; Dumuis et al., 1988), suggesting full agonist activity. In cortical tissue, 8-OH-DPAT produces a partial agonist profile (Dumuis et al., 1988), although an earlier publication from this group (Weiss et al., 1986) did in fact suggest full agonist properties of 8-OH-DPAT in cultured cortical tissue. 8-OH-DPAT has been noted to possess full agonist properties in neonatal colliculli using the stimulation of cAMP as the model to characterize 5-HT_{1A} receptor function (Hamon et al., 1984). De Vivo and Maayani (1986) only observed full agonist effects of 8-OH-DPAT in inhibiting forskolin-stimulated adenylate cyclase by 5-HT receptor agonist

action in guinea-pig and rat hippocampus, whereas Markstein et al. (1986) reported that 8-OH-DPAT was a partial agonist with respect to 5-HT in its ability to stimulate adenylate cyclase in rat hippocampal homogenates. More recent biochemical studies have suggested that one critical factor in determining the response to 8-OH-DPAT, whether full or partial agonist, is the density of 5-HT_{1A} receptor present in the tissue. Varrault and Bockeart (1992) have recently shown that at the densities of 5-HT_{1A} receptor present in rat hippocampal membranes 8-OH-DPAT acted as a full agonist in inhibiting the production of cAMP, but at much lower tissue 5-HT_{1A} receptor densities 8-OH-DPAT had only partial agonist effects. This profile of action could reflect on the existence of spare receptors. It has been shown that there is a large presynaptic 5-HT_{1A} receptor reserve where buspirone and ipsapirone are full agonists (Meller et al., 1990) but in hippocampus where there is no receptor reserve 5-HT_{1A} ligands are partial agonists (Meller et al., 1990). In electrophysiological experiments most, but not all, reports indicate that 8-OH-DPAT has both agonist and antagonist effects on the induction of hyperpolarization of membranes in the hippocampus. For example Andrade and Nicoll (1987b) reported that 30 μM 8-OH-DPAT could cause both hyperpolarization of rat hippocampal cells, although to a lesser extent than 30 μM 5-HT, and antagonize the effect of 5-HT itself. Sprouse and Aghajanian (1988) have also demonstrated partial agonist effects of 8-OH-DPAT in rat hippocampal neurones, but these effects were only

marginally less in magnitude than the action of the full agonist 5-HT. These experiments clearly suggest that 8-OH-DPAT is a partial agonist under the experimental conditions used. In contrast, purely antagonist effects of 8-OH-DPAT in the same preparation have been reported by Colino and Halliwell (1986) and by O'Connor et al. (1990). Concentrations of 30 μM 8-OH-DPAT and 5-HT as used by Colino and Halliwell (1986) and O'Connor et al. (1990) are very large indeed, and it is possible that antagonist effects at such high concentrations are artefacts.

In behavioural experiments, 8-OH-DPAT has only agonist effects on the induction of the postsynaptically-mediated 5-HT syndrome (Smith and Peroutka, 1986). In a different behavioural paradigm 5-HTP-induced inhibition of male rat sexual behaviour was antagonised by 8-OH-DPAT (Ahlenius and Larsson, 1987) suggesting antagonist action, although in female rats the induction of lordosis by 8-OH-DPAT (Ahlenius et al., 1986) would indicate agonist effects.

In summary, there is evidence from biochemical, electrophysiological and behavioural experiments which is consistent with a partial agonist profile of 8-OH-DPAT. However, the many findings of full agonist activity indicate that this topic remains controversial.

Recent work using in vivo microdialysis has shown that exposure to the elevated X-maze is associated with an increase in the extracellular concentration of 5-HT in hippocampal tissue (Rex et al., 1991) indicating an increase in the intrasynaptic 5-HT concentration as a

result of being placed on the X-maze. Exposure to the elevated X-maze also increases plasma corticosterone concentrations (Pellow et al., 1985). Together, these facts provide reasonable evidence to suggest that this may reflect a stress response, because an increase in 5-HT release is seen in response to more conventional stressors such as restraint (Kennett and Joseph, 1982).

Stress may be able to modulate intra-synaptic concentrations of 5-HT and may thereby modify the action of partial agonists for 5-HT receptors (Handley, 1991; Critchley et al., 1992). In circumstances of low 5-HT turnover, or reduced intrasynaptic 5-HT concentration, partial agonists will predominantly produce an agonist action. In circumstances of high 5-HT turnover and high intrasynaptic 5-HT concentrations, the occupancy of 5-HT_{1A} receptors by partial agonists opposes the agonist action of the full agonist 5-HT and thus will have antagonist effects. The precise net effect of 5-HT_{1A} agonists at postsynaptic 5-HT_{1A} receptors, whether agonist or antagonist, is likely to be open to modulation depending upon the intrasynaptic concentration of 5-HT.

The reported effects of 5-HT_{1A} receptor ligands in the elevated X-maze model of anxiety were summarised in the preceding chapter with the conclusion that "anxiolytic", "anxiogenic" and inactive effects have been reported for most of the commonly used ligands.

This evidence may suggest that the action of 5-HT_{1A} ligands in behavioural models may be influenced by stress through

an increase in synaptic 5-HT concentrations. Behavioural studies have confirmed that the effect of 5-HT_{1A} partial agonists on behaviour is influenced by stress. Several authors have demonstrated that restraint produces changes in the behaviour of animals subsequently tested in a number of different behavioural situations (Kennett et al., 1985a; Stone, 1979). The characteristic changes that occur are a reduction in exploratory behaviour and in locomotor activity, a reduction in the expression of rearing and reduced grooming behaviour (Berridge and Dunn, 1989; Carli et al., 1989b; Kennett et al., 1987a). 5-HT_{1A} agonists are able to counteract this behavioural deficit when administered shortly after restraint, but many hours before the behavioural test (Kennett et al., 1987a) or when given shortly before the behavioural test but many hours after the restraint (Carli et al., 1989b). These effects are well established in the open field and have also been investigated in a 2 compartment exploratory test (Carli and Samanin, 1988), but there has been no demonstration of how stressors alter behaviour in the elevated X-maze or how drugs, particularly 5-HT_{1A} partial agonists, interact with effects of stress in this test. The experiments described in this chapter were designed to investigate the effects of water deprivation and restraint on behaviour on the elevated X-maze and to assess how this changed the response to partial agonists at the 5-HT_{1A} receptor.

6.2 The effect of water deprivation on behaviour on the elevated X-maze.

Water deprivation for periods of 12 - 48 hours caused a duration-dependent increase in the open : total entry ratio (Figure 6.1a) [$F_{(1,34)} = 18.55$; $P < 0.01$] and in the time spent on the open arms (Figure 6.1b) [$F_{(1,34)} = 7.22$; $P < 0.05$]. Post hoc comparisons showed that the open : total entry ratio for the 24 hour, the 36 and the 48 hour water deprivation periods were significantly different from the non-deprived group (Figure 6.1a). Despite a significant main effect, no one group spent significantly more time on the open arms than the control group. The total number of arm entries was not affected by water deprivation (Table 6.1) [$F_{(1,34)} = 3.92$].

6.3 The effect of drugs on behaviour on the elevated X-maze in water-deprived rats.

For these experiments, rats were deprived of water for 48 hours before being given the drug 30 minutes (10 minutes for 8-OH-DPAT) before being placed on the maze.

6.3.1 Diazepam and 48 hour water deprivation.

Diazepam (2 mg/kg) given i.p. 30 minutes before exposure to the maze increased the open : total entry ratio (Figure 6.2a) [$F_{(1,20)}$ = 19.82; P < 0.01] and the time spent on the open arms (Figure 6.2b) [$F_{(1,20)}$ = 10.04; P < 0.01]. Although 48 hour water deprivation appeared to increase both the open : total entry ratio (Figure 6.2a) [$F_{(1,20)}$ = 3.39; P > 0.05] and the time spent on the open arms (Figure 6.2b) [$F_{(1,20)}$ = 0.15; P > 0.05], these increases were not significant. In rats that had been deprived of water for 48 hours, diazepam further raised the entry ratio (Figure 6.2a) and the time spent on the open arms (Figure 6.2b), consistent with the lack of a significant interaction term for both the open : total entry [$F_{(1,20)}$ = 0.007; P > 0.05] and the open arm time [$F_{(1,20)}$ = 0.41; P > 0.05]. Neither treatment, nor the combination, influenced the total number of arm entries made (Table 6.2).

6.3.2 8-OH-DPAT and 24 hour or 48 hour water deprivation.

Two experiments assessing the effects of 8-OH-DPAT in rats that had been deprived of water for either 24 or 48 hours were conducted. The first experiment used 24 hour water deprivation. 8-OH-DPAT (0.1 mg/kg) given i.p. 10 minutes before exposure to the elevated X-maze resulted in a reduction in both the open : total entry ratio (Figure

6.3a) [$F_{(1,20)}$ = 32.90; P < 0.01] and the time spent on the open arms (Figure 6.3b) [$F_{(1,20)}$ = 5.83; P < 0.05], whereas 24 hour water deprivation significantly increased both the open : total entry ratio (Figure 6.3a) [$F_{(1,20)}$ = 53.39; P < 0.01] and the time spent on the open arms (Figure 6.3b) [$F_{(1,20)}$ = 7.69; P < 0.05]. In combination, 8-OH-DPAT decreased both the entry ratio and the open arm time, notwithstanding the increase in these parameters in response to water deprivation, as reflected in the lack of a significant interaction term [$F_{(1,20)}$ = 0.24 P > 0.05 for open : total entry ratio and $F_{(1,20)}$ = 0.23; P > 0.05 for open arm time]. Neither treatment influenced the total number of entries (Table 6.3).

In the second experiment, 48 hour water deprivation increased both the open : total entry ratio (Figure 6.4a) [$F_{(1,20)}$ = 16.06; P < 0.01] and the time spent on the open arms (Figure 6.4b) [$F_{(1,20)}$ = 12.12; P < 0.01]. 8-OH-DPAT (0.1 mg/kg) given i.p. 10 minutes before exposure to the elevated X-maze caused a reduction in the entry ratio (Figure 6.4a) [$F_{(1,20)}$ = 5.56] and the time on the open arms (Figure 6.4b) [$F_{(1,20)}$ = 4.42; P < 0.05]. In combination, 8-OH-DPAT decreased both the entry ratio (Figure 6.4a) and the open arm time (Figure 6.4b), notwithstanding the increase in these parameters in response to water deprivation, again consistent with the lack of a significant interaction term [$F_{(1,20)}$ = 0.05; P > 0.05 for open : total entry ratio and $F_{(1,20)}$ = 0.23; P > 0.05 for open arm time]. In this experiment, 8-OH-DPAT was without effect on the number of

entries, although there was a main effect of water deprivation [$F_{(1,20)}$ = 4.42; P < 0.05]. Their combination resulted in a significant increase in total entries (Table 6.4), despite a non-significant interaction term [$F_{(1,20)}$ = 3.95; P > 0.05].

6.3.3 Buspirone and 48 hour water deprivation.

Buspirone (1 mg/kg) given i.p. 30 minutes before exposure to the maze did not change either the open: total entry ratio [$F_{(1,20)}$ = 0.14; P > 0.05] or the time spent on the open arms [$F_{(1,20)}$ = 0.02; P > 0.05] (Figure 6.5; Table 6.5). 48 hour water deprivation significantly increased both these measures [$F_{(1,20)}$ = 22.02; P < 0.01 for open : total arm entry ratio and $F_{(1,20)}$ = 11.65; P < 0.01 for open arm time] (Figure 6.5; Table 6.5). In combination, buspirone did not alter the increase in entry ratio and open arm time seen in response to water deprivation [interaction terms $F_{(1,20)}$ = 1.03; P > 0.05 for open : total entry ratio and $F_{(1,20)}$ = 0.236; P > 0.05 for open arm time] (Figure 6.5; Table 6.5). Total entries were not changed by any manipulation (Table 6.5).

6.3.4 Ipsapirone and 48 hour water deprivation.

Ipsapirone (2.5 mg/kg) given i.p. 30 minutes before exposure to the maze did not change either the open : total entry ratio (Figure 6.6) [$F_{(1,20)}$ = 0.07; P > 0.05] or the

time spent on the open arms (Table 6.6) [$F_{(1,20)}$ = 0.02; P > 0.05]. 48 hour water deprivation significantly increased both these indicators (Figure 6.6; Table 6.6) [$F_{(1,20)}$ = 14.66; P < 0.01 for entry ratio and $F_{(1,20)}$ = 9.73; P < 0.01 for time]. In combination, ipsapirone did not alter the increase in entry ratio and open arm time seen in response to water deprivation (Figure 6.6; Table 6.6) consistent with the lack of interaction terms for both entry ratio [$F_{(1,20)}$ = 0.19; P > 0.05] and open arm time [$F_{(1,20)}$ = 0.01; P > 0.05]. Total entries were not changed by any manipulation (Table 6.6).

6.3.4 Gepirone and 48 hour water deprivation.

Gepirone (5 mg/kg) given i.p. 30 minutes before exposure to the maze did not change either the open: total entry ratio (Figure 6.7) [$F_{(1,20)}$ = 2.71; P > 0.05] or the time spent on the open arms (Table 6.7) [$F_{(1,20)}$ = 3.41; P > 0.05]. 48 hour water deprivation significantly increased both the entry ratio [$F_{(1,20)}$ = 9.20; P < 0.01] and the open arm time [$F_{(1,20)}$ = 5.59: P < 0.05] (Figure 6.7; Table 6.7). In combination, the interaction term was not significant for either the entry ratio [$F_{(1,20)}$ = 1.45; P > 0.05] or the open arm time [$F_{(1,20)}$ = 0.53; P > 0.05], indicating that gepirone did not alter the increase in entry ratio and open arm time seen in response to water deprivation (Figure 6.7; Table 6.7). Total entries were not changed by either manipulation (Table 6.7).

6.4 The effect of restraint on behaviour on the elevated X-maze.

For testing the effects of restraint immediately prior to exposure to the X-maze, rats were removed from their home cage and restrained for either 15 minutes or one hour. They were placed immediately on the elevated X-maze, before being returned to their home cage. Control animals for these experiments were taken from their home cage either 15 minutes or one hour and left in a cage on their own before being placed on the maze. For the third experiment in this group, control animals were left in their home cage at all times except for conducting the test. Isolated rats were taken from their home cage and housed singly for 24 hours before testing on the maze. Restrained rats were removed from their home cage, restrained and tested on the maze (immediate group) or were taken from their home cage, restrained and housed singly until testing on the maze 24 hours later. These elaborate conditions were used because of evidence suggesting that the impairing effects of restraint can be countered by group housing (Dourish et al., 1989).

6.4.1 15 minute period of restraint

Rats were restrained for 15 minutes and tested in the elevated X-maze immediately after release. This procedure did not significantly influence the open : total entry

ratio, the time spent on the open arms or the total number of arm entries (Experiment 1, Table 6.8). The experiment was repeated, but again restraint did not significantly influence the open : total entry ratio, the time spent on the open arms or the number of total arm entries (Experiment 2, Table 6.8). A longer time period for restraint was therefore investigated.

6.4.2 1 hour period of restraint - tested immediately.

Rats were restrained for a period of 1 hour, released and then immediately tested on the elevated X-maze. This procedure significantly reduced the open : total entry ratio (Figure 6.8a) and the time spent on the open arms of the maze (Figure 6.8b) and also significantly decreased the total number of arm entries made (Table 6.9).

6.4.3 1 hour period of restraint - tested 24 hours later.

Rats were restrained for a period of one hour and then either tested on the elevated X-maze immediately or were housed on their own in a cage for 24 hours before being tested on the maze. A further group were housed on their own in a cage for 24 hours before testing on the maze and a fourth group acted as controls and were left in their home cage.

Restraint for 1 hour reduced the open : total entry ratio both when tested immediately or with an interval of 24

hours compared to controls (Figure 6.9a) [$F_{(3,19)}$ = 17.49; P < 0.01]. Restraint also reduced the time spent on the open arms both immediately and 24 hours after the end of restraint (Figure 6.9b) [$F_{(3,19)}$ = 7.61; P < 0.01]. Restraint did notinfluence the total number of arm entries [$F_{(3,19)}$ = 1.57; P > 0.05] (Table 6.10). Isolation for 24 hours did not produce any changes in behaviour on the maze (Figures 6.9a,b; Table 6.10).

6.5 The effect of drugs on behaviour on the elevated X-maze in rats restrained for 1 hour.

Interactions between drug treatment and restraint were investigated in the next series of experiments. Aminoglutethimide was administered 1 hour before the start of a 1 hour restraint. 8-OH-DPAT was given 10 minutes before maze exposure, either immediately after the end of a 1 hour period of restraint, or 24 hours after a 1 hour period of restraint.

6.5.1 The effect of aminoglutethimide.

Aminoglutethimide at a dose of 5 mg/kg and given i.p. 2 hours before exposure to the maze, did not influence the open : total entry ratio (Figure 6.10) [$F_{(1,28)}$ = 1.54; P > 0.05] the time spent on the open arms (Table 6.11) [$F_{(1,28)}$ = 2.07; P > 0.05] or the number of entries made (Table 6.11) [$F_{(1,28)}$ = 0.06; P > 0.05]. There was a significant effect of

restraint on the open : total entry ratio (Figure 6.11) [$F_{(1,28)}$ = 5.82; P < 0.05], although for both saline-treated and for aminoglutethimide-treated rats, restraint did not significantly alter the open : total entry ratio. Restraint did reduce the time spent on the open arms (Table 6.11) [$F_{(1,28)}$ = 29.89; P < 0.01] and the number of entries made (Table 6.11) [$F_{(1,20)}$ = 39.45: P < 0.01]. The combination of aminoglutethimide given 1 hour before the start of a 1 hour restraint, resulted in an open : total entry ratio (Figure 6.10), open arm time (Table 6.11) and total entries (Table 6.11) that were not significantly different from those of saline treated restrained rats. There was no interaction between restraint and aminoglutethimide treatment on the open : entry ratio ($F_{(1,28)}$ = 0.50; P > 0.05), the time spent on the open arms ($F_{(1,28)}$ = 0.38; P > 0.05) or the total entries ($F_{(1,28)}$ = 1.89; P > 0.05).

6.5.2 The effect of 8-OH-DPAT. (a) Testing 10 minutes after the end of 1 hour restraint.

In unrestrained animals 8-OH-DPAT, at the dose of 0.2 mg/kg given i.p. 10 minutes prior to the maze, caused a significant (P < 0.05) reduction in the open : total entry ratio (Figure 6.11) and a non-significant reduction in the time spent on the open arms (Table 6.12) without significantly influencing the total number of entries (Table 6.12) [$F_{(1,20)}$ = 2.57; P > 0.05]. Restraint also reduced the open : total entry ratio (P < 0.01) (Figure

6.11) [interaction $F_{(1,20)} = 14.53$; $P < 0.01$], time spent on the open arms ($P < 0.05$) (Table 6.12) [interaction $F_{(1,20)} = 4.35$; $P = 0.05$] and reduced the total number of arm entries (Table 6.9) [$F_{(1,20)} = 28.02$; $P < 0.01$]. There was a significant interaction between restraint and treatment with 8-OH-DPAT [open : total entry ratio interaction term $F_{(1,20)} = 14.53$; $P < 0.01$: open arm time interaction term $F_{(1,20)} = 4.34$; $P = 0.05$] with the consequence that, in restrained rats 8-OH-DPAT significantly ($P < 0.01$) increased the open : total entry ratio (Figure 6.11) although the increase in the time spent on the open arms was not significant (Table 6.12).

b) Testing on the X-maze 24 hours after the end of restraint.

In unrestrained animals 8-OH-DPAT, at the dose of 0.2 mg/kg given i.p. 10 minutes prior to exposure to the maze, caused a significant ($P < 0.05$) reduction in the open : total entry ratio (Figure 6.12) and a significant ($P < 0.01$) reduction in the time spent on the open arms (Table 6.13) without influencing the total number of entries (Table 6.13). In saline treated rats, restraint also reduced the open : total entry ratio ($P < 0.05$) (Figure 6.12) and the time spent on the open arms (Table 6.13), although in this experiment the total number of arm entries was not influenced by restraint (Table 6.13) [$F_{(1,20)} = 1.72$; $P > 0.05$]. The significant interaction between restraint and

treatment with 8-OH-DPAT [open : total entry ratio interaction term $F_{(1,20)} = 5.34$; $P < 0.05$: open arm time interaction term $F_{(1,20)} = 7.00$; $P < 0.05$] was evidenced by the fact that, in restrained rats 8-OH-DPAT did not significantly influence either the open : total entry ratio (Figure 6.12) or the time spent on the open arms (Table 6.13).

6.6 Discussion.

The apparent "anxiolytic" effect of water deprivation seen in the elevated X-maze was an unexpected finding; however, the effect was specifically related to "anxious" behaviour and was not non-specific behavioural activation, because the number of total arm entries made was not increased. Also the effect was very reliable, being present virtually every time it was tried and its magnitude was clearly dependent upon the duration of deprivation.

This apparent "anxiolytic" action of water deprivation might not necessarily indicate a global reduction of anxiety by water deprivation. Montgomery (1955) argued that in the Y-maze, the single open arm presents a greater number of novel stimuli and so elicits a greater exploratory drive than the enclosed arms, but that the elevation and relative exposure inhibit exploration of the open arm. Water deprivation might promote the desire to explore in order to find water and because of the greater number of novel stimuli on the open arm, there is greater exploration of this region of the maze. It is not possible to discover whether this is accompanied by a reduction in the 'emotion' of anxiety. In another exploration-based model of anxiety, the open field with a shaded corner, water deprivation did not show any "anxiolytic" properties (Carli and Samanin, 1988).

Testing water deprivation in animal models of anxiety that have no spatial exploratory component, such as social

interaction, might indicate whether the effect observed in the elevated X-maze is a true "anxiolytic" effect.

An "anxiolytic" effect was still observed in response to diazepam in rats that had been water-deprived. This indicated that, although there was an increase in the open : total entry ratio in response to water deprivation, it was still possible to see further increases in response to treatment with an "anxiolytic" drug.

Water deprivation for 24 or 48 hours did not significantly alter the "anxiogenic"-like response to 8-OH-DPAT in the X-maze (despite the elevated baseline), as demonstrated by the lack of any significant interaction term in the two-way analyses of variance for these experiments. These results with 8-OH-DPAT are in contrast to the results of Carli and Samanin (1988) who found that while water deprivation per se did not influence "anxious" behaviour, it could alter the action of 8-OH-DPAT in a two compartment exploratory test. The difference observed between the two sets of experiments may lie in the fact that in these experiments water deprivation per se had strong effects on the behavioural measures being assessed, whereas Carli and Samanin (1988) found that this was not the case for their experiments.

8-OH-DPAT was not found to influence water intake in water-deprived rats (Engel et al., 1984; Higgins et al., 1988; Moser et al., 1990), suggesting that 8-OH-DPAT does not act to inhibit thirst in water-deprived rats. This would suggest that the effect of 8-OH-DPAT in water-deprived rats

is not to counteract thirst and by this mechanism normalize behaviour on the elevated X-maze.

Single doses of other 5-HT_{1A} ligands, chosen on the basis of published work from this laboratory (Critchley et al., 1992), were not found to influence behaviour on the elevated X-maze and these results were not altered by 48 hour water deprivation. That previously active doses of 5-HT_{1A} ligands were without effect here, with a different observer is further testament to the variability of these drugs reported in the literature. It was anticipated that an effect would become apparent in water-deprived animals, in line with the view of Carli et al. (1989b) who suggested that prior stress experienced by an animal causes greater effects to be seen with 5-HT_{1A} ligands. Unfortunately, no effect could be observed with buspirone, ipsapirone or gepirone in control or water deprived animals in the X-maze. However, in each of these experiments, the "anxiolytic" effect of water deprivation was present.

15 minutes restraint did not influence behaviour on the elevated X-maze. This was a very surprising finding because the behaviour of animals during the stress indicated that the experience was stressful, as did the initial biochemical experiments described in chapter 3. Restrained rats typically screeched and writhed for the first 10 or 20 minutes of restraint, although this sort of behaviour was not present in the latter half of the one hour restraint procedure. These behavioural changes in response to restraint and their biphasic nature are similar to those

described by Ushijima et al. (1992). Despite these indicators that the experience was stressful, restraint did not change behaviour on the elevated X-maze after 15 minutes. This has recently been confirmed by Falter et al. (1992).

A longer period of restraint did alter the behaviour of animals on the maze. The appearance of these animals was not similar to that of animals who had received treatment with an "anxiogenic" drug. Restrained animals usually made fewer total arm entries and, in enclosed arms, were slow to turn round in the arm. Thus, in contrast to all other animals that experienced the X-maze for this thesis, restrained animals were more reluctant to face outwards when in the enclosed arm. Their overall profile suggested that they were not exhibiting behaviour indicative of heightened "anxiety", but that they were simply not interested in their surroundings. Further investigations with antidepressant drug treatment might reveal whether this effect of restraint on the X-maze reflects a depressive response.

It is possible also that restrained animals were less active because the restraint procedure caused rats, on their release, to be stiff and perhaps in pain. This possibility could be assessed experimentally using the rotarod, which is presumed to test coordination and where if it were possible for rats to walk, they presumably would. If, however, rats were unable to stay on the rotarod, this would still not distinguish between learned

helplessness and physical incoordination resulting from the restraint procedure. On release from restraint, it was common for rats to run across the bench as if to escape from the locality where restraint took place, suggesting that it was physically possible for rats to move if they wished.

These results are not entirely consistent with the classical hypothesis that increased serotonergic activity produces "anxiety" (eg. Iversen, 1984) because restraint for 15 minutes did not change X-maze behaviour but did increase 5-HT turnover. Behaviour was changed after one hour of restraint, when 5-HT turnover presumably is still elevated (Joseph and Kennett, 1983), yet 24 hours after restraint there is a recovery of 5-HT turnover (Dickinson et al., 1985) in the presence of altered X-maze behaviour. It remains quite possible that the "anxiogenic"- / depressant- like effect of prolonged restraint is not a consequence of activating serotonergic mechanisms. For instance, stimulation of locus coeruleus neurones produces behavioural changes indicative of increased "anxiety" (Redmond, 1979) and restraint is known to elevated noradrenaline turnover (Tanaka et al., 1990).

Previous reports have found an "anxiogenic" / depressant - like effect of restraint in an open field when tested 24 hours after the end of restraint (Kennett et al., 1985a; Carli et al., 1989b). Results with the X-maze confirm that this model is also able to detect an impairment of performance in response to restraint.

Isolation for a 24 hour period did not significantly alter the behaviour of animals in the maze. It was necessary to do this experiment because some authors have demonstrated that behavioural impairment after restraint is not present in group housed animals (Dourish et al., 1989). The experiments of Kennett et al. (1985 a, b, 1986, 1987 a, b) used rats that had been housed singly and those of Carli et al. (1989) used rats housed in pairs. These observations have lead Deakin (1991b) to conclude that the effects of restraint are reversed by group housing, or phrased in a different way, that social interaction protects against the performance impairing effects of restraint. In animals, housing conditions are known to impact upon the kinetics of 5-HT_1 receptors (Popov and Petkov, 1990), the activity of 5-HT systems (Crespi et al., 1992) and the behaviour of rodents (Francès et al., 1990), providing a potential mechanism for explaining altered behavioural responses to drugs which act on 5-HT systems.

That isolation per se did not influence behaviour on the maze is evidence that the alterations in behaviour seen 24 hours after a 1 hour restraint were not due to the fact that the animals had been kept on their own for the 24 hours prior to the maze exposure.

Aminoglutethimide inhibits the synthesis of all steroid molecules from cholesterol and causes a reduction in plasma corticosterone concentration in the rat (Bitar and Weiner, 1984). Its failure to influence the response to restraint is consistent with a previous report that metyrapone did

not influence the behavioural response to restraint in an open field test (Kennett et al., 1985a). This suggests that corticosterone produced in response to stress is not of major importance in determining acute behavioural impairments, at least in the elevated X-maze. Alternatively, as plasma corticosterone concentrations were not assessed in this experiment or after the same treatment with aminoglutethimide, it is possible that treatment did not influence plasma corticosterone concentrations. In this case, the lack of alteration of response to restraint after aminoglutethimide would result because of a failure to compromise the corticosterone response fully.

Restraint altered the behavioural response to 8-OH-DPAT. When tested immediately after the end of restraint, 8-OH-DPAT switched from being "anxiogenic" to being "anxiolytic" and when there was a 24 hour interval between the end of restraint and the test, the "anxiogenic" action switched to one of no effect. The ability of restraint to reveal an "anxiolytic"-like effect of 8-OH-DPAT, in contrast to the "anxiogenic"-like action of this substance seen in control animals, is evidence that this 5-HT_{1A} agonist can produce opposite effects in the elevated X-maze depending on the conditions of the experiment.

There are several reports that treatment with 5-HT_{1A} receptor agonists in stressed animals results in different behavioural responses. Carli and Samanin (1988) reported that 2 hours restraint ending 24 hours before testing inhibited exploration of the dark compartment of a two

compartment arena. This deficit could be abolished by 8-OH-DPAT given 1 hour before the behavioural test. These results are very similar to those found in the present experiments in that restraint-induced deficits in X-maze exploration were abolished by 8-OH-DPAT given shortly before the behavioural test. This seems to be a general property of 5-HT_{1A} receptor agonists as similar results have been reported for buspirone (Carli et al., 1989b).

Mention has already been made of the fact that the failure of water deprivation to switch the response to 8-OH-DPAT in the elevated X-maze is not consistent with a previous result in a two compartment exploration test (Carli and Samanin, 1988).

This discrepancy between the effects of restraint and water deprivation and an interaction with 8-OH-DPAT may lie in the fact that in the present work, there was an "anxiolytic" effect of water deprivation and an "anxiogenic" / depressant effect of restraint. As 5-HT_{1A} agonists appear to act to reduce the magnitude of restraint-induced impairments in performance in the open field and water deprivation had an "anxiolytic" effect, this may be one reason why water deprivation did not have the same effects as restraint. Incidentally, the doses of 8-OH-DPAT used by Carli and Samanin were approximately 10 fold higher than those used here. This may also have been a contributory factor to the observed differences.

These results would suggest that 5-HT_{1A} receptor function is altered in rats that have been previously stressed. A

series of very similar experiments have suggested that the 5-HT_{1A} receptor is intricately involved in behavioural responses to restraint. After repeated restraint stress, there is an enhancement of forepaw treading induced by the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Dickinson et al., 1985). These behaviours arise from agonist action at postsynaptic 5-HT_{1A} receptors (see Chapter 1 for references) and this finding suggests that repeated restraint alters 5-HT_{1A} receptor sensitivity.

Treatment with a 5-HT_{1A} receptor agonist shortly after a single restraint results in the attenuation of the behavioural deficit observed 24 hours later in the open field test (Kennett et al., 1987a). This has been demonstrated to occur with 8-OH-DPAT, ipsapirone and buspirone (Kennett et al., 1987a) and also with the antidepressants sertraline and desipramine, but not by diazepam or chlordiazepoxide (Kennett et al., 1987a), suggesting that this action of the 5-HT_{1A} ligands reflects an antidepressant, rather than an "anxiolytic" effect. This effect is abolished by pCPA pretreatment (Beer et al., 1990), indicating that the antidepressant effect of 8-OH-DPAT, buspirone and ipsapirone in the open field test is mediated by presynaptic 5-HT_{1A} receptors.

The protocol of the experiments reported by Kennett et al. (1985-1990) differs from that used here and that of Carli and Samanin (1988) and Carli et al. (1989) in that in the former experiments drugs were administered shortly after the restraint, whereas the latter gave drugs shortly before

the behavioural test. Because of this difference, the experiments cannot be directly compared.

In summary, the experiments presented here showed that although water deprivation did not influence the action of any agent tested in the elevated X-maze, it was possible to detect opposite effects of 8-OH-DPAT in the elevated X-maze in rats that had been restrained.

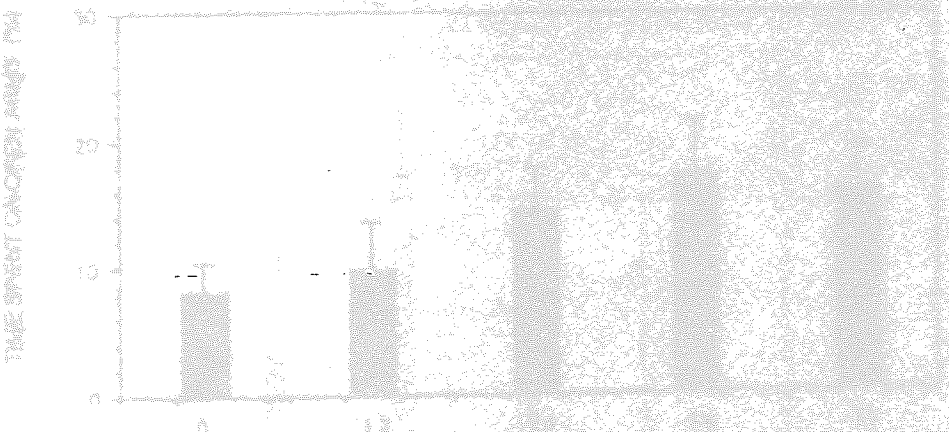


Figure 6.1a. The effect of water deprivation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group (control n = 12). Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control.

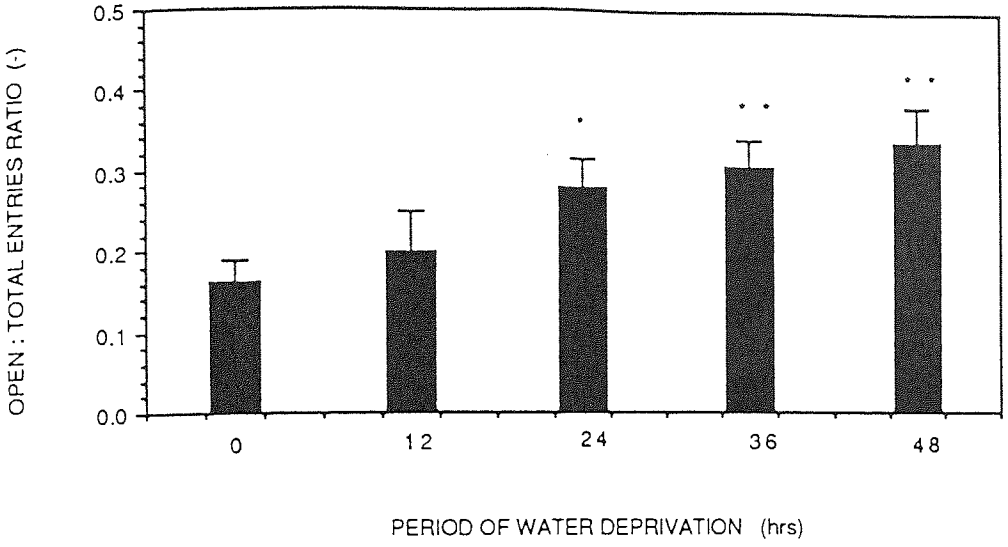


Figure 6.1b. The effect of water deprivation on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group (control n = 12). Comparisons by Dunnett's t test.

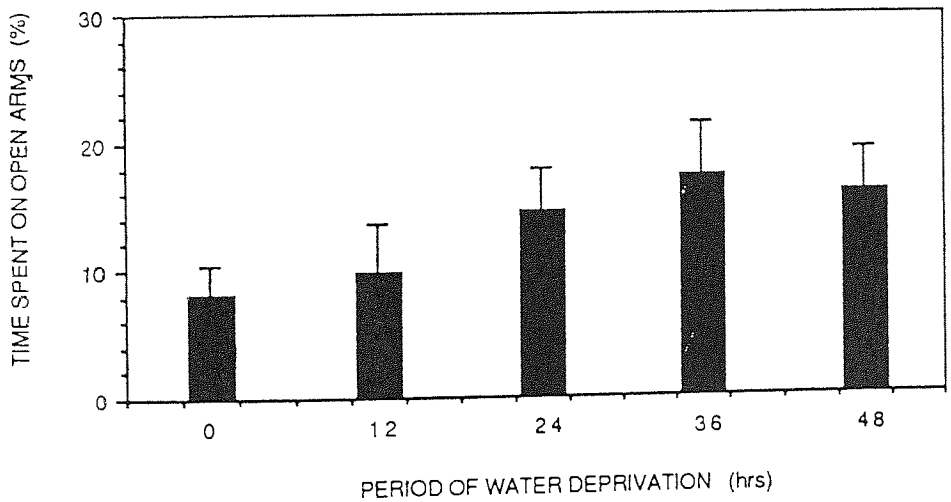


Table 6.1. The effect of water deprivation on the total number of arm entries made in the elevated X-maze.

Duration of Deprivation (hrs)	0	12	24	36	48
Total Entries	23.1 ± 1.7	23.5 ± 3.4	26.3 ± 2.0	23.8 ± 1.9	19.3± 2.3

Results were analysed by regression ANOVA - no main effect.

Figure 6.2a. The effect of diazepam and 48 hour water deprivation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control ∞∞ P < 0.01 compared to water-deprived control.

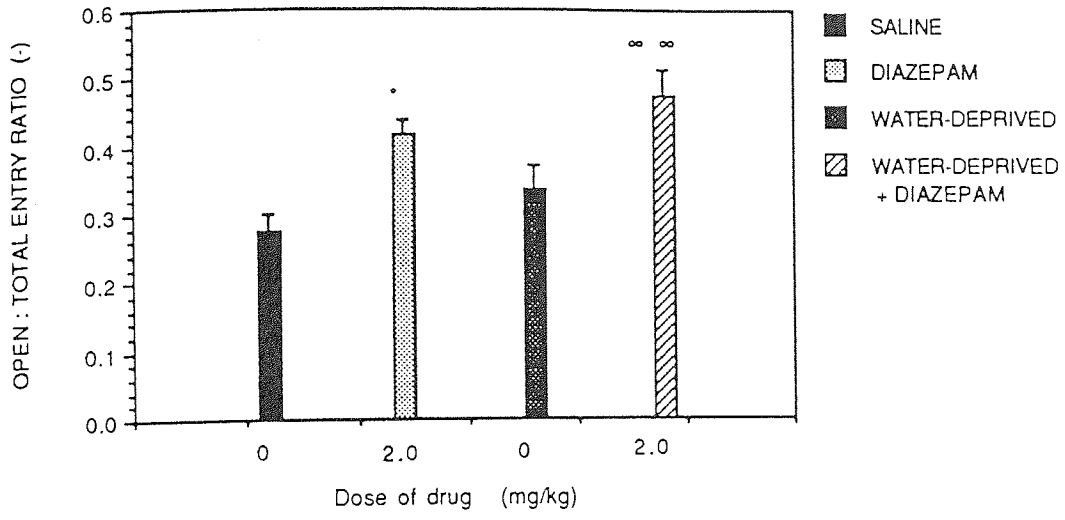


Figure 6.2b. The effect of diazepam and 48 hour water deprivation on the time spent on the open arms in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test ** P < 0.01 compared to control ∞∞ P < 0.01 compared to water-deprived control.

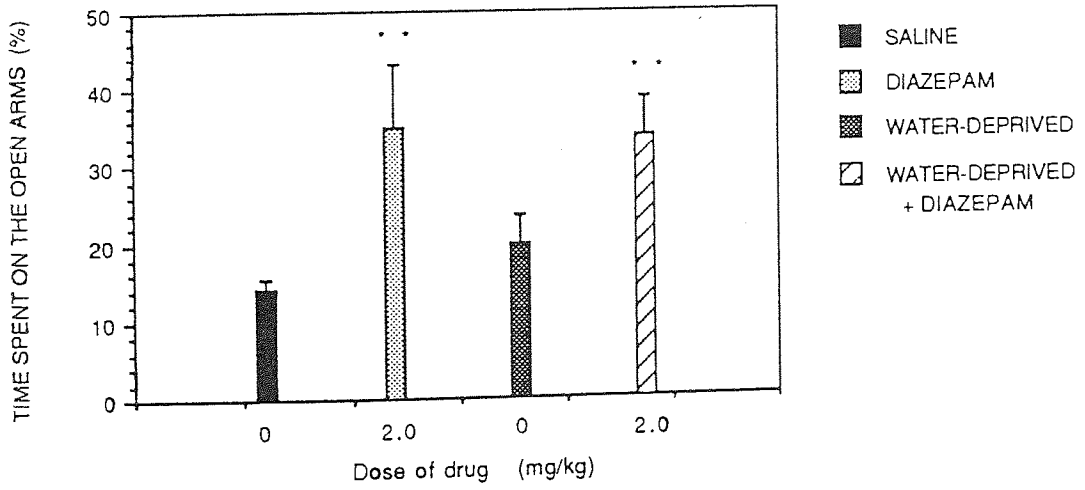


Table 6.2. The effect of 48 hour water deprivation and treatment with diazepam on behaviour on the elevated X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Total Entries
Control	0.0	6	23.3 ± 3.3
Diazepam	2.0	6	24.7 ± 3.9
Water-deprived	0.0	6	19.5 ± 1.6
Diazepam	2.0	6	29.2 ± 4.0

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA.

Figure 6.3a. The effect of 24 hour water deprivation and 8-OH-DPAT on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control.

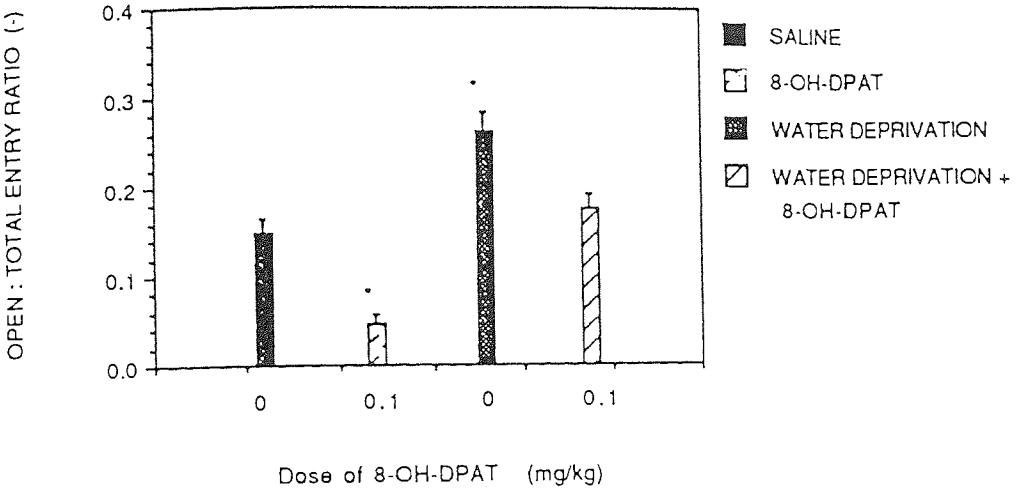


Figure 6.3b. The effect of 24 hour water deprivation and 8-OH-DPAT on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control.

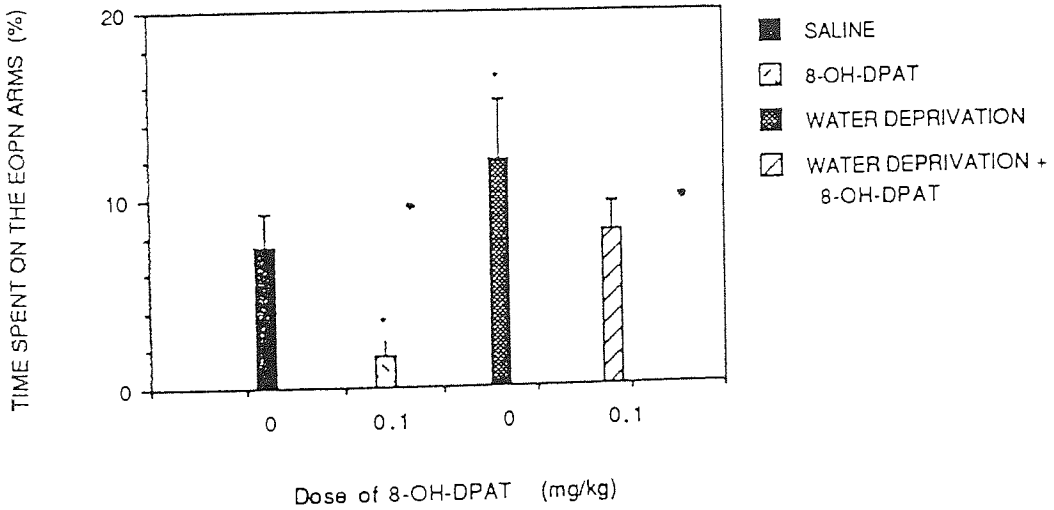


Table 6.3. The effect of 24 hour water deprivation and 8-OH-DPAT on the total entries made on the elevated X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Total Entries
Control	0.0	6	25.2 ± 2.2
8-OH-DPAT	0.1	6	20.3 ± 1.9
Water-deprived	0.0	6	25.5 ± 4.7
8-OH-DPAT	0.1	6	24.3 ± 2.6

Statistical comparisons were made by 2-way ANOVA.

Figure 6.4a. The effect of 48 hour water deprivation and 8-OH-DPAT on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparison by Tukey's test * P < 0.05 compared to control.

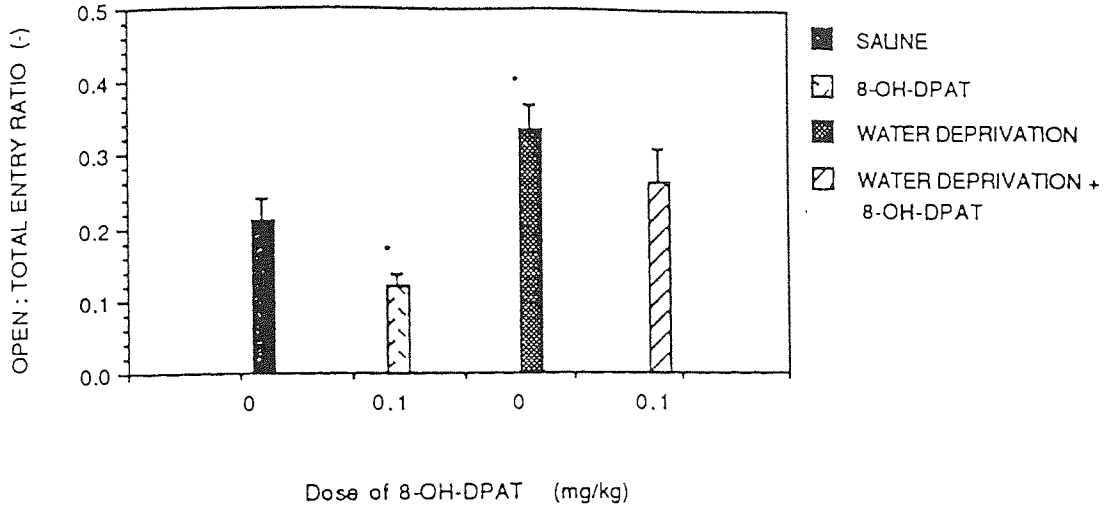


Figure 6.4b. The effect of 48 hour water deprivation and 8-OH-DPAT on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control.

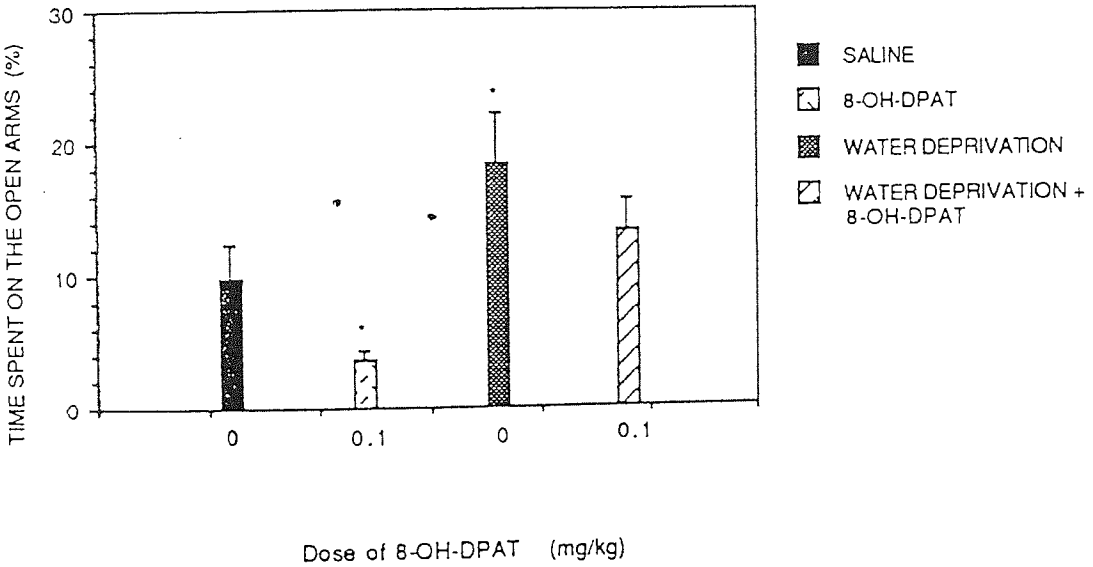


Table 6.4. The effect of 48 hour water deprivation and 8-OH-DPAT on the total entries made on the elevated X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Total Entries
Control	0.0	6	21.0 ± 3.3
8-OH-DPAT	0.1	6	20.5 ± 2.1
Water-deprived	0.0	6	21.3 ± 1.8
8-OH-DPAT	0.1	6	32.3 ± 3.6**

Statistical comparisons were made using Dunnett's t test after a 2-way ANOVA. ** P < 0.05 compared to control.

Figure 6.5. The effect of buspirone and 48 hour water deprivation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
** P < 0.01 compared to control ∞∞ P < 0.01 compared to buspirone group.

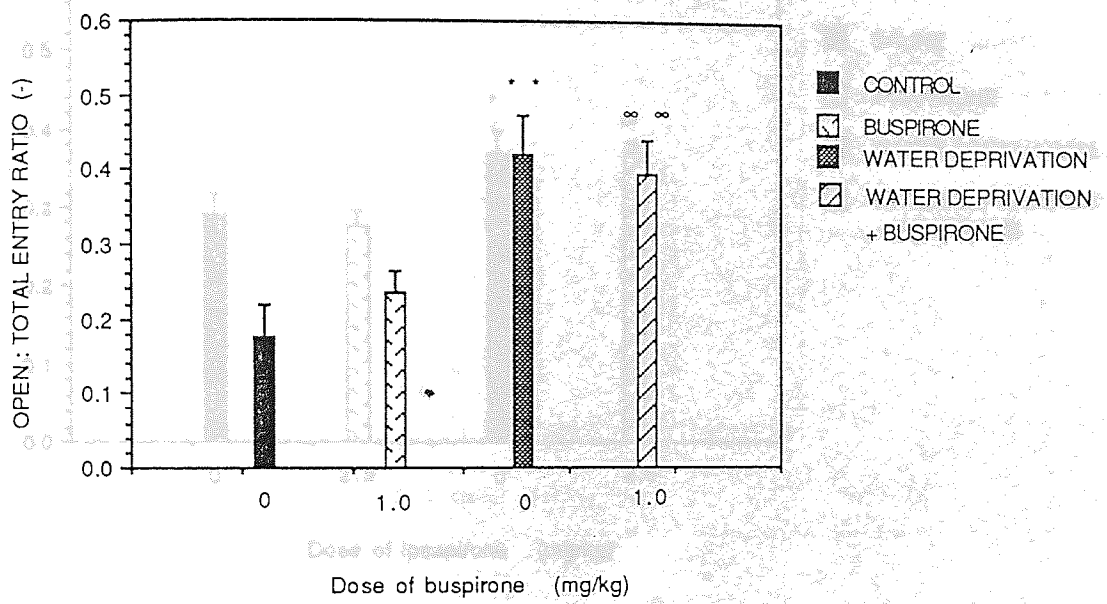


Table 6.5. The effect of 48 hour water deprivation and treatment with buspirone on behaviour on the X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Time on Open Arms (%)	Total Entries
Control	0.0	6	5.7 ± 1.8	14.2 ± 2.2
Buspirone	1.0	6	8.6 ± 2.1	19.8 ± 2.1
Water-deprived	0.0	6	29.4 ± 8.9**	19.0 ± 2.3
Buspirone	1.0	6	26.2 ± 7.3∞∞	18.2 ± 2.4

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA. ** P < 0.01 compared to non-water-deprived control; ∞∞ P < 0.01 compared to non-water-deprived buspirone.

Figure 6.6. The effect of Ipsapirone and 48 hour water deprivation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test

* P < 0.05 compared to control ∞ P < 0.05 compared to Ipsapirone group.

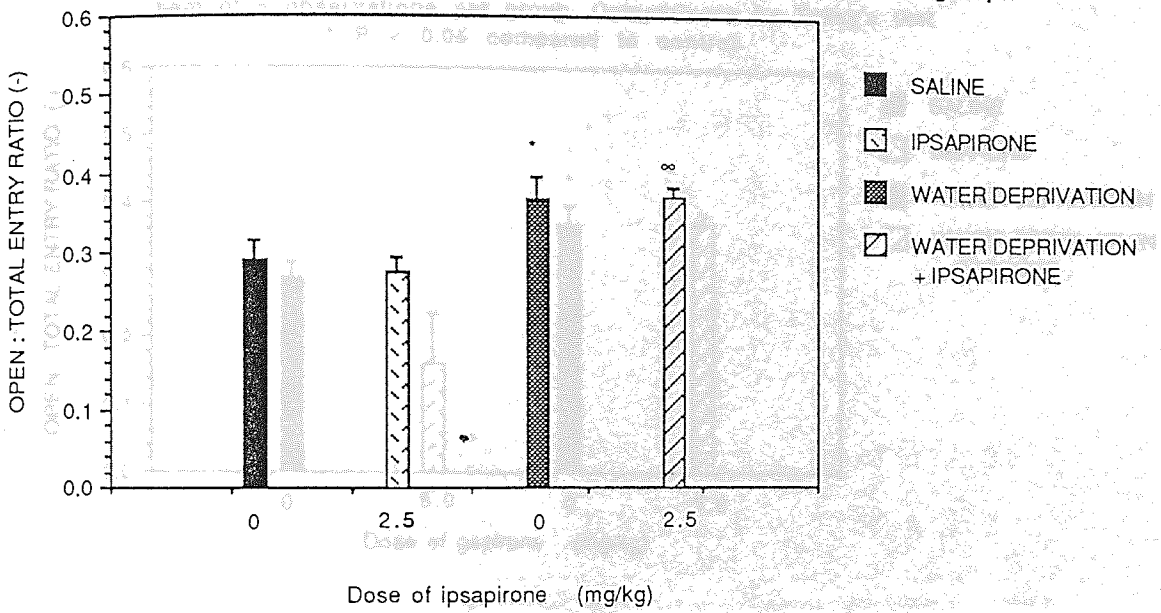


Table 6.6. The effect of 48 hour water deprivation and treatment with ipsapirone on behaviour on the X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Time on Open Arms (%)	Total Entries
Control	0.0	6	11.5 ± 3.1	18.5 ± 3.0
Ipsapirone	2.5	6	11.6 ± 2.0	22.3 ± 2.2
Water-deprived	0.0	6	19.4 ± 2.9*	18.2 ± 1.5
Ipsapirone	2.5	6	20.1 ± 2.0∞	19.3 ± 1.1

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA. * P < 0.05 compared to non-water-deprived control; ∞ P < 0.05 compared to non-water-deprived ipsapirone.

Figure 6.7. The effect of gepirone and 48 hour water deprivation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control.

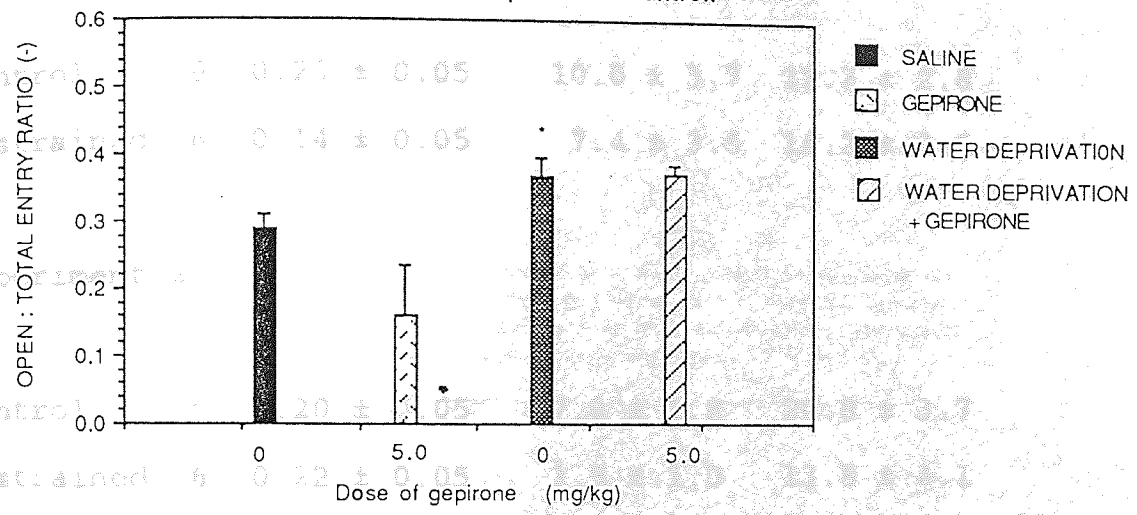


Table 6.7. The effect of 48 hour water deprivation and treatment with gepirone on behaviour on the X-maze.

TREATMENT DRUG	DOSE (mg/kg)	N	Time on Open Arms (%)	Total Entries
Control	0.0	6	12.5 ± 3.2	20.5 ± 4.0
Gepirone	5.0	6	8.7 ± 4.9	15.0 ± 3.2
Water-deprived	0.0	6	23.7 ± 3.2**	20.8 ± 1.3
Gepirone	5.0	6	12.5 ± 3.3	15.7 ± 1.9

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA. ** P < 0.01 compared to non-water-deprived control.

Table 6.8. The effect of 15 minutes restraint on behaviour on the elevated X-maze.

TREATMENT	N	OPEN:TOTAL RATIO (-)	TIME ON OPEN ARMS (%)	TOTAL ENTRIES
Experiment 1				
Control	6	0.20 ± 0.05	10.0 ± 3.7	19.2 ± 2.8
Restrained	6	0.14 ± 0.05	7.4 ± 3.5	16.2 ± 2.5
Experiment 2				
Control	6	0.20 ± 0.05	7.0 ± 1.8	20.8 ± 3.7
Restrained	6	0.22 ± 0.05	3.9 ± 1.0	13.8 ± 4.1

Comparisons were made using Student's unpaired t test.

Figure 6.8a. The effect of a 1 hour period of restraint on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test ** P < 0.01.

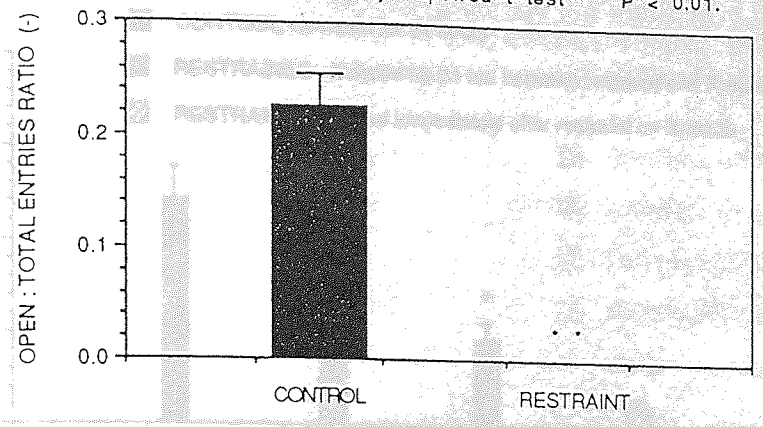


Figure 6.8b. The effect of a 1 hour period of restraint on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparison by unpaired t test ** P < 0.01.

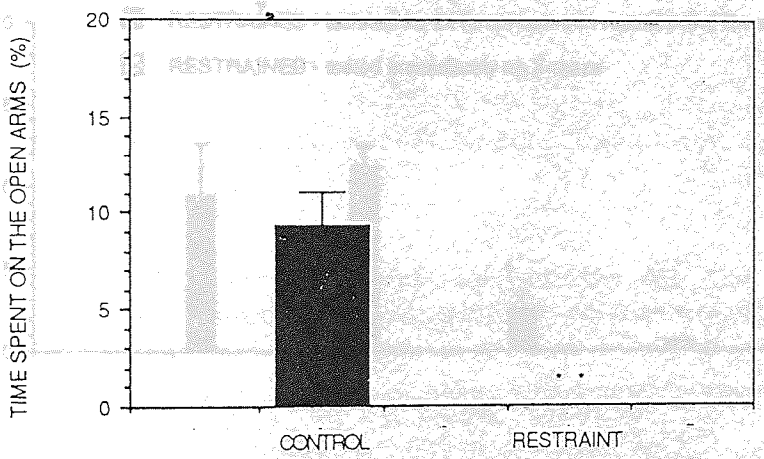


Table 6.9. The effect of 1 hour restraint on behaviour on the elevated X-maze.

TREATMENT	N	Total Entries
Control	6	26.0 ± 1.3
Restraint	6	3.0 ± 1.0**

Comparisons were made using Student's unpaired t test. ** P < 0.01 compared to control.

Figure 6.9.a. The effect of isolation, restraint and restraint with isolation on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 5 or 6 observations per group. Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control - group housed.

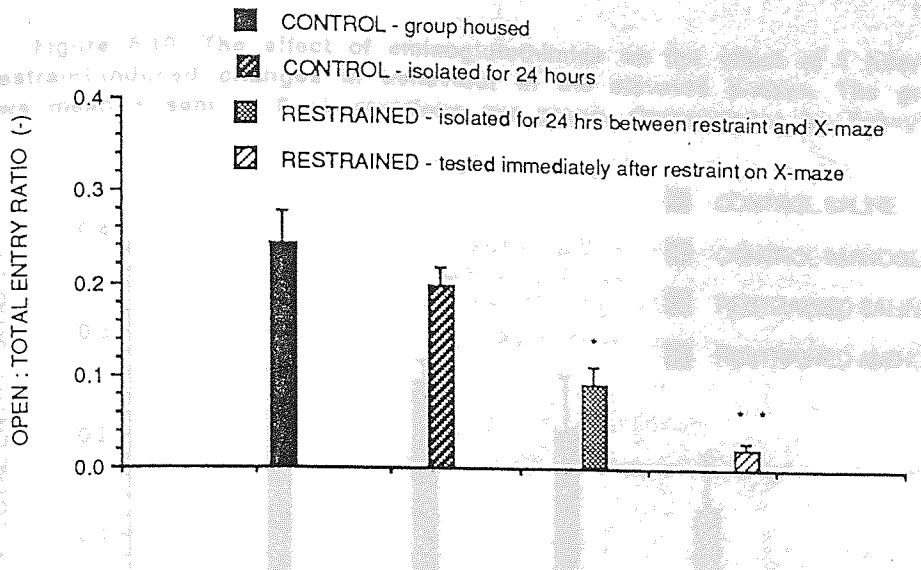


Figure 6.9b. The effect of isolation, restraint and restraint and isolation on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 5 or 6 observations per group. Comparisons by Dunnett's t test * P < 0.05 ** P < 0.01 compared to control - group housed

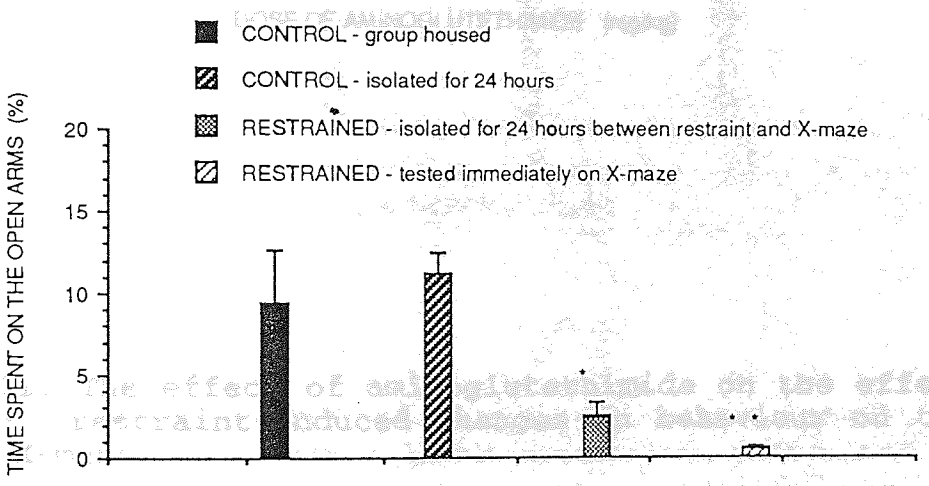


Table 6.10. The effect of isolation and 1 hour restraint tested immediately or 24 hour later on behaviour on the elevated X-maze.

TREATMENT	N	Total Entries
Control	6	18.3 ± 3.1
Isolation	6	22.3 ± 2.3
Restraint (immediately)	6	14.4 ± 3.2
Restraint + 24 hours Isolation	5	18.4 ± 2.5

Statistical comparisons were made by 1-way ANOVA.

Figure 6.10. The effect of aminoglutethimide on the effect of 1 hour restraint-induced changes in behaviour in the elevated X-maze. The graph shows mean ± sem of 8 observations per group. Comparisons by Tukey's test.

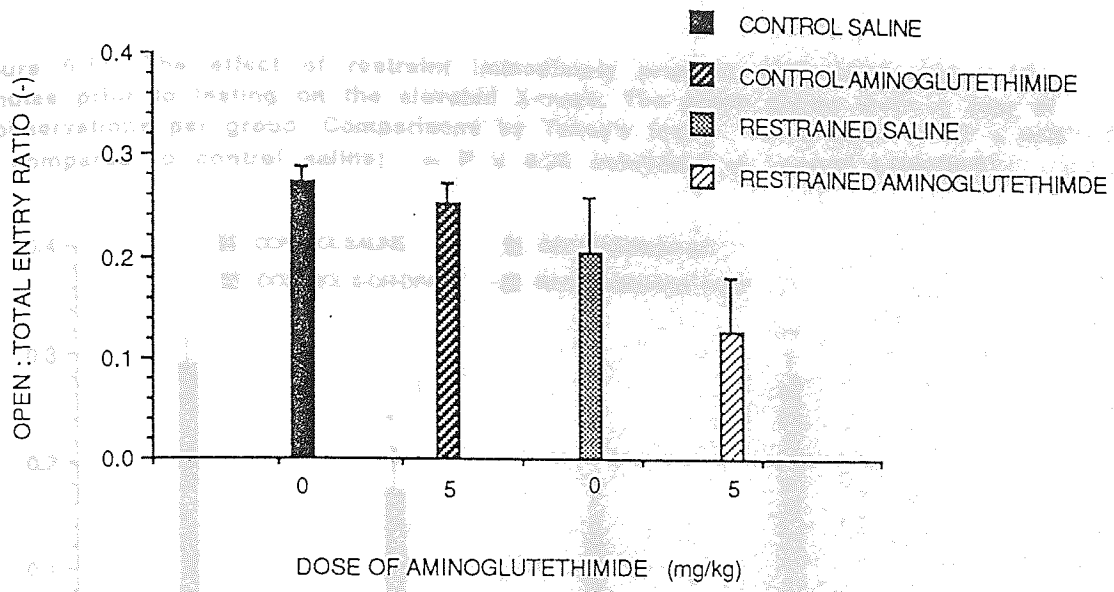


Table 6.11. The effect of aminoglutethimide on the effect of 1 hour restraint-induced changes in behaviour on the elevated X-maze.

TREATMENT	N	Time on Open Arms (%)	Total Entries
CONTROL			
Saline	8	11.1 ± 1.5	22.1 ± 0.5
5 mg/kg Aminoglutethimide	8	8.4 ± 1.9	19.0 ± 2.4
RESTRAINED			
Saline	8	3.3 ± 1.1**	8.5 ± 2.6**
5 mg/kg Aminoglutethimide	8	2.2 ± 0.9°°	8.7 ± 2.3°°

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA ** $P < 0.01$ compared to saline control. °° $P < 0.01$ compared to aminoglutethimide control.

Figure 6.11. The effect of restraint immediately prior to 8-OH-DPAT, given 10 minutes prior to testing on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 ** P < 0.01 compared to control saline; ∞ P < 0.05 compared to control 8-OH-DPAT.

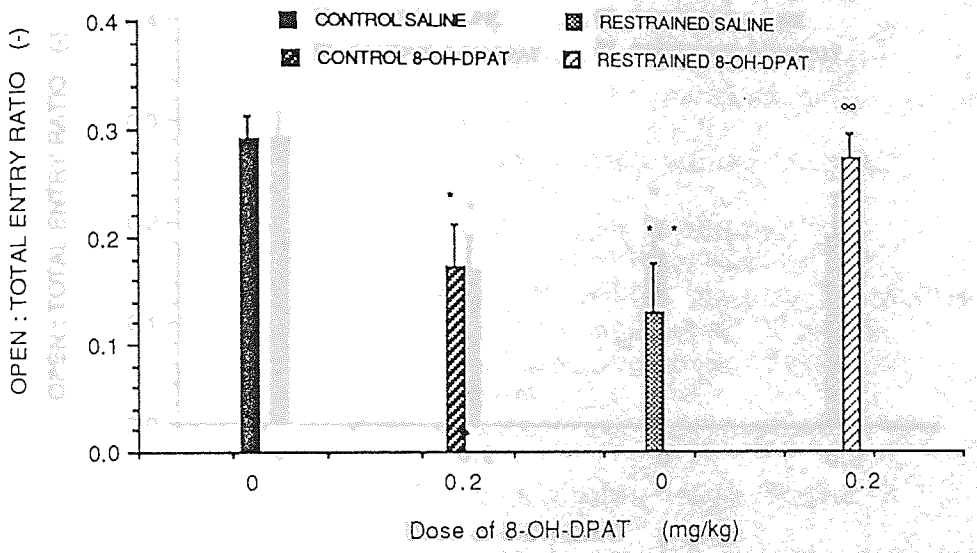


Table 6.12. The effect of restraint stress immediately prior to 8-OH-DPAT given 10 minutes prior to testing on the elevated X-maze.

TREATMENT	N	Time on Open Arms (%)	Total Entries
CONTROL			
Saline	6	11.2 ± 1.7	29.0 ± 3.7
0.2 mg/kg 8-OH-DPAT	6	7.4 ± 1.8	24.8 ± 2.5
RESTRAINED			
Saline	6	3.8 ± 1.9*	7.7 ± 2.7**
0.2 mg/kg 8-OH-DPAT	6	7.9 ± 2.1	13.2 ± 3.2∞

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA. * P < 0.05; ** P < 0.01 compared to saline control. ∞ P < 0.05 compared to control 8-OH-DPAT.

Figure 6.12. The effect of restraint 24 hours prior to 8-OH-DPAT, given 10 minutes prior to testing on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 compared to control saline.

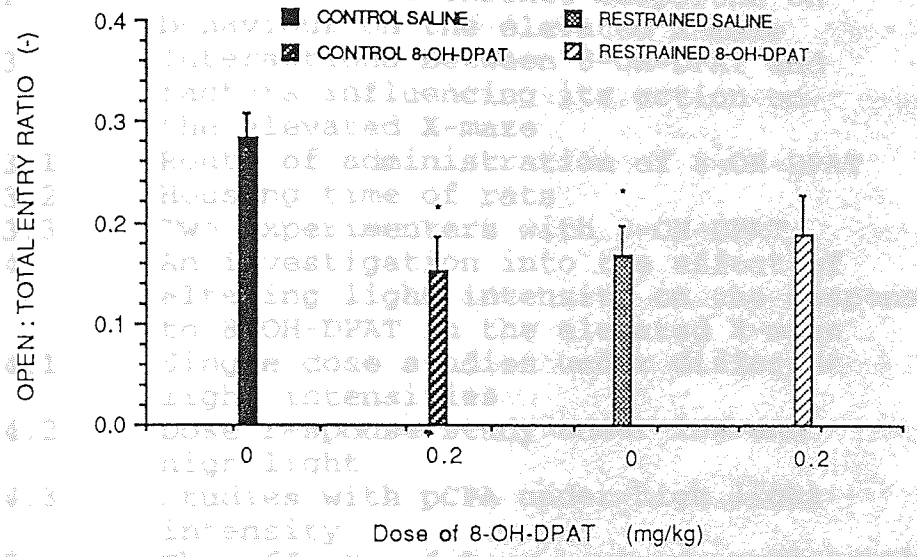


Table 6.13. The effect of 1 hour restraint stress 24 hours prior to 8-OH-DPAT given 10 minutes prior to testing on the elevated X-maze.

TREATMENT	N	Time on Open Arms (%)	Total Entries
CONTROL			
Saline	6	14.0 ± 2.5	31.5 ± 2.5
0.2 mg/kg 8-OH-DPAT	6	5.1 ± 1.7**	24.3 ± 3.6
RESTRAINED			
Saline	6	6.0 ± 1.8*	23.0 ± 2.8
0.2 mg/kg 8-OH-DPAT	6	8.1 ± 2.3	25.5 ± 1.6

Statistical comparisons were made using Tukey's test after a significant 2-way ANOVA * P < 0.05; ** P < 0.01 compared to saline control.

Chapter 7

An investigation of factors that may interact with drug response in the elevated X-maze model of anxiety.

Contents

7.1 Introduction.

At the start of the work conducted for this thesis there were good reasons to believe that it was possible to be able to detect differing responses to 5-HT_{1A} receptor ligands depending on the precise experimental conditions used and upon prior stressful experience. This has been discussed in the previous chapter. The work described in this chapter details some other sources of potential modulators of drug action, which for various reasons were thought to be able to interact with treatment with a 5-HT_{1A} receptor agonist. Most of the experiments were conducted with 8-OH-DPAT, because, as reflected in the previous two chapters, the 5-HT_{1A} partial agonists buspirone, ipsapirone and gepirone were not active in the elevated X-maze model under the operating conditions used.

There is a delay of two to three weeks in the therapeutic action of the 5-HT_{1A} receptor agonists in the treatment of anxiety and depression (Goa and Ward, 1986; Feighner and Boyer, 1989). There are very few reports of the effect of the long term administration of 5-HT_{1A} receptor ligands in behavioural models of anxiety or depression despite this being necessary for their clinical anxiolytic or antidepressant effect and therefore this is a clear source of potential disparity between the preclinical pharmacology and therapeutic action of this group of compounds. Moser (1989) reported that the chronic administration of buspirone did not change the response to acutely

administered buspirone in the elevated X-maze. However, Moser usually describes an "anxiogenic" effect of buspirone (Moser, 1989; Moser et al., 1990), but this acute "anxiogenic" effect was not detected in the control study for the chronic experiment reported by Moser (1989). Operating from a standard no response to buspirone, it was thought that chronic treatment with buspirone might produce an "anxiolytic" profile consistent with the delay in its clinical effect.

The brain concentration of 8-OH-DPAT after a single dose is dependent upon the route of administration (Perry and Fuller, 1989). This concentration is approximately tenfold higher following a subcutaneous than an intraperitoneal injection, largely because of extensive metabolism on passage through the liver (Perry and Fuller, 1989). Söderpalm et al. (1989) have attributed the variability of the 5-HT_{1A} receptor ligands in the elevated X-maze to a complex dose-response relationship, in which low doses act preferentially at presynaptic 5-HT autoreceptors to decrease 5-HT release and thus produce an "anxiolytic" effect, whereas at higher doses a postsynaptic action prevails and causes an "anxiogenic" response. This gives grounds to believe that the same dose of 8-OH-DPAT might have opposite effects in the elevated X-maze as a function of the route of administration of the compound. This possibility was tested experimentally using both routes and two doses of the drug.

Transport of animals from a supplier to the animal

facilities of the institution where the work is carried out is probably a major stressful event. The time allowed for recovery from this stress may be a critical factor in determining how an animal responds in any test to which it is subjected. Given the association between stress and the serotonergic system, of which some indication has been given previously, the interval between the delivery and the use of the animal in the elevated X-maze was perceived to be a potential source of the variability in drug response and this possibility was directly tested.

The possibility that a different response to treatment with 8-OH-DPAT was dependent upon the person conducting the experiment was also assessed. A comparison between many of the control open : total entry ratios with those of an immediate predecessor (Njung'e, 1989) clearly indicate that those obtained by the present author are appreciably lower than those obtained previously. This may reflect a difference in the strain of rat used (PVG Hooded v Wistar), but it is possible that the difference lies in the handling of the animal immediately prior to being placed on the maze. The effect of two experimenters was assessed on the response to 8-OH-DPAT on the maze.

The behaviour of rodents in models of anxiety is known to be influenced by changes in light intensity (Crawley et al., 1981; File and Peet, 1980). Benjamin et al. (1990) reported that light intensity was able to modulate the behaviour of control animals in an elevated X-maze adapted for use in the mouse and Morato and Castrechini (1989) have

reported that approximately tenfold changes in light intensity influence the behaviour of rats in the X-maze. A chance observation during the course of experiments with 8-OH-DPAT suggested that, while attempting to establish an "anxiogenic" action of 8-OH-DPAT, on one particular day the drug produced an "anxiolytic" effect - on this day three out of four lights in the experimental room were deliberately switched off, but for another purpose. The possibility that light intensity may be able to switch the action of 8-OH-DPAT was also investigated.

There have been no investigations reported with the elevated X-maze which use test periods longer than 10 minutes. Some authors use the same individual rats repeatedly on the maze; indeed previous students in this laboratory have routinely re-used animals at weekly intervals. A loss of the "anxiolytic" response to benzodiazepines has been shown to occur on a second exposure to the maze 24 hours after an initial short (5 minutes) exposure (File, 1990). A possible explanation for the loss of "anxiolytic" response to benzodiazepine treatment is the development of a phobic response directed against the open arms of the maze.

The behavioural treatment of phobias, as opposed to their pharmacological treatment, usually follows one of two mutually exclusive lines. One method encourages a bit by bit, one step at a time approach by which the phobic individual gradually develops confidence in their ability to develop an appropriate response to the phobic stimulus

as a result of building up experience in coping with presentation of the phobic stimulus. The second approach is to flood the individual with the phobic stimulus - expose them to such an intensely fearful presentation regarding the phobic stimulus, that on a subsequent confrontation with the phobic stimulus, the phobic reaction is overcome. The final series of experiments adopted the second of these approaches using exposure to the whole of the elevated X-maze as the phobic stimulus. The effect of a long exposure to the elevated X-maze was investigated on behaviour both within the duration of the trial and on the response to a subsequent exposure to the maze. The effect of a previous long term exposure on the response to 8-OH-DPAT was also investigated.

7.2 The effect of chronic buspirone on behaviour on the elevated X-maze model.

Figure 7.1a shows that the rate of weight gain was no different between the group given buspirone in the drinking water and that given tap water. Buspirone-treated rats hardly drank in the first 24 hours (Figure 7.1b) and as a consequence did not gain weight over this period. The graph shows that this small difference in body weight was never eliminated. The fluid intake of rats given buspirone in the drinking water was no different to rats given normal tap water apart from the first day and is shown in Figure 7.1b. Acutely administered buspirone at 1 mg/kg given ip 30 minutes prior to exposure to the maze did not significantly influence the open : total entry ratio ($F_{(1,20)}$ = 1.24; P > 0.05) (Figure 7.2a), the time spent on the open arms ($F_{(1,20)}$ = 0.12; P > 0.05) (Figure 7.2b) or the number of entries ($F_{(1,20)}$ = 0.012; P > 0.05) (Table 7.1). Chronic buspirone treatment was also without effect on the open : total entry ratio ($F_{(1,20)}$ = 0.64; P > 0.05), the time spent on the open arms ($F_{(1,20)}$ = 0.02; P > 0.05) or total entries ($F_{(1,20)}$ = 0.29; P > 0.05). Neither was there any differences in X-maze behaviour in rats receiving a saline injection after the chronic administration of buspirone (Figure 7.2; Table 7.1).

7.3 Interactions between 8-OH-DPAT and factors that may influence its action on the elevated X-maze.

7.3.1 The effect of route of administration of 8-OH-DPAT.

8-OH-DPAT, at a dose of 0.2 mg/kg, was administered either intraperitoneally or subcutaneously to rats 10 minutes before exposure to the maze. At this dose four of the subcutaneously treated animals fell off the maze, and therefore the experiment was discontinued.

This procedure was repeated using 0.1 mg/kg 8-OH-DPAT. By both routes, the drug had a significant main effect on both the open : total entry ratio [$F_{(1,20)} = 47.50$; $P < 0.01$] and on open arm time [$F_{(1,20)} = 37.36$; $P < 0.01$]. Post hoc tests confirmed that 8-OH-DPAT significantly reduced the open : total entry ratio (Figure 7.3a) and the time spent on the open arms (Figure 7.3b) compared to their saline control group. There was also a main effect of route of administration on the open : total entry ratio (Figure 7.3a)[$F_{(1,20)} = 7.11$; $P < 0.05$] and on the time on the open arms (Figure 7.3b)[$F_{(1,20)} = 9.57$; $P < 0.01$] with post hoc tests confirming that the entry ratio and the time on the open arms were significantly reduced in rats injected sc compared to those receiving ip injection (Figures 7.3a and 7.3b). Total entries remained unchanged in response to either manipulation (Table 7.2).

7.3.2 The effect of housing time of rats.

The effects of 8-OH-DPAT in the X-maze were compared in animals that had been kept in the animal house for either one or three weeks. Rats kept for three weeks were significantly heavier than those kept for one week (Table 7.3). There was a significant effect of drug treatment on both open : total arm entry ratio [$F_{(1,20)} = 38.09$; $P < 0.01$] and on open arm time [$F_{(1,20)} = 24.13$; $P < 0.01$]. Post hoc testing confirmed that 8-OH-DPAT, at 0.2 mg/kg and given ip 10 minutes before exposure to the maze, significantly reduced the open : total entry ratio in both rats that had been housed for one week and those housed for three weeks (Figure 7.4a). 8-OH-DPAT also significantly reduced the time spent on the open arms in both groups of rats (Figure 7.4b), but did not influence the number of arm entries made by either group of rats (Table 7.4). There was no effect of housing time on either the open : total entry ratio [$F_{(1,20)} = 0.04$; $P > 0.05$] or the open arm time [$F_{(1,20)} = 2.73$; $P > 0.05$]. Neither the open : total arm entry ratio (Figure 7.4a) nor the time spent on the open arms (Figure 7.4b) differed between the group of rats housed for one week compared to those housed for three weeks. However, there was a significant main effect of housing time on the total number of arm entries [$F_{(1,20)} = 7.03$; $P < 0.05$]. Rats held for three weeks and given 8-OH-DPAT made significantly fewer total arm entries than those kept for one week (Table 7.4).

7.3.3 The effect of two different experimenters.

In this experiment, a single batch of rats was randomly divided into two and handled for 3 days prior to the test day only by the experimenter assigned to that group. 8-OH-DPAT was administered i.p. at a dose of 0.2 mg/kg 10 minutes prior to exposure to the maze by the two separate experimenters. Neither 8-OH-DPAT nor the experimenter produced any change in the open : total entry ratio ($F_{(1,20)}$ = 3.24; P > 0.05 [8-OH-DPAT] $F_{(1,20)}$ = 0.02; P > 0.05 [experimenter] or the time spent on the open arms ($F_{(1,20)}$ = 2.58; P > 0.05 [8-OH-DPAT] $F_{(1,20)}$ = 0.08; P > 0.05 [experimenter] (Table 7.5). 8-OH-DPAT did not influence the total number of arm entries made ($F_{(1,20)}$ = 2.69; P > 0.05), but there was an effect of experimenter in the number of total entries, although this was only apparent in saline-treated animals ($F_{(1,20)}$ = 5.02; P < 0.05; Table 7.5).

7.4 An investigation into the effect of altering light intensity on the response to 8-OH-DPAT in the elevated X-maze.

7.4.1 Single dose studies under different light intensities.

Experiment 1 - complete darkness and low light.

Administration of 8-OH-DPAT at a dose of 0.2 mg/kg in either complete darkness or at a light intensity of 170 lux resulted in both cases in a significant reduction of the open : total entry ratio ($F_{(1,20)}$ = 28.88; P < 0.01) (Figure 7.5a) and a significant reduction in the time spent on the open arms ($F_{(1,20)}$ = 41.28; P < 0.01) (Figure 7.5b) without changing the total number of arm entries ($F_{(1,20)}$ = 1.40; P > 0.05) (Table 7.6). In complete darkness, saline treated animals spent more time on the open arms than at 170 lux ($F_{(1,20)}$ = 6.18; P < 0.05) (Figure 7.5b) and also made a greater number of arm entries ($F_{(1,20)}$ = 14.38; P < 0.01) (Table 7.6), although there was no main effect of light intensity on the open : total arm entry ratio ($F_{(1,20)}$ = 0.05; P > 0.05) (Figure 7.5a).

Experiment 2 - low light and moderate light.

There was a significant interaction between treatment with 8-OH-DPAT and light intensity on the open : total entry

ratio critierion ($F_{(1,20)} = 4.37$; $P < 0.05$), although not on the open arm time criterion ($F_{(1,20)} = 3.61$; $P > 0.05$). Post hoc comparisons suggested that under a light intensity of 172 lux and at the dose of 0.2 mg/kg, 8-OH-DPAT reduced both the open : total entry ratio (Figure 7.6a) and the time spent on the open arms (Figure 7.6b). When the same dose was given under 211 lux, it did not influence either the open : total entry ratio (Figure 7.6a) or the time spent on the open arms (Figure 7.6b). Treatment with 8-OH-DPAT had a significant main effect on the total number of arm entries ($F_{(1,20)} = 5.69$; $P < 0.05$), but when the individual drug treated groups were compared against their control group, this effect was not statistically significant (Table 7.7). Light intensity did not influence the open : total entry ratio (Tukey's test, $P > 0.05$), the time spent on the open arms ($F_{(1,20)} = 0.96$; $P > 0.05$) or the total number of arm entries ($F_{(1,20)} = 0.04$; $P > 0.05$).

Experiment 3 - low light and high light.

In a third experiment, there was a significant interaction between light intensity and treatment with 8-OH-DPAT which was significant for both the open : total entry ratio criterion ($F_{(1,20)} = 21.52$; $P < 0.01$) and the open arm time criterion ($F_{(1,20)} = 4.83$; $P < 0.05$). 0.2 mg/kg 8-OH-DPAT at a light intensity of 170 lux again significantly reduced the open : total entry ratio (Tukey's test, $P < 0.01$) (Figure 7.7a) although the reduction in the time spent on

the open arms was not significant (Figure 7.7b). When tested under bright light (785 lux), 0.2 mg/kg 8-OH-DPAT significantly increased the open : total entry ratio (Tukey's test, $P < 0.01$) (Figure 7.7a) and increased the time spent on the open arms (Figure 7.7b), although this last increase was not quite significant. There was no effect of 8-OH-DPAT ($F_{(1,20)} = 0.13$; $P > 0.05$) or of light intensity ($F_{(1,20)} = 0.29$; $P > 0.05$) on the total number of arm entries made in this experiment (Table 7.8). Increasing the light intensity significantly decreased the open : total entry ratio of saline treated rats (Tukey's test, $P < 0.05$) (Figure 7.7a), but did not alter the amount of time spent on the open arms (Tukey's test, $P > 0.05$) (Figure 7.7b).

7.4.2 Dose response studies under low and high light intensity.

There was a significant interaction between 8-OH-DPAT treatment and light intensity on the open : total entry ratio ($F_{(4,50)} = 24.52$; $P < 0.01$) but not on the time spent on the open arms ($F_{(4,50)} = 3.72$; $P > 0.05$) or the total entries ($F_{(4,50)} = 0.55$). Under light intensity of 170 lux, 8-OH-DPAT over the dose range 0.025 - 0.2 mg/kg produced a significant reduction in the open : total entry ratio at the highest dose used (Tukey's test, $P < 0.05$) (Figure 7.8a) and tended to reduce the time spent on the open arms, although this was not significant ($F_{(4,50)} = 1.37$; $P > 0.05$)

(Figure 7.8b). In contrast, under the higher light intensity of 785 lux, 8-OH-DPAT over the same dose range significantly increased the open : total entry ratio at the two highest doses (Tukey's test, $P < 0.01$) (Figure 7.8a) and increased the time spent on the open arms (Tukey's test, $P < 0.01$) (Figure 7.8b). In neither case did 8-OH-DPAT influence the number of arm entries made ($F_{(4,50)} = 0.79$; $P > 0.05$) (Table 7.7).

In saline treated rats, there were no differences between the two light intensities in the open : total entry ratio (Tukey's test, $P > 0.05$) (Figure 7.8a), the time spent on the open arms (Tukey's test, $P > 0.05$) (Figure 7.8b), or the total number of entries (Tukey's test, $P > 0.05$) (Table 7.7).

7.4.3 Studies with pCPA under high light intensity (785 lux).

There were significant interactions between treatment with 8-OH-DPAT and pCPA in both experiments on the open : total entry ratio ($F_{(1,20)} = 6.58$; $P < 0.05$ [0.1] and $F_{(1,20)} = 20.71$; $P < 0.01$ [0.2]) and the time spent on the open arms ($F_{(1,20)} = 20.69$; $P < 0.01$ [0.1] and $F_{(1,20)} = 7.56$; $P < 0.05$ [0.2]). Post hoc specific group comparisons suggested that in rats given saline injections 72, 48 and 24 hours prior to testing, 8-OH-DPAT, in doses of 0.1 and 0.2 mg/kg, produced an increase in the open : total entry ratio (Figures 7.9a and 7.10a) and in the time spent on the open

arms (Figures 7.9b and 7.10b). pCPA, given at a dose of 300 mg/kg 72, 48 and 24 hours before exposure to the maze, also significantly increased the open : total entry ratio (Figures 7.9a and 7.10a) but the increases in the time spent on the open arms seen after pCPA (Figures 7.9b and 7.10b) were not significant. In rats pretreated with pCPA (300 mg/kg 72, 48 and 24 hours before testing), 8-OH-DPAT at 0.1 and 0.2 mg/kg reduced the open : total entry ratio (Figures 7.9a and 7.10a) and the time spent on the open arms (Figures 7.9b and 7.10b) compared to the pCPA saline group, although this decrease did not extend below the open : total entry ratio and open arm time of saline control treated rats. 8-OH-DPAT reduced the total number of entries ($F_{(1,20)}$ = 5.02; P < 0.05 [0.1] and $F_{(1,20)}$ = 4.59; P < 0.05 [0.2]), as did pCPA ($F_{(1,20)}$ = 53.78; : < 0.01 [0.1] and $F_{(1,20)}$ = 12.71; P < 0.01) (Tables 7.10 and 7.11).

7.5. The effects of long term exposure to the elevated X-maze.

7.5.1 A comparison of behaviour within a one hour exposure to the elevated X-maze.

In this experiment, 12 rats were exposed to the elevated X-maze for a period of one hour. The total number of arm entries made during consecutive 10 minute segments for the course of a one hour exposure to the maze showed a

reduction during the course of the exposure. Statistical analysis indicated that there was a significant reduction for all segments from the 10 - 20 minute segment onwards, compared to the initial 10 minutes (Figure 7.11a).

The time spent on the open arms of the maze was also significantly reduced in all 10 minute segments after the first 10 minute segment, when compared to that first 10 minute segment (Figure 7.11b).

7.5.2 The effect of a one hour exposure on behaviour on the elevated X-maze 24 hours later.

6 rats were left on the elevated X-maze for one hour on the first day. 24 hours after the end of the 1 hour session, rats were replaced on the maze for 10 minutes and their behaviour was compared to the first 10 minutes of their first exposure, on the previous day. Another group of rats was exposed to the maze only on the second day, for 10 minutes.

The mean open : total entry ratio of re-exposed rats was significantly less on the second day compared to the first day (Figure 7.12a), as was the time spent on the open arms (Figure 7.12b) and the total number of entries (Figure 7.12c). These measures on the second day also appeared to be less than the corresponding measures taken from animals exposed for the first time to the maze on the second day (Figures 7.12a, 7.12b and 7.12c).

7.5.3 The effect of a one hour exposure on the response to 8-OH-DPAT in the elevated X-maze 24 hours later.

The 12 animals whose behaviour was described in section 7.5.1 were re-exposed to the maze on day 2 for 10 minutes, 24 hours after the end of the first exposure, which was of a 1 hour duration. 6 were replaced on the maze 10 minutes after a saline injection and 6 were replaced on the maze 10 minutes after an injection of 0.2 mg/kg 8-OH-DPAT. In addition, rats that had not been pre-exposed to the maze before also received either saline or 8-OH-DPAT at a dose of 0.2 mg/kg.

In the group of rats not previously exposed to the maze, 8-OH-DPAT produced its expected "anxiogenic" effect. There was a reduction in the open : total entry ratio (Figure 7.13a) and no change in the total number of arm entries made (Figure 7.13b).

In the group of rats that were re-exposed to the maze, the mean open : total entry ratio after treatment with saline was significantly lower on the second exposure compared to the first 10 minutes of the 1 hour exposure the previous day (Figure 7.13c), as was the mean total number of arm entries (Figure 7.13d). In these animals, analysis of the individual scores indicated a marginal trend towards a reduction in the open : total ratio (Figure 7.13e) and a clearer reduction in total entries (Figure 7.13g).

In the group of rats that had been pre-exposed to the maze 8-OH-DPAT apparently produced a dichotomous reaction. Of

the 6 animals in the group, three animals made 0 open arm entries and thus had open : total entry ratios of 0, whereas the remaining three had open : total entry ratios that were higher than the open : total entry ratio for the same rat on the previous day, and in addition were higher than the open : total entry ratios of any of the pre-exposed saline-treated rats on the second day (Figure 7.13c). The individual values which make up this group are depicted in Figure 7.13f. 8-OH-DPAT significantly increased the mean total number of arm entries made by rats pre-exposed to the X-maze compared to those receiving saline that had also been pre-exposed (Figure 7.13d). Figure 7.13h shows that the response of individual rats to treatment with 8-OH-DPAT on the total entries made on the maze was not uniform.

7.6 Discussion.

Chronic treatment with buspirone did not influence responses to a saline injection or an acute challenge with buspirone on the elevated X-maze, an effect previously reported (Moser et al., 1989). Chronic treatment with another 5-HT_{1A} receptor ligand, ipsapirone, has also been shown not to change elevated X-maze behaviour (Wright et al., 1992). The reason for the lack of "anxiolytic" activity on chronic dosing is not clear. It may be that the therapeutic action develops with chronic treatment in humans as a result of the interaction between chronic treatment and the presence of anxiety. The analogous model in animals would be to impose repeated exposures to the elevated X-maze in the presence of continued treatment with the 5-HT_{1A} ligand.

Neither the route of administration nor the housing time of rats changed the direction of response to 8-OH-DPAT and therefore the expectations that these variables were responsible for the disparity in the published reports were not substantiated. With regard to the finding with the route of administration, it is quite possible that the dose used was already too high to detect a low dose "anxiolytic" response in line with the conclusions made by Söderpalm et al. (1989). However, a comparison across the published literature indicate that "high" doses, in the terms of Söderpalm et al. (1989), are not particularly associated with "anxiogenic" responses to 5-HT_{1A} ligands (eg Dunn et

al., 1989; Pellow et al., 1987).

One finding which arose from the experiment investigating the influence of route of administration on behaviour on the X-maze was that subcutaneous administration was associated with lower open : total entry ratios and time on the open arms, irrespective of drug treatment. Whether this reflects a real difference in the stressful nature of the two different routes of injection is not possible to say. This may reflect the fact that most of the experiments conducted to that point were with intraperitoneal injetion and thus I was more familiar with this route than the subcutaneous route.

There was no effect of housing time on the "anxiety" - related measures on the elevated X-maze, but rats that had been housed longer were less active.

The failure to detect any effects of either drug treatment or of experimenter in the final experiment in this section demonstrates a common finding in behavioural experiments, namely that there are occasions when experiments do not work for reasons which cannot be identified.

Empirical experimental evidence suggested that light intensity was able to influence the effect of 8-OH-DPAT in the elevated X-maze, at least in the albino strain. Under low to moderate light intensities, 8-OH-DPAT had an "anxiogenic"-like effect on both the open : total entry ratio and open arm time criteria. There was some variation in the control open : total entry ratio under the standard 170 lux light intensity, but it is possible to attribute

some of these differences to the fact that experiments were done at both Aston University and at the Wellcome Foundation. Despite these baseline variations, 8-OH-DPAT produced "anxiogenic" effects in each case. The response to 8-OH-DPAT changed depending upon the light intensity, but this change was not due to altering the baseline response. Thus, an "anxiolytic" effect of 8-OH-DPAT arose in the third single dose experiment (785 lux) when the baseline, indicated by the saline-treated group, was sufficient to be able to detect an "anxiogenic" effect, found in the second of these experiments, done at 172 lux.

As the finding that light intensity was able to govern the response to treatment with a 5-HT_{1A} receptor ligand seemed rather improbable, an experiment was devised to conduct a dose-response study under conditions where "anxiogenic" responses were anticipated and where "anxiolytic" responses were anticipated. This study confirmed that at low light intensities, 8-OH-DPAT had a dose-dependent "anxiogenic" effect, whereas at high light intensity there was a dose-related "anxiolytic" effect. These changes were again independent of changes in total arm entries made.

At this stage it seemed that light intensity had been identified as being a factor able to modulate the response to 8-OH-DPAT and furthermore, this could explain why it had been difficult to establish any effect of 8-OH-DPAT in the initial stages of experiments for this thesis. There are no reports in the literature to the effect that light intensity is able to interact with 5-HT_{1A} receptor ligands

in the elevated X-maze. Luscombe et al. have reported "anxiolytic" effects of several 5-HT_{1A} receptors ligands, including 8-OH-DPAT, in the elevated X-maze (Luscombe et al., 1992) but this group uses low light intensities. Therefore this finding is not able to be fully integrated with the opposing reports of "anxiolytic" and "anxiogenic" effects from 5-HT_{1A} ligands in the elevated X-maze in the elevated X-maze. However, the effect seemed to be real and further investigations were warranted. It was possible at this stage to look at whether this effect was found with other 5-HT_{1A} receptor ligands, or to investigate the mechanism of the effect which was produced by 8-OH-DPAT. The latter route was chosen because there was a positive effect to be manipulated, thus bearing a greater liklihood of positive results, given imposed time constraints.

pCPA pretreatment, consistent with previous reports (Critchley et al., 1992), had an "anxiolytic" effect in the elevated X-maze. This effect was tainted by the fact that pCPA pretreatment also decreased total entries. pCPA abolished the "anxiolytic" effect of 8-OH-DPAT under high light intensities and 8-OH-DPAT reduced the open : total entry ratio in pCPA pretreated rats, indicating that the "anxiolytic" effect may originate from a presynaptic mechanism. The "anxiogenic" effect of 8-OH-DPAT in the elevated X-maze is also mediated by presynaptic means, being abolished by pCPA lesions and by 5,7-DHT lesions of the dorsal Raphé nucleus (Critchley et al., 1992).

Together, these results indictate that agonist action at

presynaptic $5\text{-}HT_{1A}$ receptors may be able to give rise to both "anxiolytic" and "anxiogenic" effects in the X-maze. The precise mechanism of how light intensity influences the response to 8-OH-DPAT was not elucidated by the present set of experiments. There is some disagreement regarding the use of photopic versus scotopic measurements when conducting experiments with rats. Photopic (cone) vision is used under conditions of bright light, whereas scotopic (rod) vision is predominant in dim lighting. As rats are nocturnal animals, it is not surprising that their visual system is geared towards scotopic vision. However, although the rat eye is predominantly possessed of rods (Dowling, 1963), and is thus scotopically sensitive, the rat does possess functional vision within the photopic range (Messing, 1972). Behavioural studies have indicated that albino rats can make fine discriminations in illumination over the photopic range (Messing, 1972) and so the use of a photopic measurement in the present study seems justified. Additionally, increasing light intensity did not alter the behaviour of control animals within any single experiment, which implies that vision was not differentially impaired under the higher light intensity with respect to the lower light intensity.

There is evidence that lighting conditions influence the activity of serotonergic neurones in the aneasthetized rat (Mosko and Jacobs, 1974). There was a tendency towards there being an increase in firing rates in light conditions compared to dark conditions. Alternatively, the 5-HT

turnover rate is thought to be higher in the dark phase of a 24 hour cycle, corresponding with the period of greater general activity (Hutson et al., 1985). Whether these two findings are of relevance to the present results indicating that acute changes in lighting conditions influences the effect of 8-OH-DPAT in the elevated X-maze, is a matter for conjecture at the present time.

During the course of a 1 hour exposure to the elevated X-maze rats made progressively less exploration. Most animals eventually went to sleep during this exposure, although the time that this occurred was different between different individuals. 24 hours after a 1 hour exposure, rats showed behavioural changes that indicated that there was reduced exploration. There were fewer arm entries than for both the first 10 minutes of X-maze exposure, and for rats tested for the first time on the second day. The open : total entry ratio was also significantly reduced.

Habituation might be expected to have two different aspects: habituation to the novelty of the maze might result in reduced curiosity leading to a reduced total exploration. Habituation to the aversive nature of the maze might be expected to result in an increase in the open : total entry ratio. In fact a reduction in this measure was apparent. This interpretation might suggest that there was habituation to curiosity evoked by the maze, but that aversion to the maze increased, indicating that different features of the maze produce different adaptive responses. The significance of the apparent dichotomous reaction to 8-

OH-DPAT in rats that had been previously exposed to the maze is not clear. Exposure to the maze might be affecting the animals in the same way as a stressor which may influence the subsequent action of a 5-HT_{1A} receptor agonist (Kennett et al., 1987; Carli et al., 1989). This could give rise to the "anxiolytic" response to 8-OH-DPAT in three animals, but does not explain why there were both "anxiolytic" and "anxiogenic" responses to 8-OH-DPAT in animals that had received the same treatment.

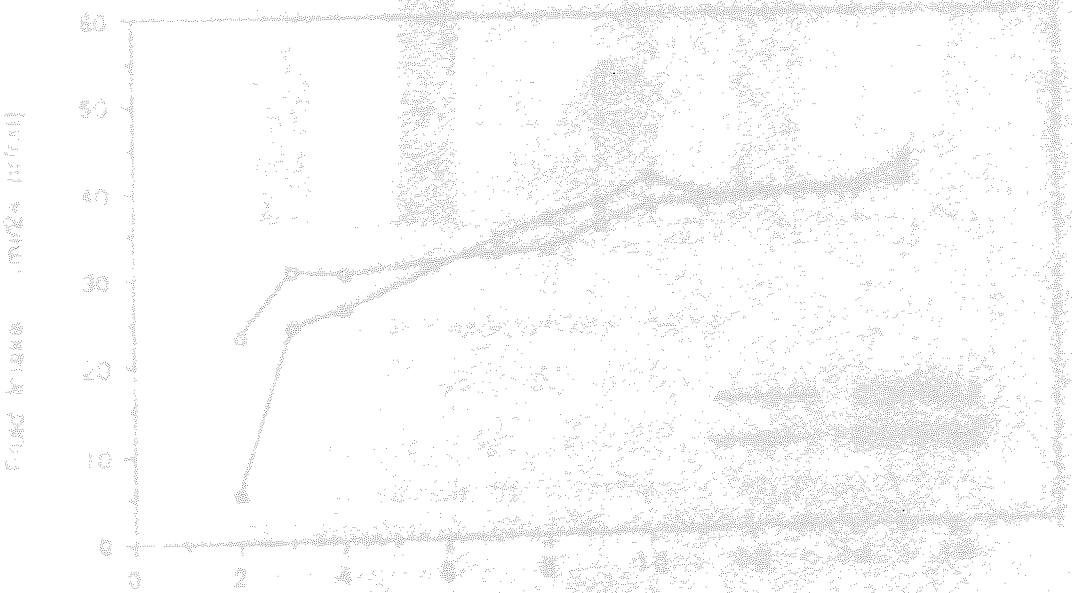


Figure 7.1a. The increase in body weight of rats given buspirone in the drinking water. The graph shows mean + sem of 12 rats per group.

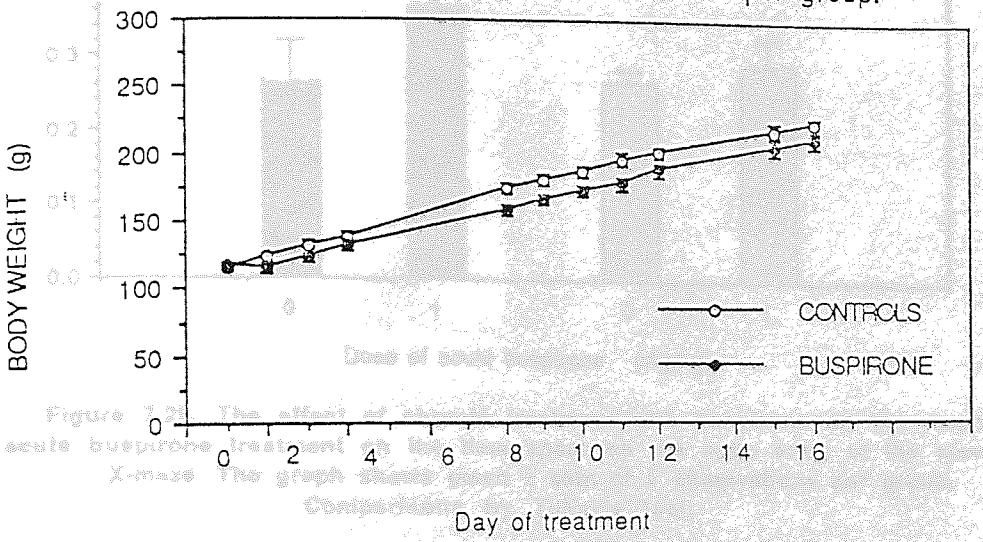


Figure 7.1b. The fluid intake of rats given buspirone in the drinking water or normal tap water. The graph shows mean daily fluid intake from 12 rats per group.

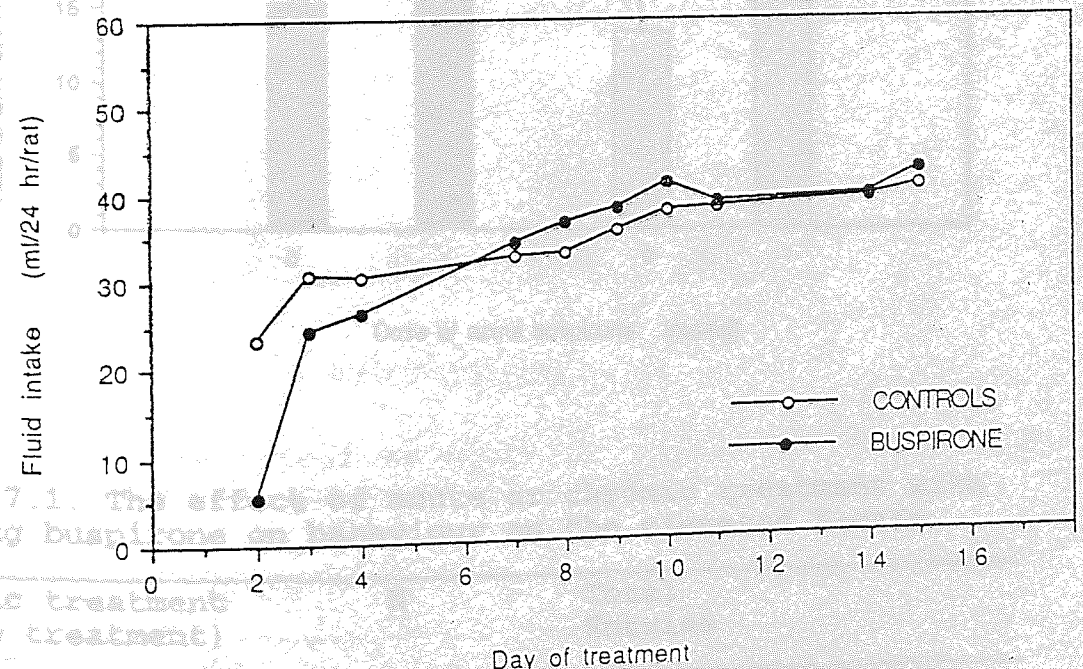


Figure 7.2a. The effect of chronic treatment with buspirone on the response to acute treatment with buspirone on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test.

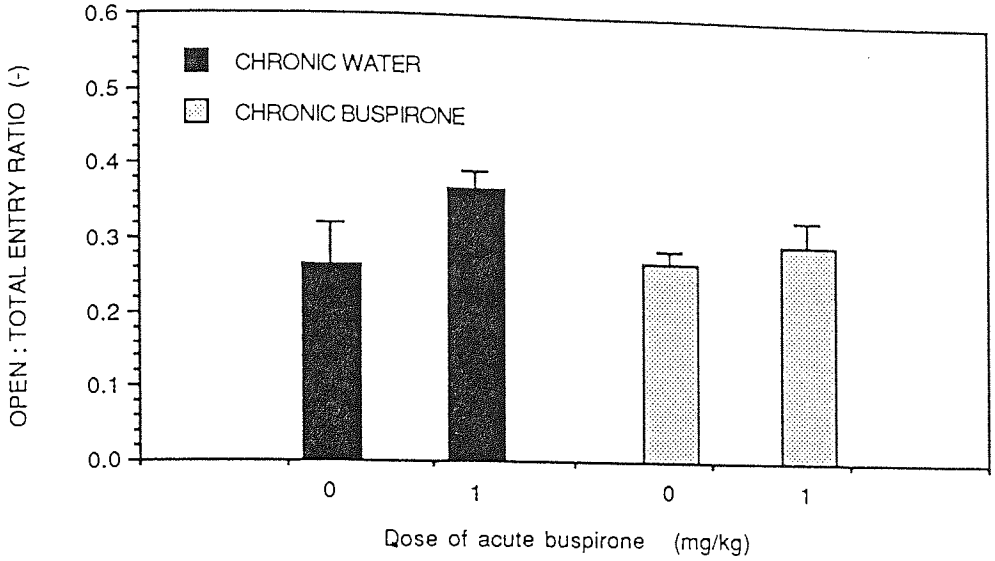


Figure 7.2b. The effect of chronic treatment with buspirone on the response to acute buspirone treatment on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test.

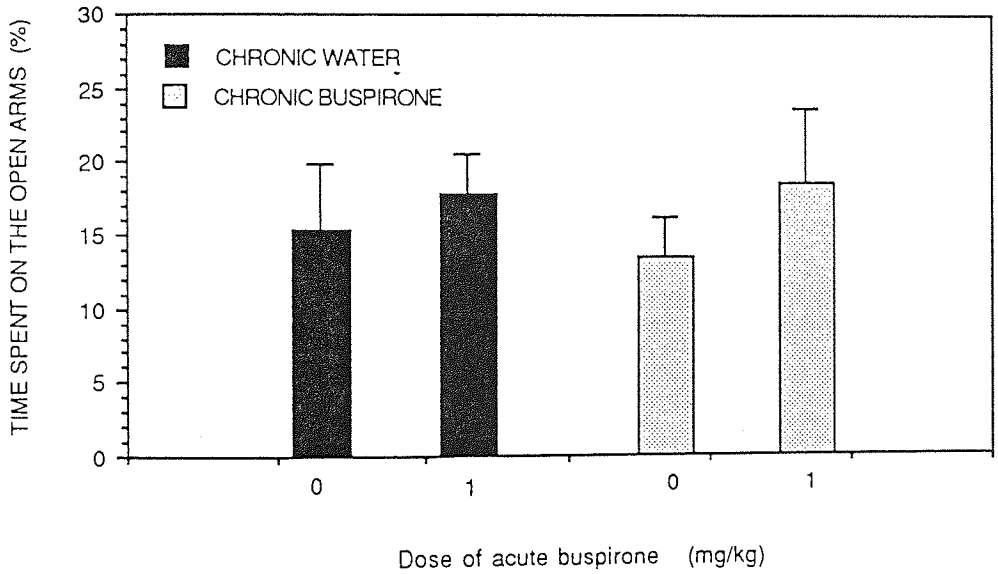


Table 7.1. The effect of acute or chronic treatment with 1 mg/kg buspirone on behaviour on the elevated X-maze.

Chronic treatment (Acute treatment)	N	Total Entries
water (saline)	6	18.3 ± 2.2
water (buspirone)	6	19.3 ± 0.9
buspirone (saline)	6	20.8 ± 3.6
buspirone (buspirone)	6	19.3 ± 1.4

Statistical comparisons were made by two way ANOVA.

Figure 7.3a. The effect of route of administration of 8-OH-DPAT on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 ** P < 0.01 compared to ip saline; ◊◊ P < 0.01 compared to ip 8-OH-DPAT.

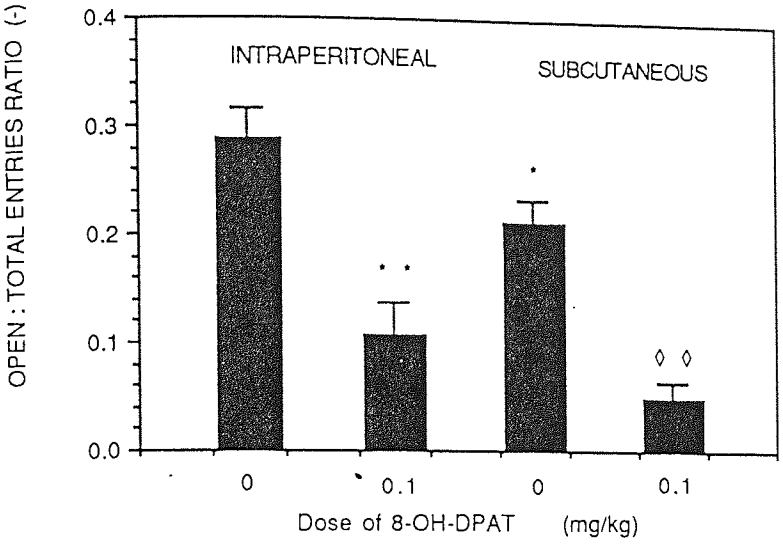


Figure 7.3b. The effect of route of administration of 8-OH-DPAT on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test ** P < 0.01 compared to ip saline; ◊ P < 0.05 compared to ip 8-OH-DPAT.

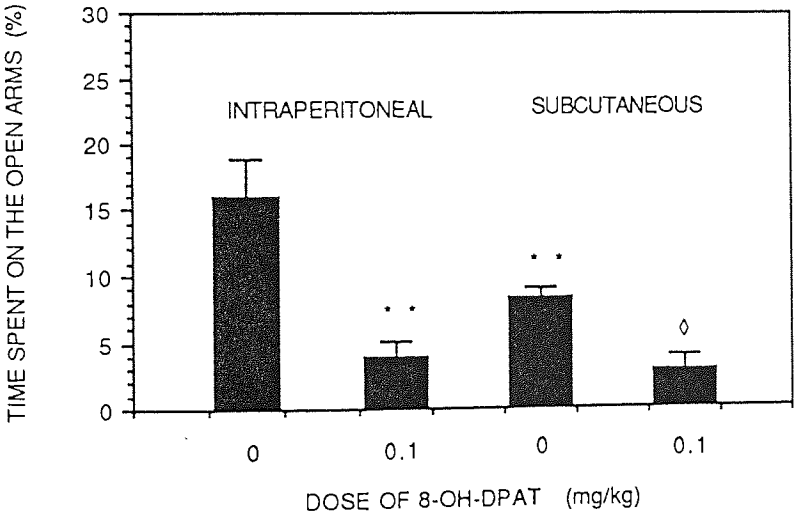


Table 7.2. The effect of route of administration of 0.1 mg/kg 8-OH-DPAT in the elevated X-maze.

Treatment	Route	N	Total Entries
saline	ip	6	28.0 ± 5.2
8-OH-DPAT	ip	6	22.0 ± 2.6
saline	sc	6	28.3 ± 6.0
8-OH-DPAT	sc	6	33.6 ± 6.4

Statistical comparisons were made by two way ANOVA.

Figure 7.4a. The effect of housing time on the open : total entry ratio in response to 8-OH-DPAT in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 compared to one week saline;
◊ P < 0.05 compared to three week saline.

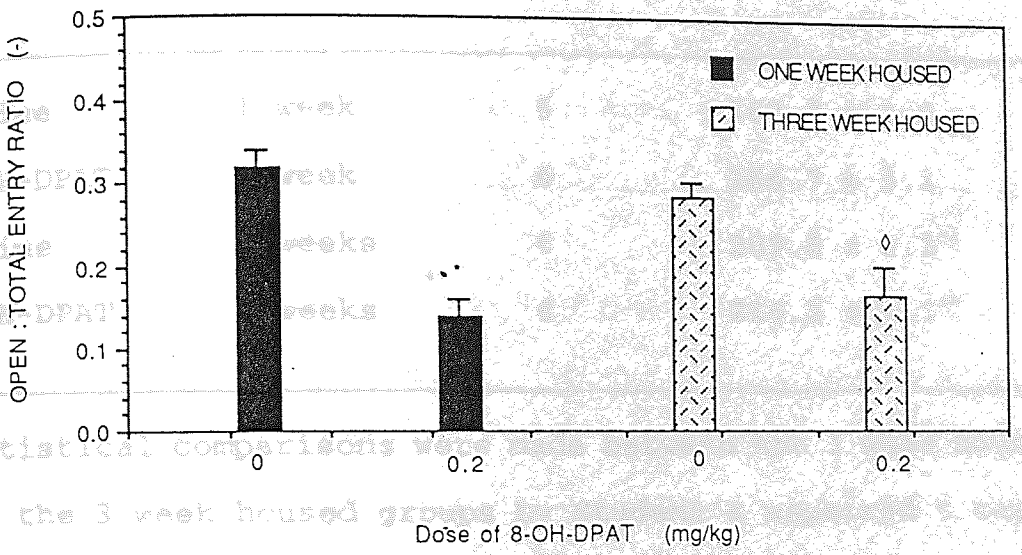


Figure 7.4b. The effect of housing time on the time spent on the open arms of the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 compared to one week housed saline;
◊ P < 0.05 compared to three week housed saline.

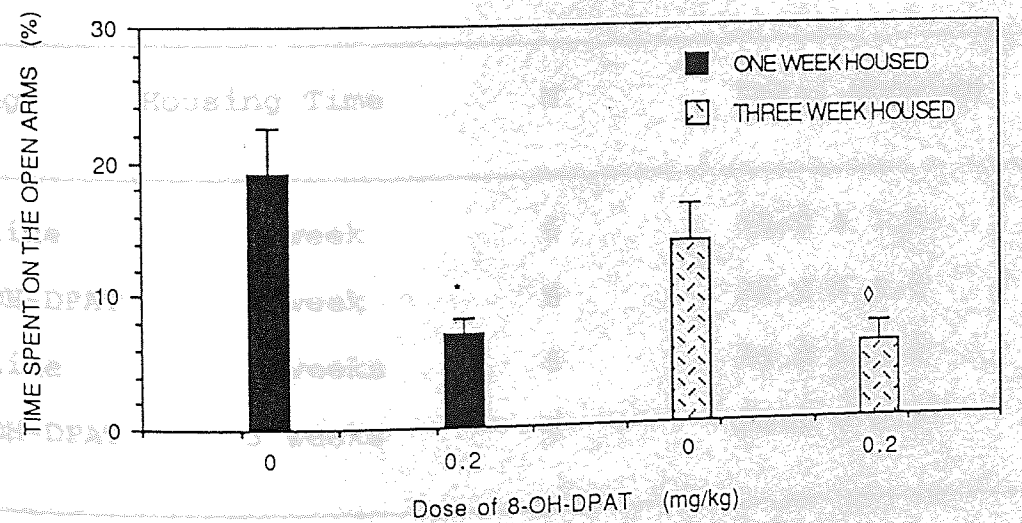


Table 7.3. The body weight of rats used to test the effect of housing time on the response to 0.2 mg/kg 8-OH-DPAT in the elevated X-maze.

Drug	Housing Time	N	Weight (g)
Saline	1 week	6	167.8 ± 2.9
8-OH-DPAT	1 week	6	166.7 ± 3.1
Saline	3 weeks	6	209.8 ± 3.3**
8-OH-DPAT	3 weeks	6	216.2 ± 6.1**

Statistical comparisons were made between the 1 week housed and the 3 week housed groups by Student's unpaired t test. ** $P < 0.01$ compared to 1 week housed.

Table 7.4. The effect of housing time on the response to 0.2 mg/kg 8-OH-DPAT in the elevated X-maze.

Drug	Housing Time	N	Total Entries
Saline	1 week	6	30.0 ± 1.1
8-OH-DPAT	1 week	6	35.0 ± 4.8
Saline	3 weeks	6	24.0 ± 2.5
8-OH-DPAT	3 weeks	6	24.0 ± 2.9*

Statistical comparisons were made by two way ANOVA followed by Tukey's test. * $P < 0.05$ compared to 1 week 8-OH-DPAT.

Table 7.5. The effect of two experimenters with 0.2 mg/kg 8-OH-DPAT on the elevated X-maze.

Treatment Drug	N	Open : Total Entry Ratio	Time on Open Arms (%)	Total Entries
Experimenter A				
Saline	6	0.16 ± 0.05	9.0 ± 2.8	31.2 ± 6.9
8-OH-DPAT	6	0.14 ± 0.02	6.4 ± 1.8	24.0 ± 4.1
Experimenter B				
Saline	6	0.15 ± 0.02	5.7 ± 1.4	18.7 ± 1.5*
8-OH-DPAT	6	0.14 ± 0.03	5.6 ± 1.3	22.2 ± 2.4

Statistical comparisons were made by two way ANOVA followed by Tukey's test. * $P < 0.05$ compared to experimenter A saline treated group.

Figure 7.5a. The effect of light intensity on the open : total entry ratio in the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test ** P < 0.01 compared 170 lux control; ◊ P < 0.05 compared to 0 lux control.

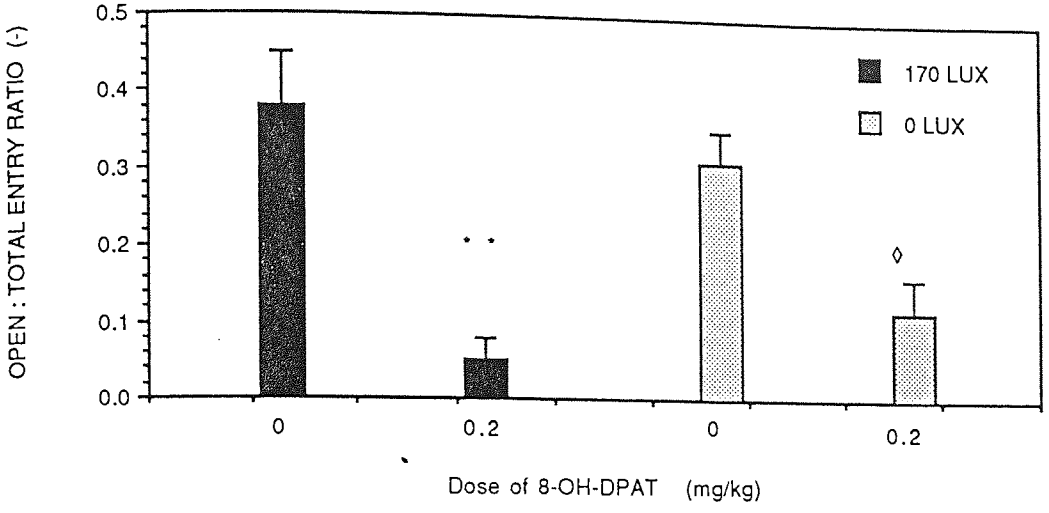


Figure 7.5b. The effect of light intensity on the time on the open arms of the elevated X-maze in response to 8-OH-DPAT.

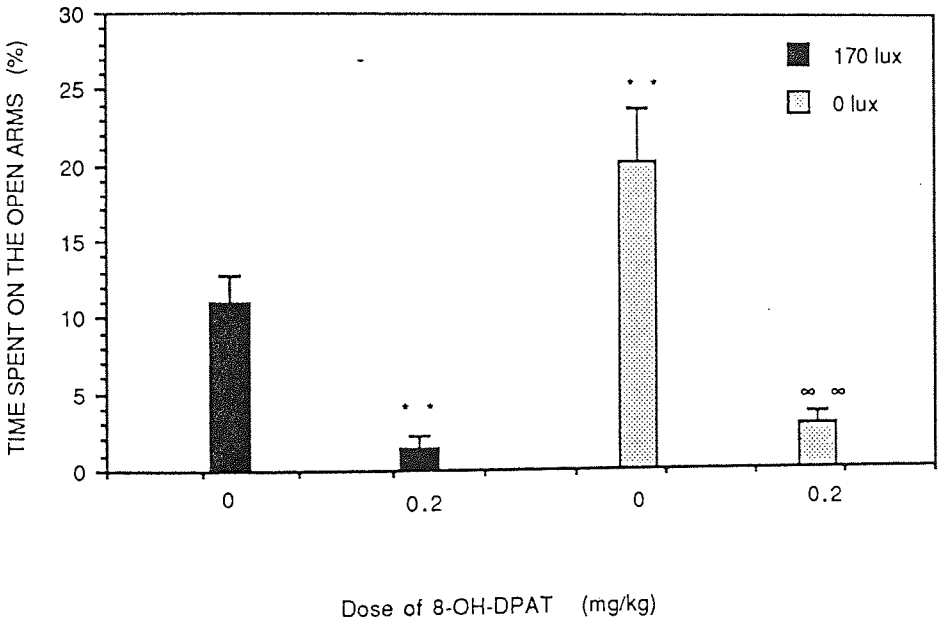


Table 7.6. Light intensity and 8-OH-DPAT on the total entries.

	170 LUX	0 LUX
saline	13.7 ± 3.4	25.5 ± 1.5**
8-OH-DPAT	11.8 ± 3.5	20.8 ± 1.5

Statistical comparisons were made by two way ANOVA. ** P < 0.01 compared to 170 lux saline.

Figure 7.6a. The effect of light intensity on the open : total entry ratio in the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test ** P < 0.01 compared to 172 lux saline.

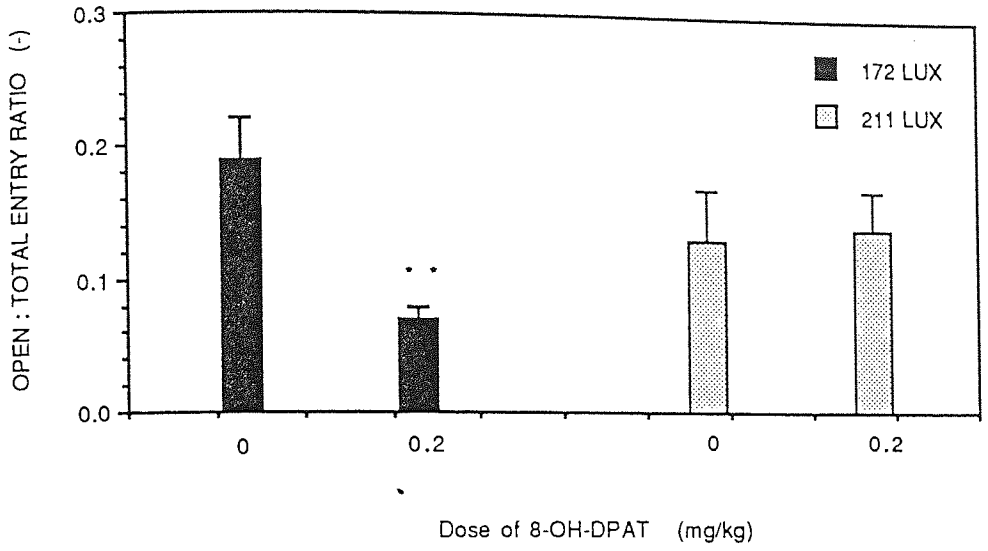


Figure 7.6b. The effect of light intensity on the open : total entry ratio in the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations. Comparisons by Tukey's test * P < 0.05 compared to 172 lux saline.

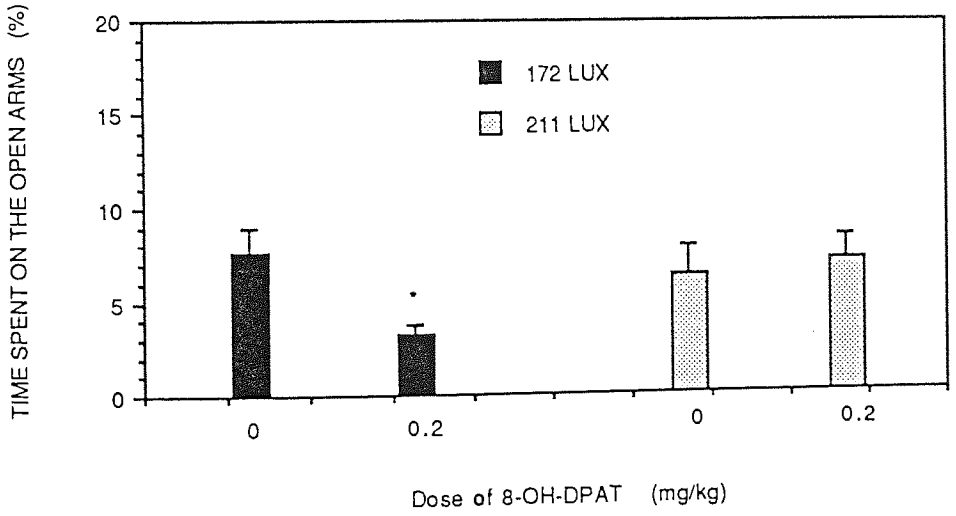


Table 7.7. Light intensity and 8-OH-DPAT on the total entries.

	172 LUX	211 LUX
saline	24.3 ± 2.2	24.2 ± 0.8
8-OH-DPAT	29.3 ± 2.4	30.5 ± 3.2

Statistical comparisons were made by two way ANOVA.

Figure 7.7a. The effect of light intensity on the open : total entry ratio on the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test * P < 0.05 ** P < 0.01 compared to 170 lux saline; ◊◊ P < 0.01 compared to 785 lux saline.

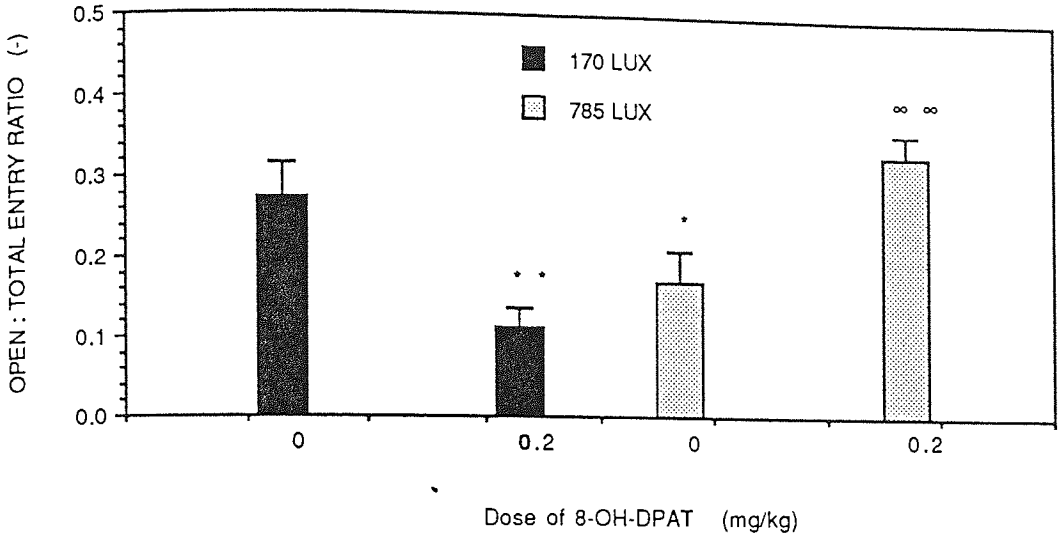


Figure 7.7b. The effect of light intesnity on the time spent on the open arms of the elevated X-maze in response to 8-OH-DPAT. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test.

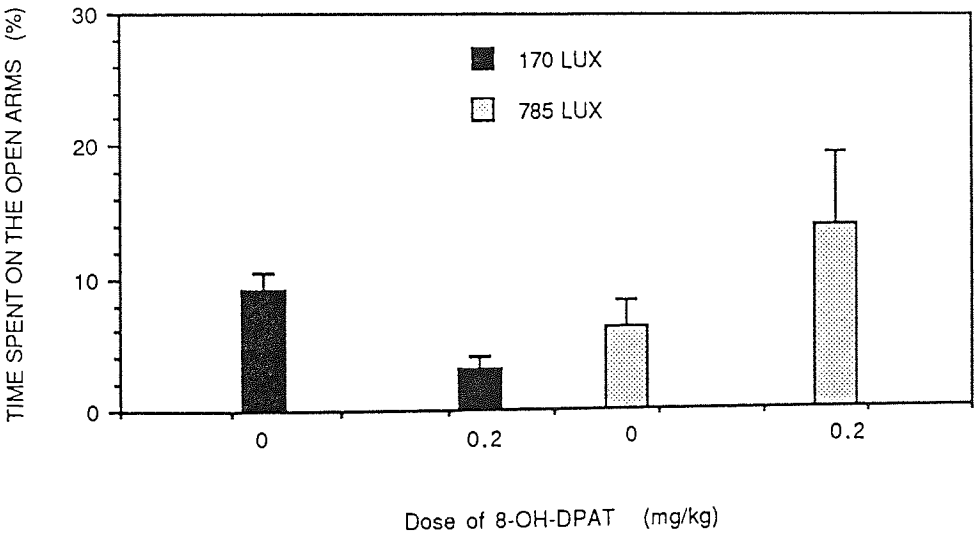


Table 7.8. Light intensity and 8-OH-DPAT on the total entries.

	170 LUX	785 LUX
saline	24.2 ± 3.7	19.2 ± 2.2
8-OH-DPAT	19.8 ± 1.0	20.8 ± 4.9

Statistical comparisons were made by two way ANOVA.

Figure 7.8a. The effect of light intensity on the response to 8-OH-DPAT on the open : total entry ratio on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 ** P < 0.01 compared to own control group.

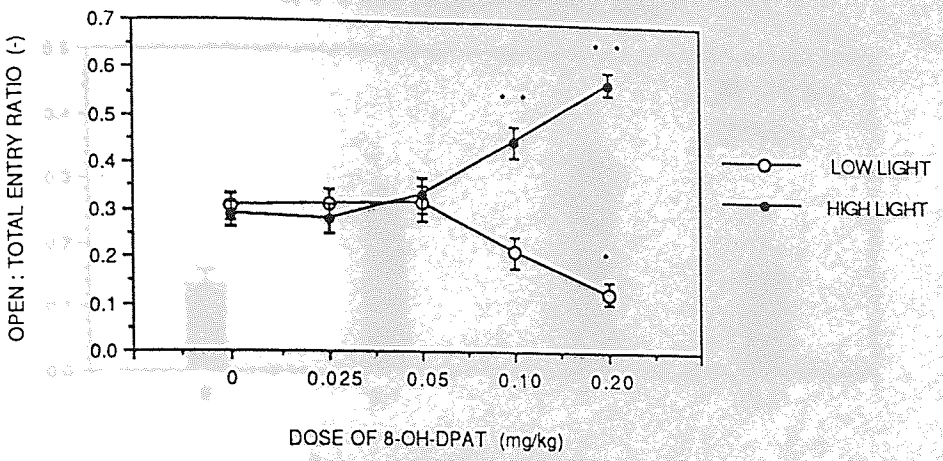


Figure 7.8b. The effect of light intensity on the response to 8-OH-DPAT on the time spent on the open arms of the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
** P < 0.01 compared to own control group.

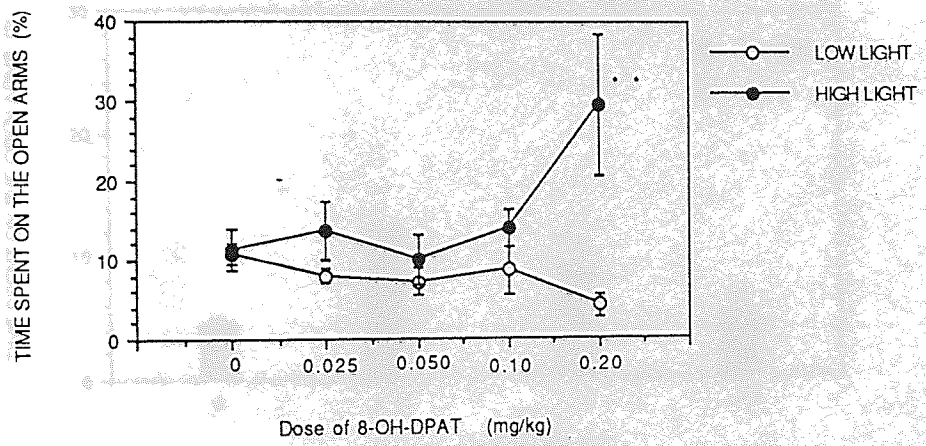


Table 7.9. The effect of light intensity on the total number of arm entries made on the elevated X-maze after 8-OH-DPAT.

Light Intensity	Dose of 8-OH-DPAT (mg/kg)	N	Total Entries
170 lux	0.0	6	20.8 ± 1.5
	0.025	6	19.5 ± 1.6
	0.050	6	16.2 ± 3.4
	0.10	6	23.0 ± 4.1
	0.2	6	18.2 ± 3.2
785 lux	0.0	6	19.8 ± 2.4
	0.025	6	20.8 ± 2.1
	0.050	6	19.8 ± 2.6
	0.10	6	24.2 ± 1.4
	0.20	6	26.7 ± 6.5

Statistical comparisons were made by two way ANOVA, but there was no significant effect.

Figure 7.9a. The effect of pCPA on the response to 8-OH-DPAT in the elevated X-maze under high light intensity. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 ** P < 0.01 compared to control saline;
◊◊ P < 0.01 compared to pCPA saline.

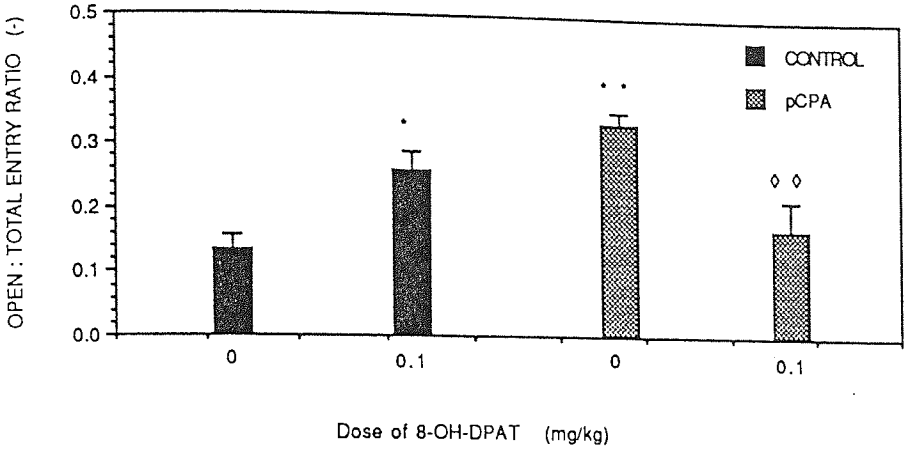


Figure 7.9b. The effect of pCPA on the response to 8-OH-DPAT in the elevated X-maze under high light intensity. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 compared to control saline.

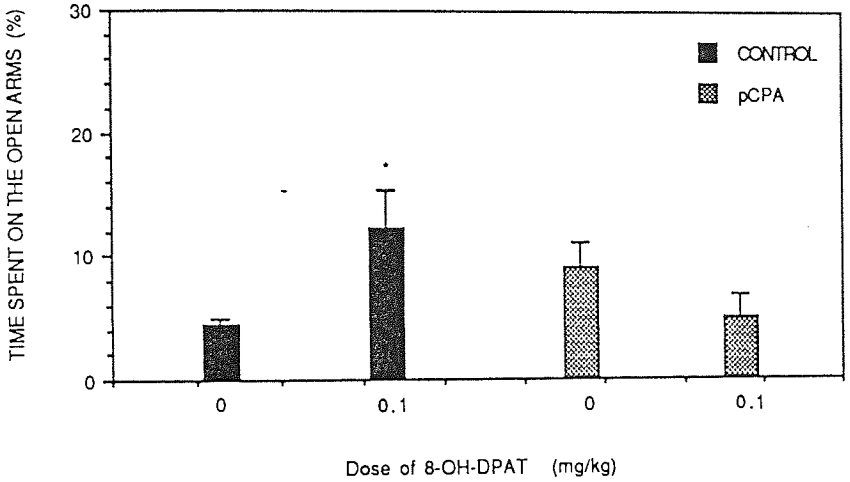


Table 7.10. The effect of pCPA on the total entries made on the elevated X-maze after 8-OH-DPAT under high light.

Pretreatment [Treatment]	Dose (mg/kg)	N	Total Entries
Saline [Saline]	0 0	6	24.0 ± 3.8
Saline [8-OH-DPAT]	0 0.1	6	22.2 ± 2.5
pCPA [Saline]	300 0	6	13.0 ± 2.4*
pCPA [8-OH-DPAT]	300 0.1	6	12.3 ± 2.4∞

Statistical comparisons were made by two way ANOVA. * P < 0.05 compared to saline control; ∞ P < 0.05 compared to 8-OH-DPAT control.

Figure 7.10a. The effect of pCPA on the response to 8-OH-DPAT on the elevated X-maze under high light intensity. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 ** P < 0.01 compared to control saline;
◊ P < 0.05 compared to pCPA saline.

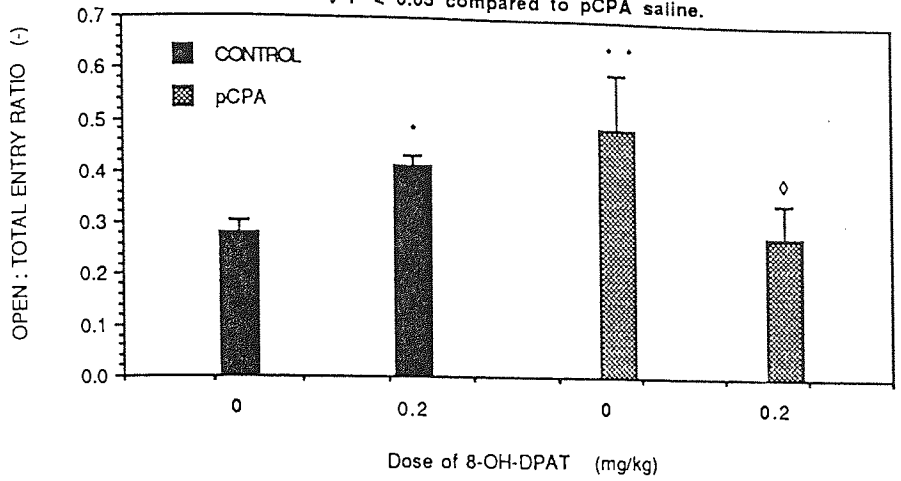


Figure 7.10b. The effect of pCPA on the response to 8-OH-DPAT on the elevated X-maze under high light intensity. The graph shows mean ± sem of 6 observations per group. Comparisons by Tukey's test
* P < 0.05 compared to control saline;
◊◊ P < 0.01 compared to pCPA saline.

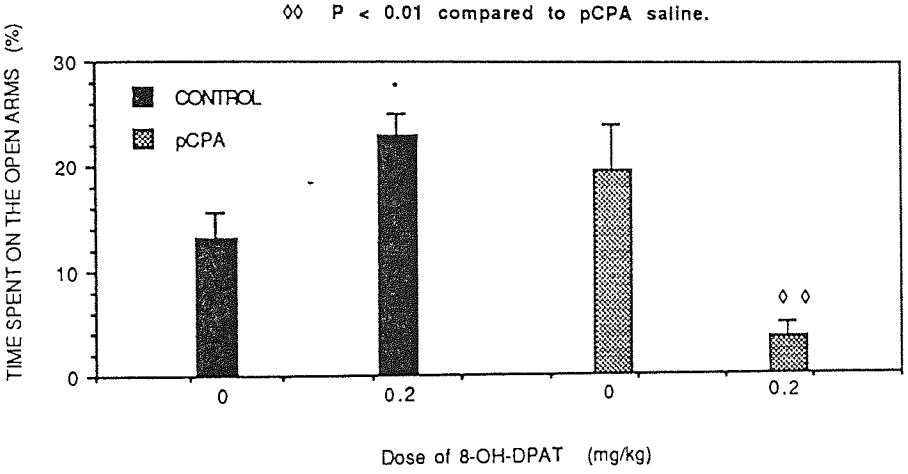


Table 7.11. The effect of pCPA on the total entries made on the elevated X-maze after 8-OH-DPAT under high light.

Pretreatment [Treatment]	Dose (mg/kg)	N	Total Entries
Saline [Saline]	0 0.0	6	24.0 ± 2.4
Saline [8-OH-DPAT]	0 0.2	6	21.8 ± 1.6
pCPA [Saline]	300 0.0	6	12.2 ± 1.2**
pCPA [8-OH-DPAT]	300 0.2	6	5.8 ± 2.0◊◊

Statistical comparisons were made by two way ANOVA. ** P < 0.01 compared to saline control; ◊◊ P < 0.01 compared to 8-OH-DPAT control.

Figure 7.11a. The number of arm entries made in 10 minutes within a one hour exposure to the elevated X-maze. The graph shows mean ± sem of 12 rats for each period. Comparisons by Dunnett's t test
* P < 0.05 ** P < 0.01 compared to 0 - 10 minutes segment.

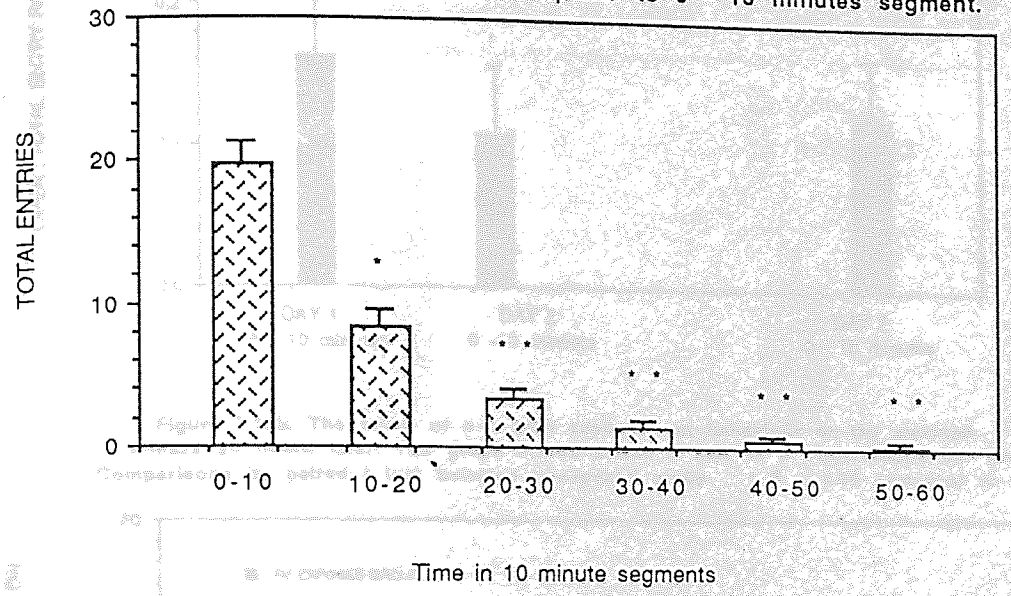


Figure 7.11b. The time spent on the open arms of the elevated X-maze during a one hour exposure. The graph shows mean ± sem of 12 observations per group.
Comparisons by Dunnett's t test *P < 0.05
** P < 0.01 compared to 0 - 10 minutes segment.

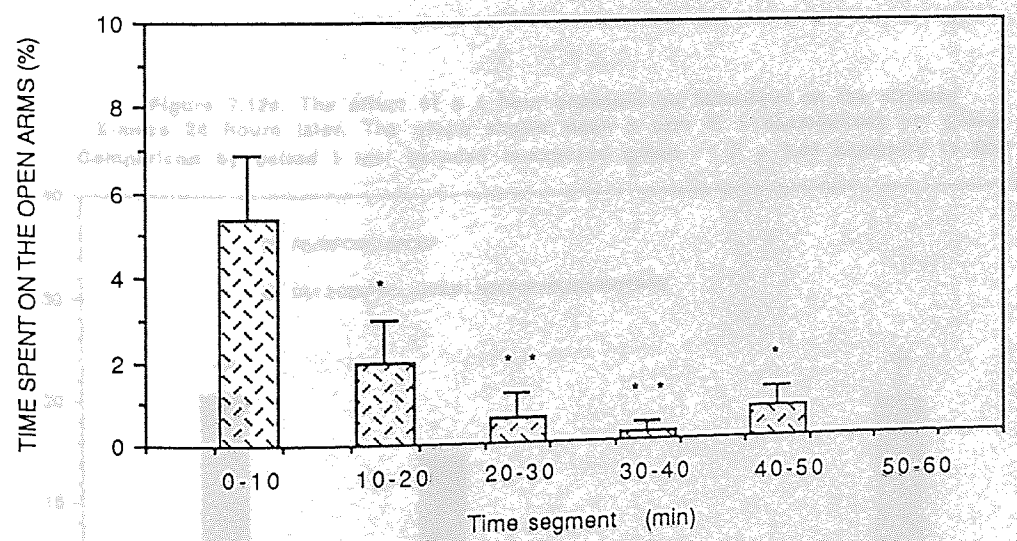


Figure 7.12a. The effect of a 1 hour exposure on behaviour on the elevated X-maze 24 hours later. The graph shows mean ± sem of 6 observations per group. Comparisons by paired t test between re-exposed group * P < 0.05 compared to Day 1.

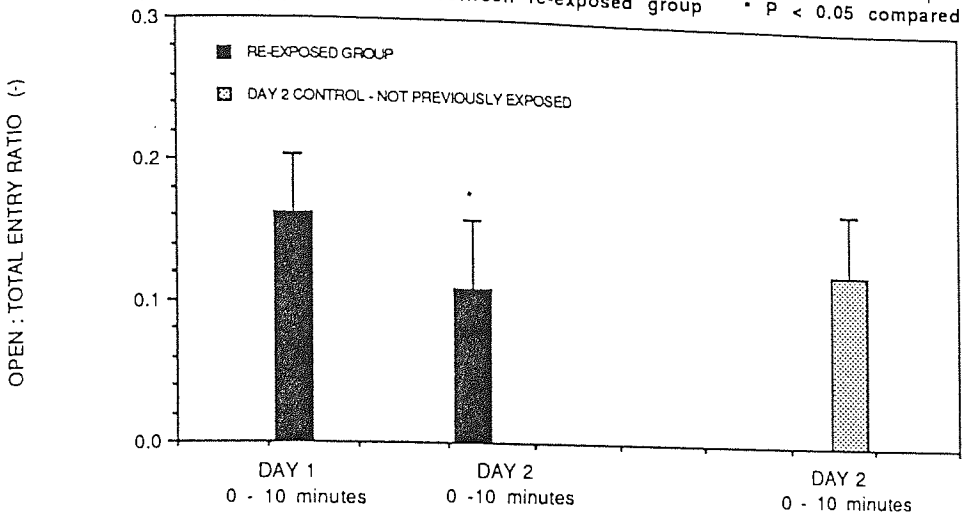


Figure 7.12b. The effect of a 1 hour exposure on behaviour on the elevated X-maze 24 hours later. The graph shows maean ± sem of 6 observations per group. Comparisons by paired t test between re-exposed group * P < 0.05 compared to Day 1.

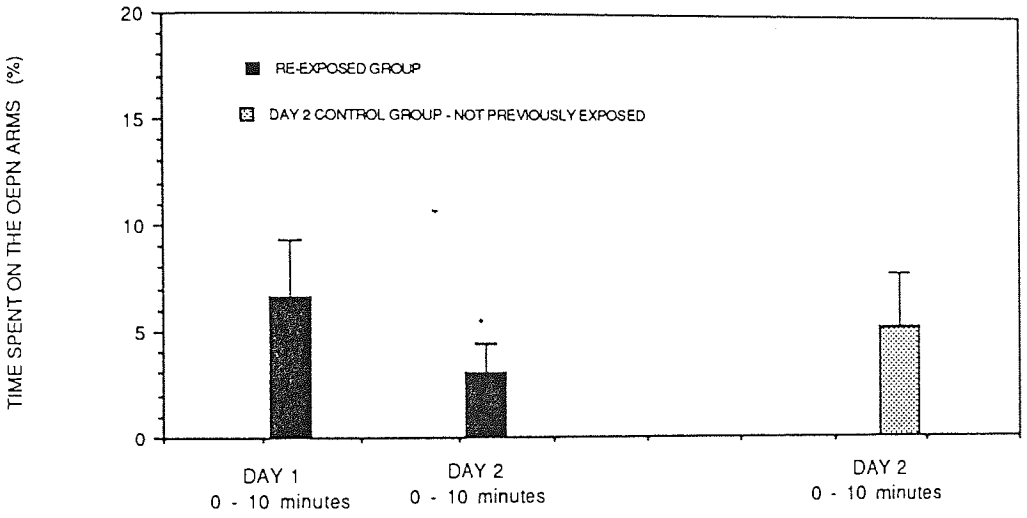


Figure 7.12c. The effect of a 1 hour exposure on behaviour on the elevated X-maze 24 hours later. The graph shows mean ± sem of 6 observations per group. Comparison by paired t test between re-exposed group * P < 0.05 compared to Day 1.

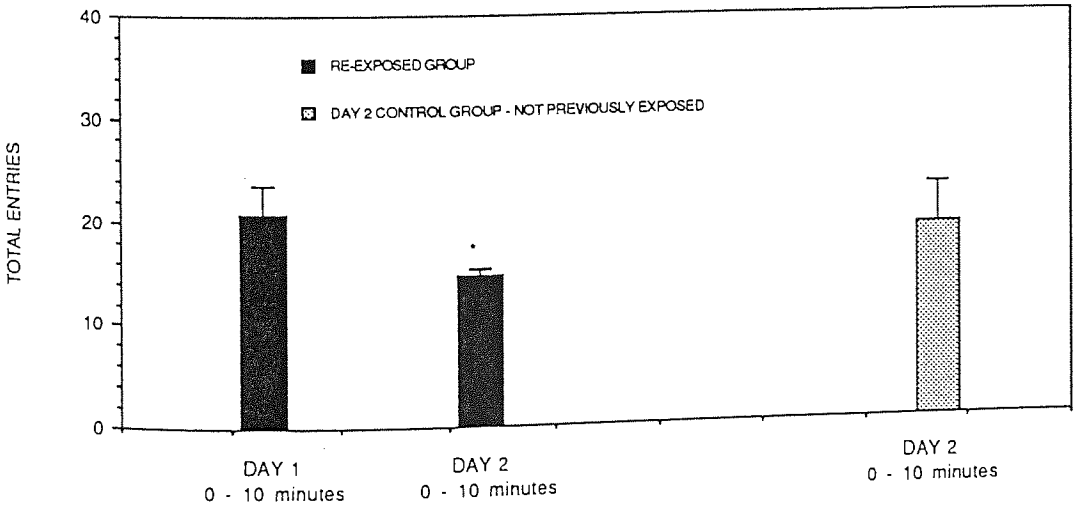


Figure 7.13a. The effect of 8-OH-DPAT in rats not previously exposed to the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test * P < 0.05 compared to control. (a) open : total entry ratio.

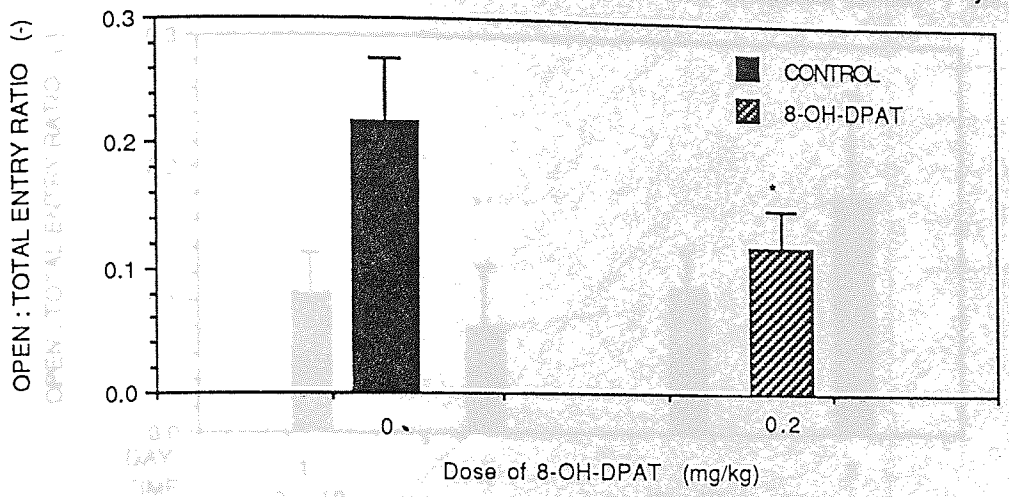


Figure 7.13b. The effect of 8-OH-DPAT in rats not previously exposed to the elevated X-maze. The graph shows mean ± sem of 6 observations per group.
Comparisons by unpaired t test compared to control * P < 0.05.

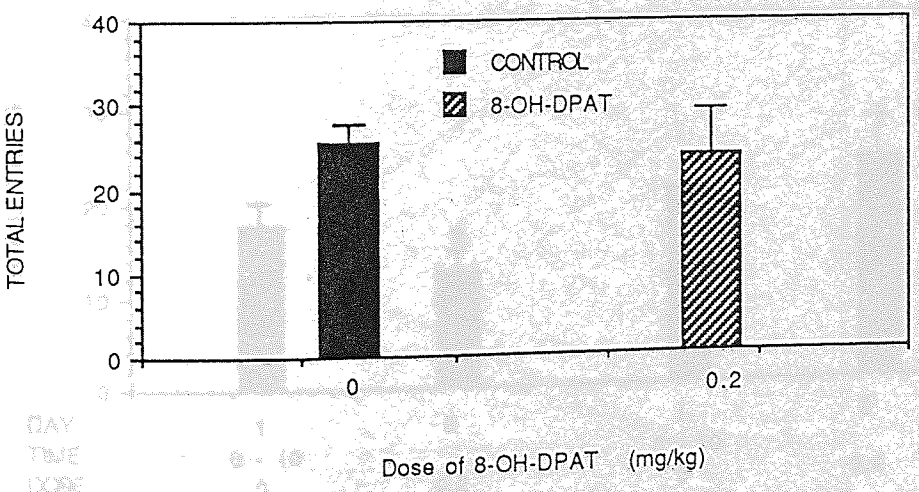


Figure 7.13c. The effect of a 1 hour exposure to the elevated X-maze on the response to 8-OH-DPAT 24 hours later. The graph shows mean ± sem of 6 observations per group. Comparisons by paired t test * P < 0.05 compared to Day 1 saline.

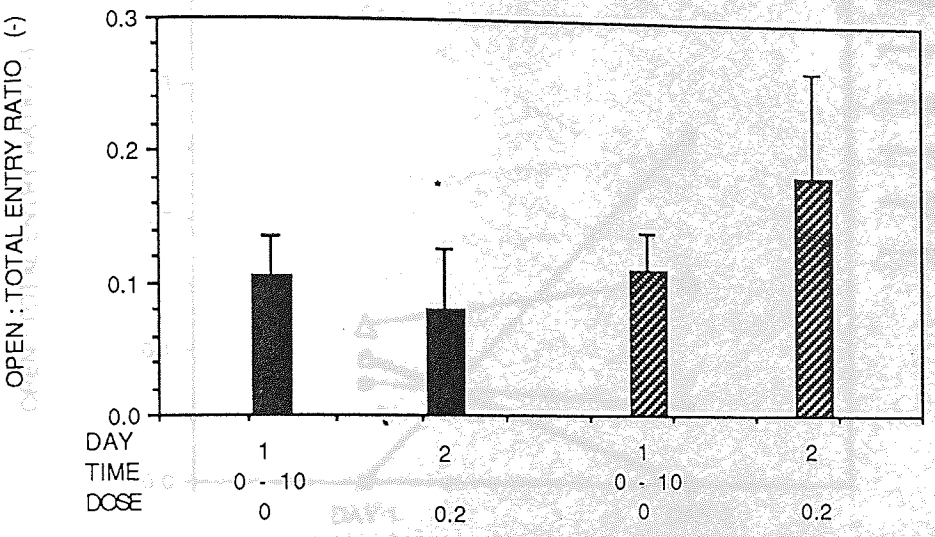


Figure 7.13d. The effect of a 1 hour exposure on the elevated X-maze on the response to 8-OH-DPAT 24 hours later. The graph shows mean ± sem of 6 observations per group. Comparisons by paired t test * P < 0.05 compared to Day 1 saline.

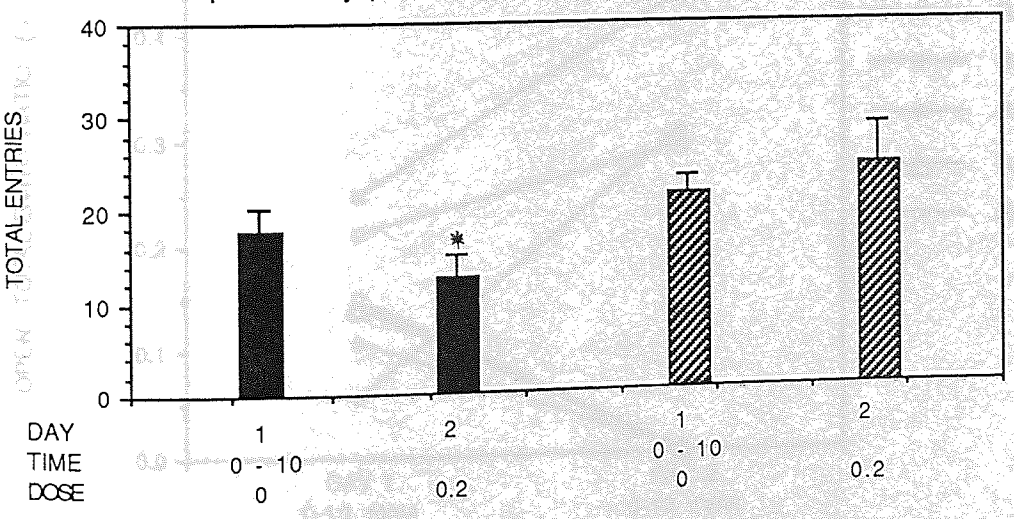


Figure 7.13e. Open : total entry ratio on the elevated X-maze of individual rats given a 10 minute exposure 10 minutes after a saline injection and 24 hours after a 1 hour exposure.

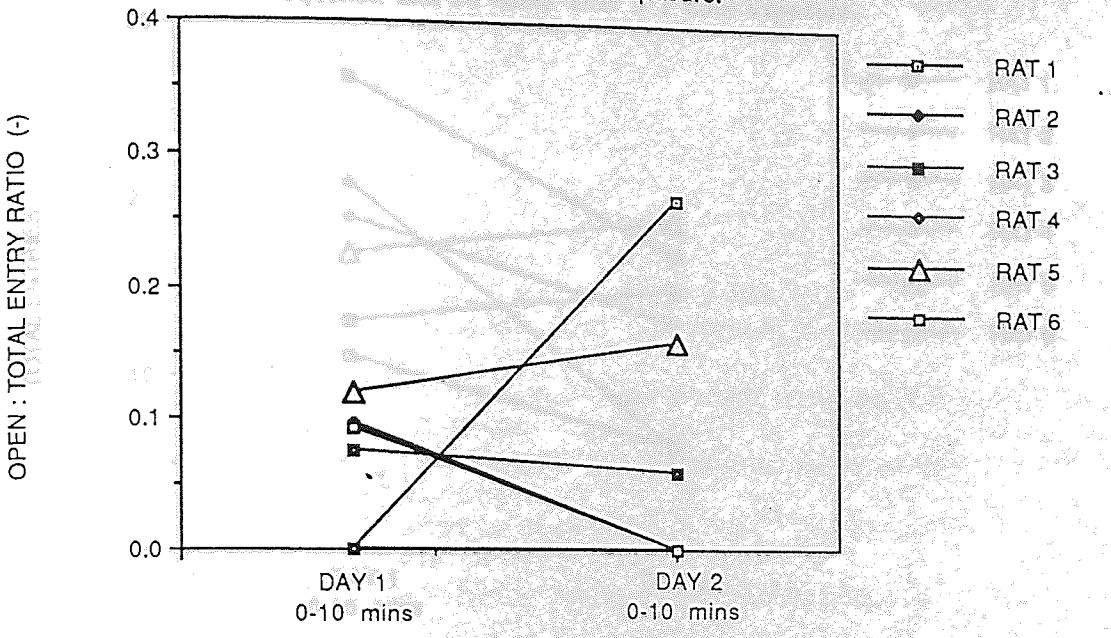


Figure 7.13f. Open : total entry ratio on the elevated X-maze of individual rats given a 10 minute exposure 10 minutes after an injection of 0.2 mg/kg 8-OH-DPAT and 24 hours after a 1 hour exposure.

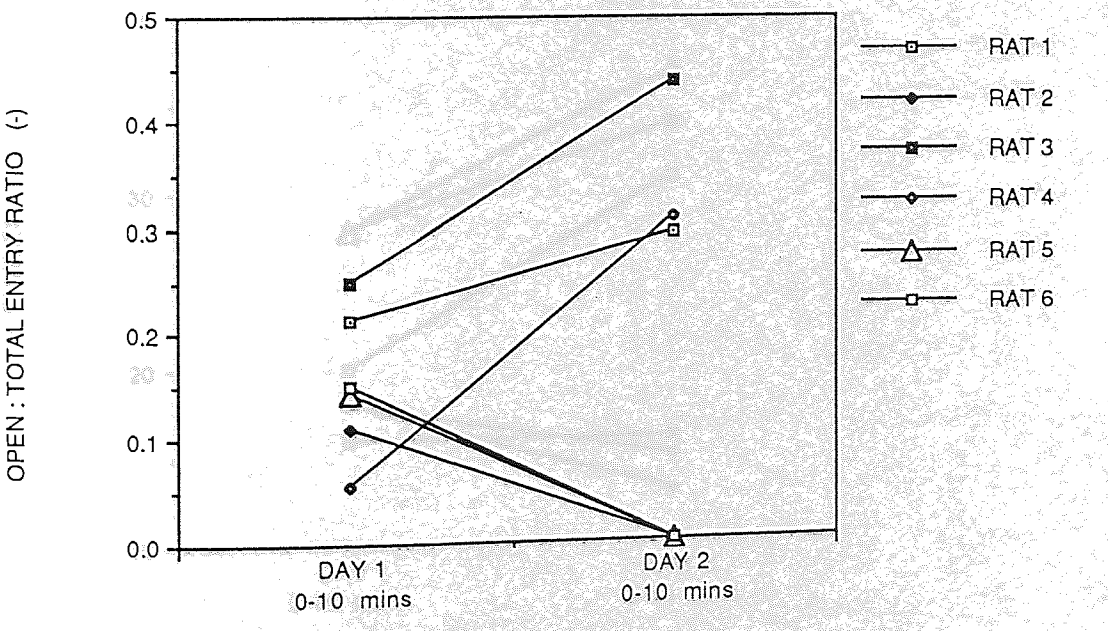


Figure 7.13g. The total number of arm entries made on the elevated X-maze by individual rats given a 10 minute exposure 10 minutes after a saline injection and 24 hours after a 1 hour exposure.

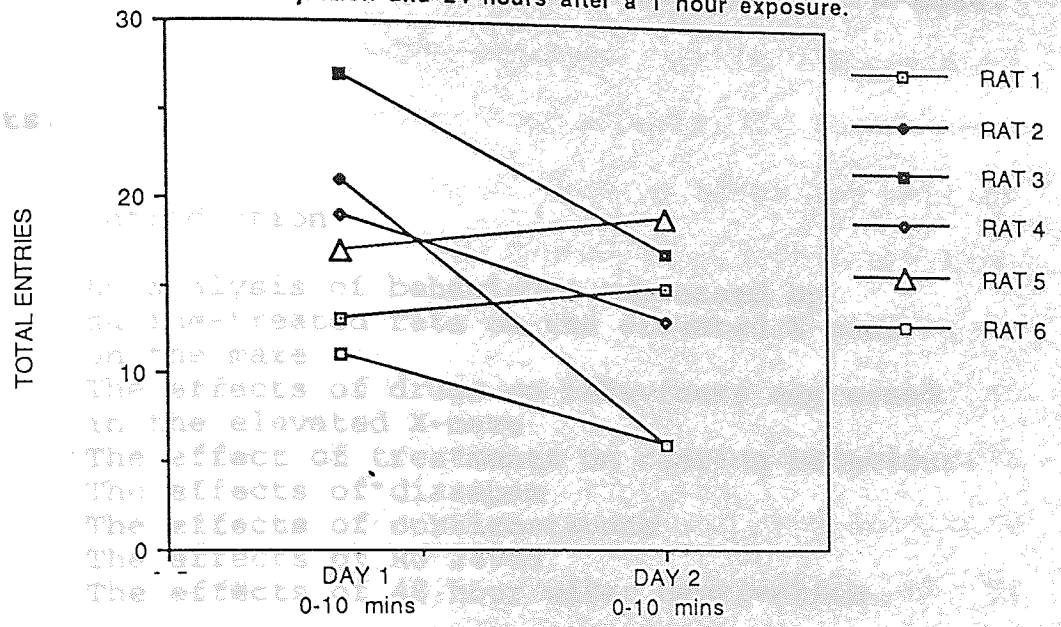
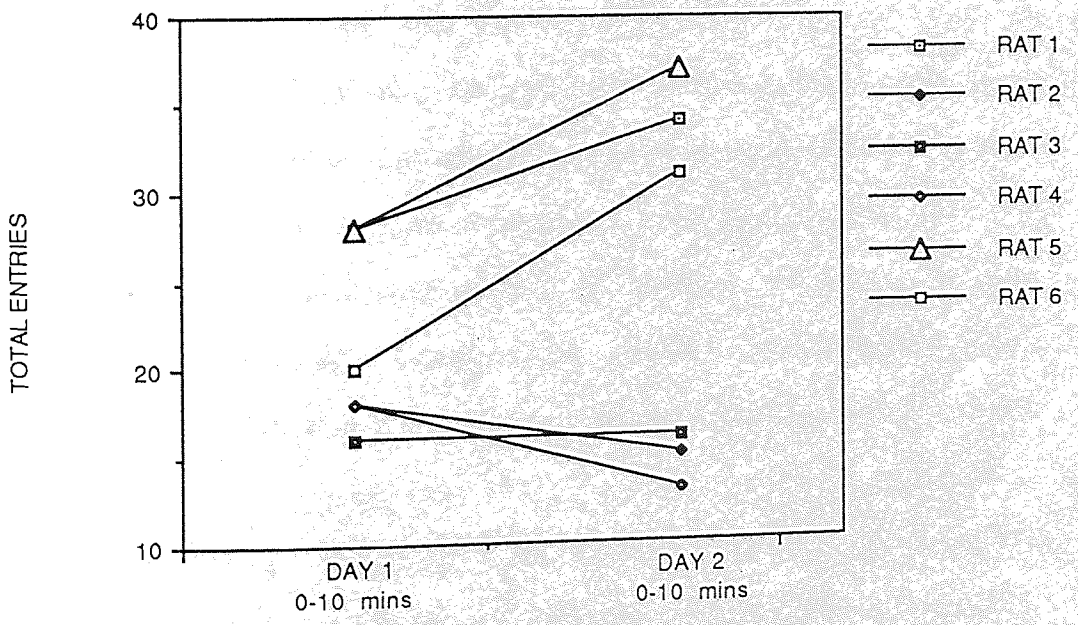


FIGURE 7.13h. The total number of arm entries made on the elevated X-maze by individual rats given a 10 minute exposure 10 minutes after an injection of 0.2 mg/kg 8-OH-DPAT and 24 hours after a 1 hour exposure.



Chapter 8

A descriptive analysis of behaviour on the elevated X-maze.

Contents.

8.1 Introduction.

The elevated X-maze is often employed for the detection of "anxiolytic" and "anxiogenic" drug effects. The most common measures taken are the ratio of open to total arm entries (Handley and Mithani, 1984; Pellow et al., 1985), the time spent on the open arms (Pellow et al., 1985) or the number of open arm entries given no change in the total number of arm entries (Söderpalm et al., 1989), but there has been little investigation into what behaviours are occurring during the test or how these are modulated by pharmacological challenges. The importance of studying the expression of behaviour in anxiety tests has been discussed recently (see Rogers, 1991). The purpose of the work described in this chapter was to describe, in a quantitative manner, the structure of behaviour of animals on the elevated X-maze and to try to identify changes in behaviour expressed by the animal that were consistent with changes noted in the "anxiety"-related measures (open : total entry ratio and time spent on the open arms) in the same animals. The occurence of different behaviours and their modulation by treatments with "anxiolytic" or "anxiogenic" effects were studied within different regions of the maze.

Results from this chapter are derived from experiments reported in other chapters which were video taped. Behavioural categories are defined in the methods chapter.

8.2 An analysis of the behaviour of saline-treated rats on the elevated X-maze.

Saline-treated rats reared and groomed only in the enclosed arms (Figure 8.1). Peering was substantially greater in the centre square than in enclosed arms and was virtually non-existent in the open arms (Figure 8.1). Sniffing was the predominant behaviour in the open and enclosed arms, but was expressed only to a small extent in the centre square, where, after peering, locomotion was the predominant behaviour (Figure 8.1).

8.3.1 The effects of drugs on behaviours expressed in the elevated X-maze

The behaviour of drug-treated animals in the three different regions of the elevated X-maze were quantified and compared with the corresponding score for saline treated animals for each region. As the time spent in each region was influenced by "anxiolytic" and "anxiogenic" treatments, an absolute measure of the time spent in each behaviour in each region was not appropriate. Instead, the proportion of time spent in each behaviour for each region was calculated as a % of the total time spent in each area.

8.3.2 The effect of treatments on rearing behaviour.

Diazepam (1.5 mg/kg) significantly increased rearing in both the enclosed arms and the centre square, although there was still no rearing on the open arms (Figure 8.2a). Corticosterone, (10 mg/kg), did not influence rearing in any region (Figure 8.2b). In contrast, the "anxiogenic" agent RU 24969 (1 mg/kg) decreased rearing in the enclosed arms (Figure 8.2c) and 48 hour water deprivation, which had an "anxiolytic" effect, increased rearing in all regions of the maze (Figure 8.2d).

8.3.3 The effects of diazepam.

Apart from rearing, diazepam (1.5 mg/kg) did not alter the occurence of any variable in any region (Figure 8.3).

8.3.4 The effects of corticosterone.

10 mg/kg corticosterone, a dose which increased both the open : total entry ratio and total entries treatment did not influence the occurence of any behaviour on the elevated X-maze (Figure 8.5).

8.3.5 The effects of RU 24969.

The 5 animals which had showed a strong "anxiogenic" response to 1 mg/kg RU 24969 were included in the analysis, with 1 rat who did not appear to respond being excluded. RU 24969 treated rats spent a greater proportion of their time on the open arms in locomotor behaviour than did saline treated rats (Figure 8.6). No other changes in behaviour were recorded on the open arms. In the centre square, RU 24969 increased locomotor behaviour and decreased the time spent peering (Figure 8.6). In the enclosed arms, RU 24969 increased sniffing and decreased both grooming and peering (Figure 8.6).

8.4 The effects of 48 hour water deprivation.

Rearing apart, the behaviour of 48 hour water deprived rats on the elevated X-maze was similar to their control group. The only significant change was a reduction in the occurrence of locomotor behaviour in the centre square (Figure 8.7).

8.5 Discussion.

The study has shown that the three different regions of the X-maze are associated with a differential expression of behaviour in rats that have received a saline injection. Grooming and rearing behaviours were only expressed in the enclosed arms, suggesting that this analysis is capable of detecting changes in behaviour due to "anxiety"-provoking situations of differential intensity (Handley and McBlane, 1991; Pellow et al., 1985). The results suggested that peering behaviour is strongest in intensity in the centre square, followed by the enclosed arms, which would accord with these areas being more likely to be associated with conflict regarding entry into another region of the maze, in particular the open arm (Handley and McBlane, 1991). However, no effect of drug treatment was observed on peering behaviour. The implication of this finding is that behaviour is modulated more by X-maze region than by drug treatment. The most surprising finding to arise from the analysis of the effects of drugs on behaviour in the elevated X-maze was that the structure of behaviour was very resistant to alteration by treatments which did produce major changes in the overall "anxiety" ratings of the same animals. Thus, for most treatments, there was no change in the relative expression of the different behaviours in the different regions of the maze.

The analysis of behavioural changes brought about by an "anxiolytic" dose of diazepam did suggest, however, that

rearing behaviour was increased. This change, along with those found in different regions of the maze in saline treated animals, tend to confirm previous suggestions that rearing behaviour could be inversely related to the state of "anxiety" (eg Moser, 1989).

In conclusion, these results demonstrate that the structure of behaviour expressed on the elevated X-maze is heavily influenced by where the animal is on the maze. Differential expression of these behaviours can be detected in response to treatment which changes anxiety-related scores in the X-maze, although the most appropriate method for the detection of these changes deserves further study.

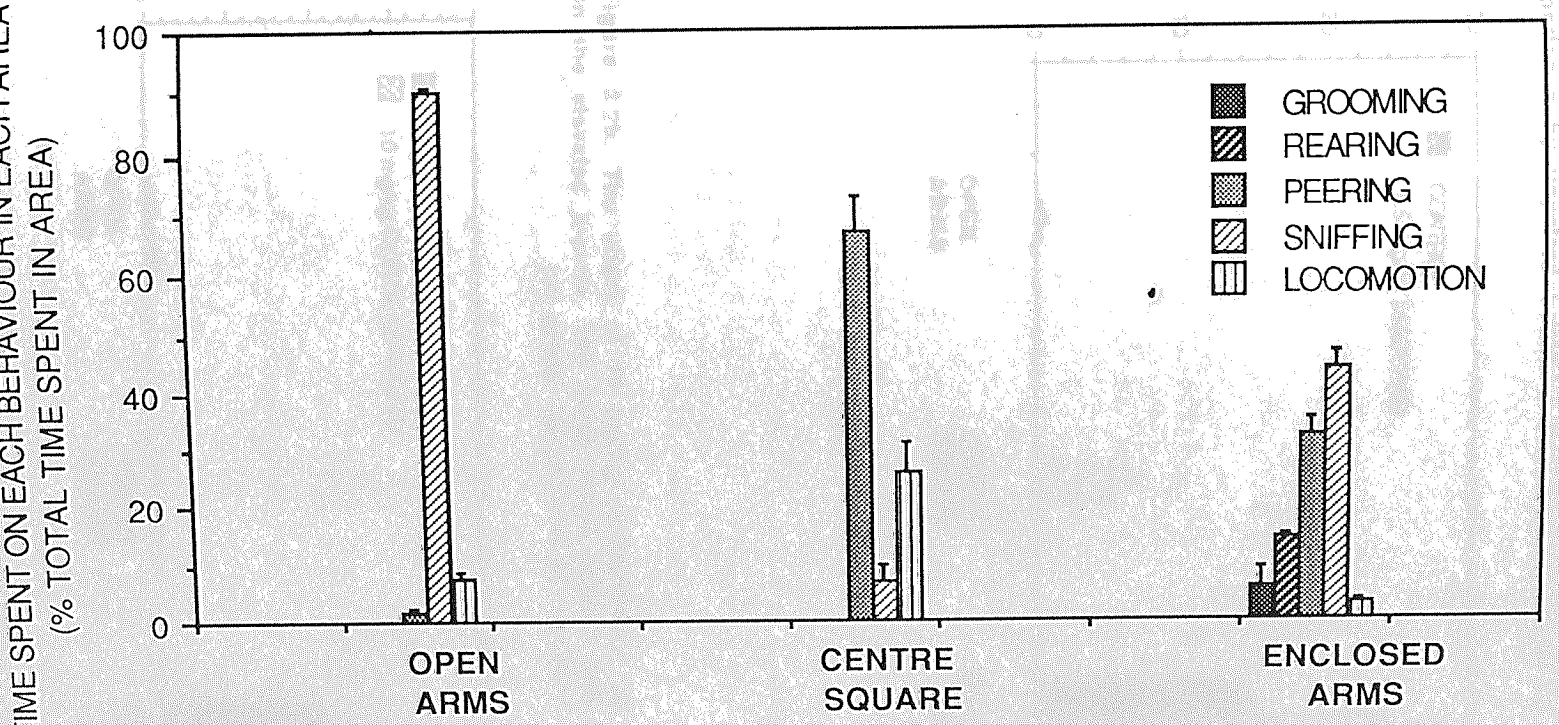


Figure 8.1. Behaviour of saline-treated rats on different regions of the elevated X-maze. The graph shows mean ± sem of 6 observations per group.

Figure 8.2a. The effect of 1.5 mg/kg diazepam on rearing behaviour in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test * P < 0.05 comopared to same area control.

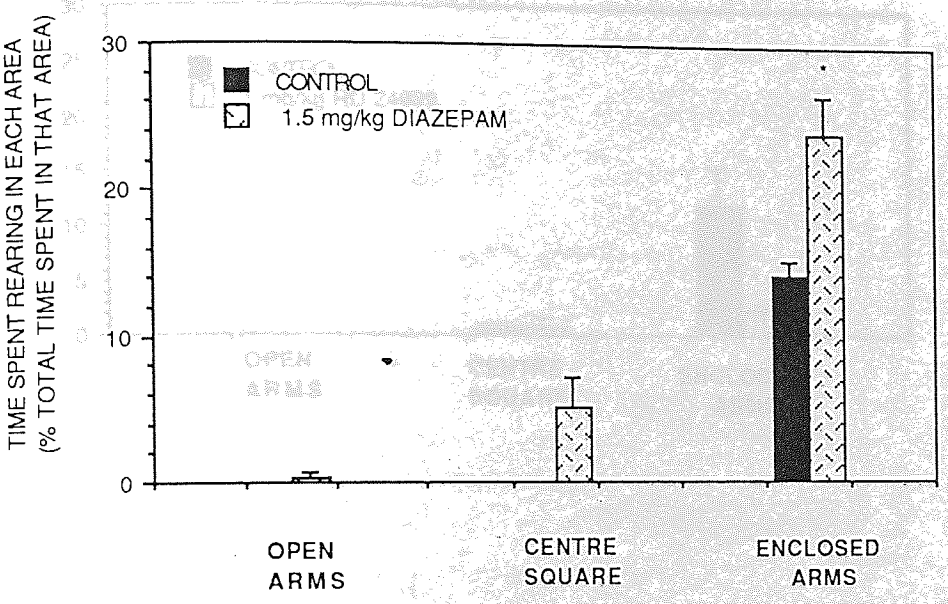


Figure 8.2b. The effect of 10 mg/kg corticosterone on rearing behaviour on the elevated X-maze. The graph shows mean ± sem of 12 observations per group. Comparisons by unpaired t test.

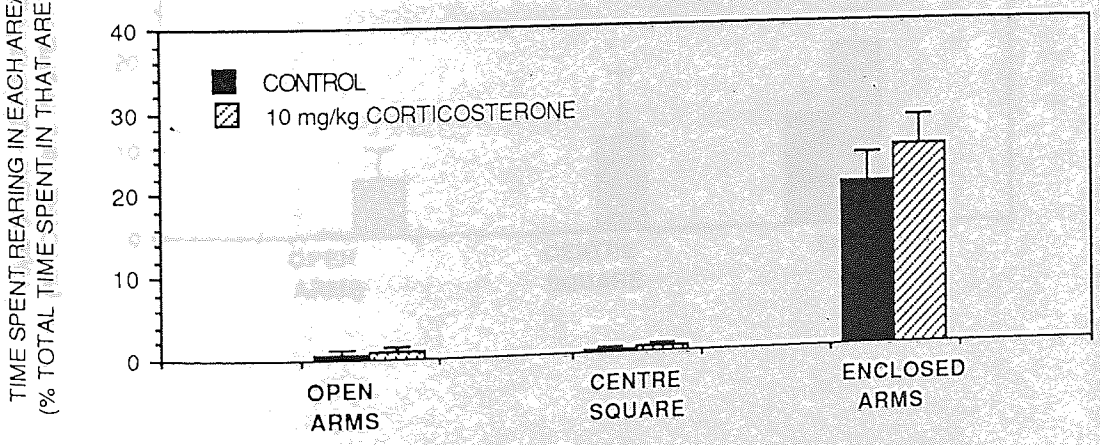


Figure 8.2c. The effect of 1 mg/kg RU 24969 on rearing behaviour on the elevated X-maze. The graph shows mean ± sem of 5 observations per group. Comparison by unpaired t test * P < 0.05 compared to same are control.

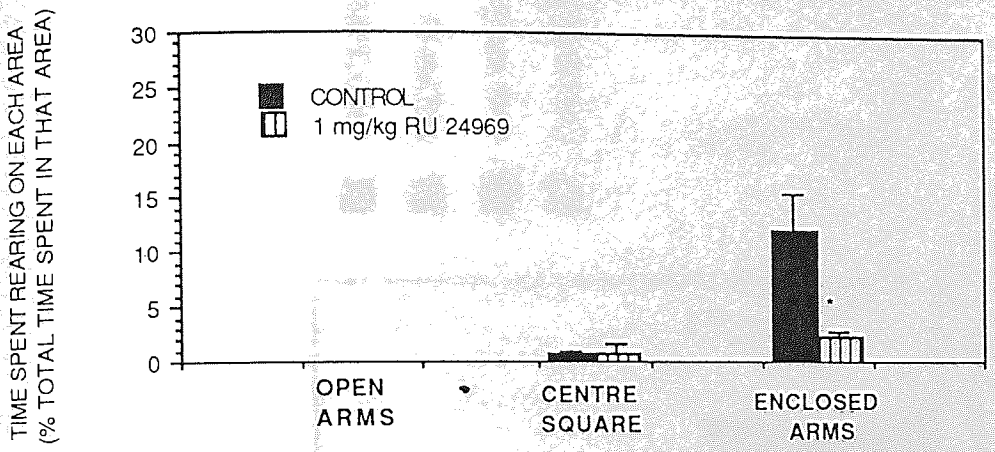
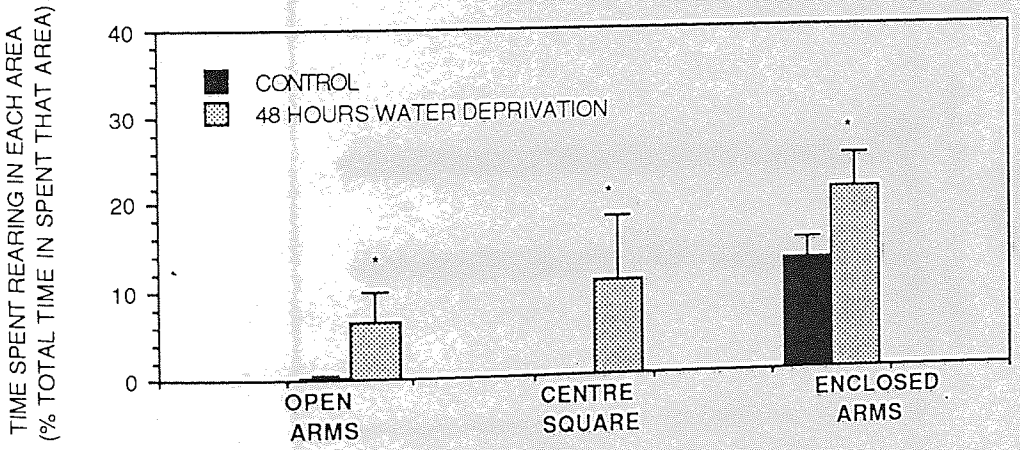


Figure 8.2d. The effect of 48 hour water deprivation on rearing in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test * P < 0.05 compared to same area control.



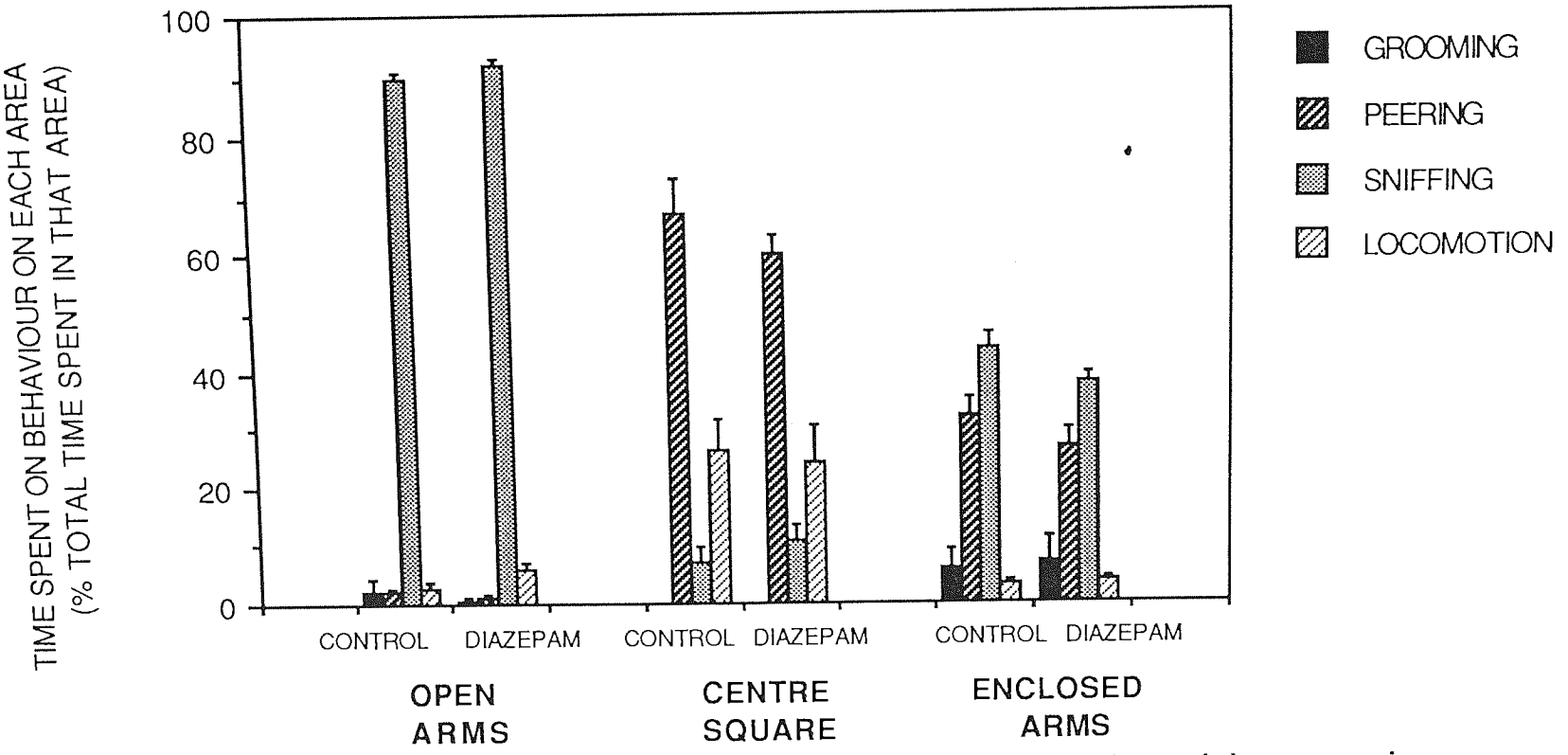


Figure 8.3. The effect of 1.5 mg/kg diazepam on the differential expression of behaviour. The graph shows mean ± sem of 6 observations per group.

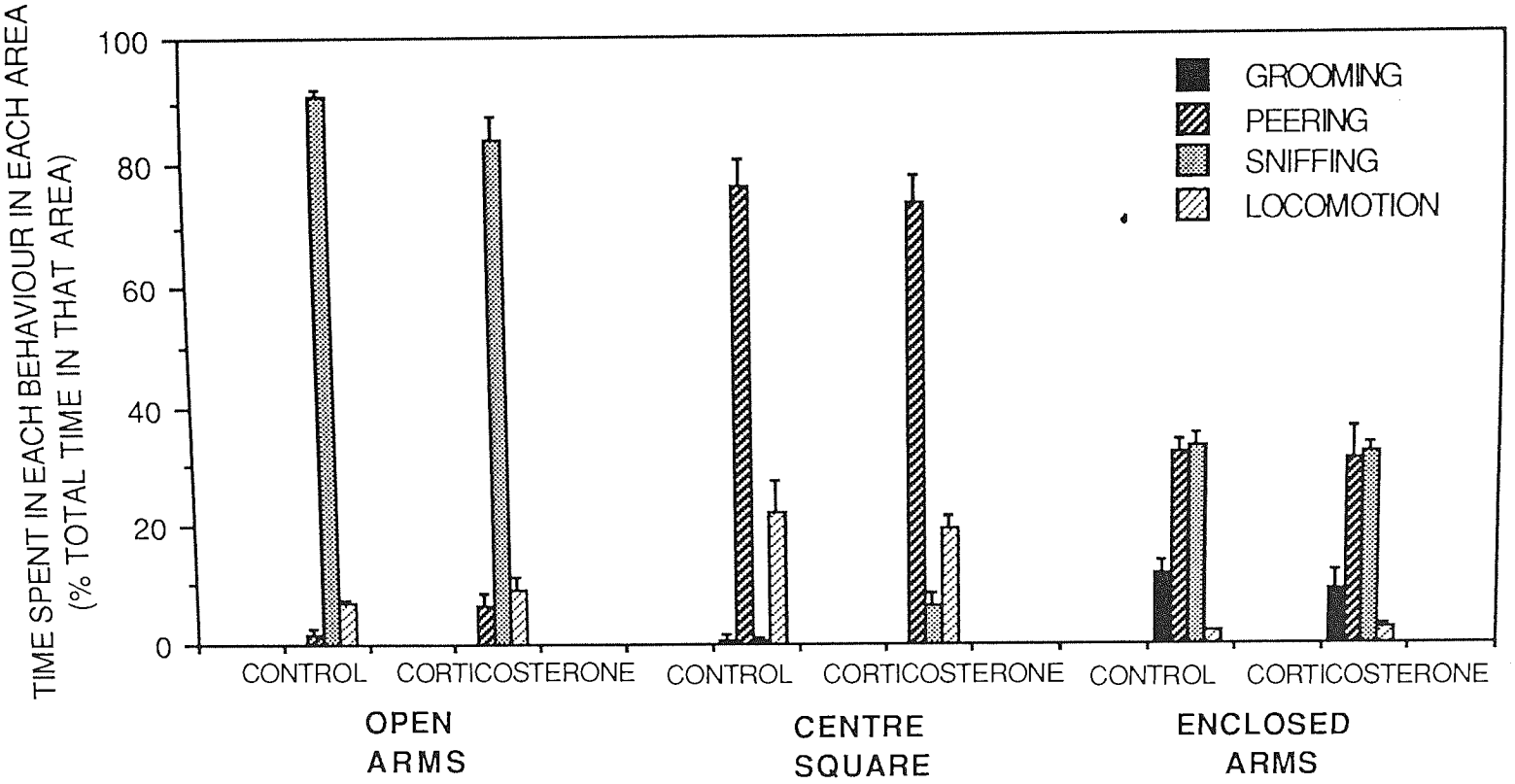


Figure 8.4. The effect of 10mg/kg corticosterone on the differential expression of behaviour in the elevated X-maze. The graph shows mean ± sem of 12 observations per group.

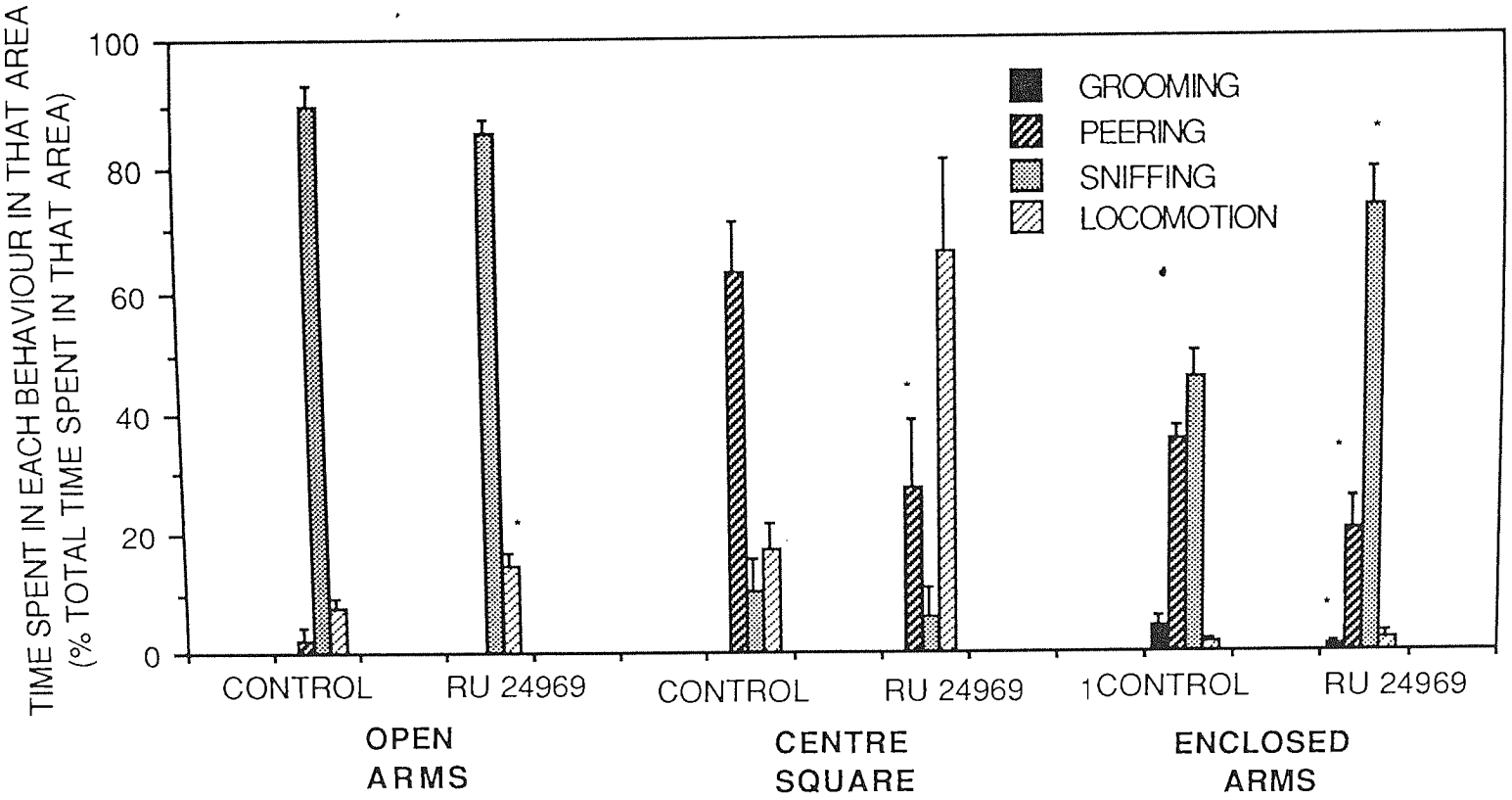


Figure 8.5. The effect of 1 mg/kg RU 24969 on the differential expression of behaviour in the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test * $P < 0.05$ compared to control.

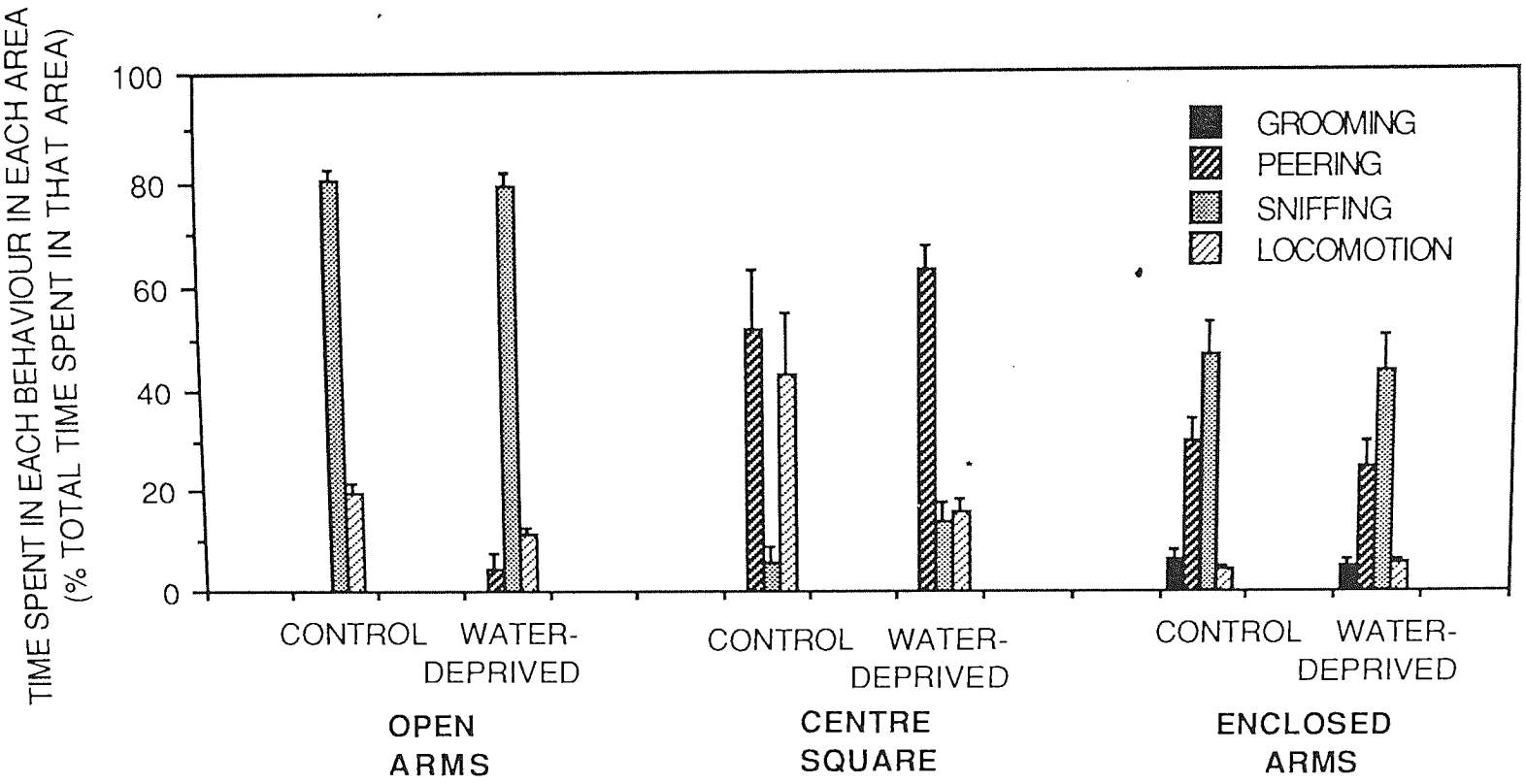


Figure 8.6. The effect of 48 hour water deprivation on the differential expression of behaviour on the elevated X-maze. The graph shows mean ± sem of 6 observations per group. Comparisons by unpaired t test
* $P < 0.05$ compared to control.

Chapter 9 General Discussion

Introduction.

The main theme of the work described in this thesis was to try to identify factors which may be responsible for producing the variety of conflicting results obtained in different animal models of "anxiety" with drugs that act through serotonergic mechanisms. In particular, the aim was to identify why ligands for the 5-HT_{1A} receptor produce highly volatile results in the elevated X-maze model of anxiety (see Introduction to Chapter 5) and to compare effects in this model with those in the Vogel test, where these agents have a more consistent "anxiolytic" effect. One possible means of explaining the variability in 5-HT_{1A} effects in the elevated X-maze lies in the fact that these agents have partial agonist properties at postsynaptic 5-HT_{1A} receptors and because of this, their mode of action, whether agonist or antagonist, could be dependent upon intrasynaptic 5-HT concentrations. Stressors associated with activation of the hypothalamic-pituitary-adrenal axis elevate brain 5-HT turnover; such stressors include footshock (Bliss et al., 1968; Thierry et al., 1968), restraint (Kennett et al., 1985; Mitchell and Thomas, 1988; Morgan et al., 1975) and food deprivation (Knott and Curzon, 1972; Knott et al., 1973). One of the differences between the Vogel test and the elevated X-maze is the degree of stress integral to the model. On the basis of the ability of the experimental protocol to increase plasma corticosterone concentration, the conclusion has been

reached that punished drinking is a more stressful procedure than the elevated X-maze (File et al., 1988). The stressfulness of an animal model might be relevant to 5-HT related drug effects, through an ability to activate 5-HT systems. This may have relevance to 5-HT_{1A} partial agonists. At the outset of the project, good evidence existed to indicate that the behaviour in the open field of animals that had been previously stressed could be modulated by 5-HT_{1A} receptors ligands (eg Kennett et al., 1987a). However, there had been no investigations into the effects of stress on behaviour of animals on the X-maze, nor any reports that the variability of 5-HT_{1A} ligands in the X-maze was explicable in terms of stress activating 5-HT systems.
The purpose of these experiments was, therefore, to characterize responses to 5-HT_{1A} ligands in the elevated X-maze under 'basal' conditions, that is in the absence of any deliberate stressor; to investigate whether restraint and/or water deprivation influences 5-HT_{1A} ligand responses in the elevated X-maze; to compare these with effects of the same drugs in the Vogel test and finally to ascertain the effect of water deprivation on central 5-HT systems.

Principal Findings.

Water deprivation was able to elevate 5-HT turnover and thus was shown to have a similar action to restraint on this measure. Water deprivation did not influence the action of any drug treatment on behaviour on the X-maze,

but did, itself, have quite a powerful "anxiolytic"-like effect on maze exploration. In contrast, restraint reduced maze exploration and was able to modify the behavioural response to the only 5-HT_{1A} agent tested in this paradigm, 8-OH-DPAT. The 'basal' "anxiogenic" effect of this compound was switched to an "anxiolytic" effect in rats restrained immediately before behavioural testing, and was abolished in rats restrained for 1 hour, 24 hours prior to testing. Increasing light intensity also switched the "anxiogenic" effect of 8-OH-DPAT to an "anxiolytic" effect.

Interpretation.

1 - The measurement of 5-HT turnover.

It was assumed in these experiments that a rise in the 5-HIAA:5-HT ratio is consonant with an increase in the activity of the serotonergic system in response to stress and that this is evidence of an increase in the synaptic concentration of 5-HT. The experiments reported here do not prove that this is so because it is not possible from whole brain studies to identify the origin of the indoles.
There is some evidence that the measurement of 5-HIAA concentrations do not accurately reflect the release of 5-HT, but primarily reflect intraneuronal metabolism of 5-HT (Kalén et al., 1988; Kalén et al., 1989a). For example, pinching the tail of rats lead to an increase in the extracellular 5-HT concentration in the hippocampus, but

did not change 5-HIAA concentration in the same animals (Kalén et al., 1989b). It is possible that increases in the extracellular concentration of 5-HIAA arises from internal metabolism of 5-HT and so is not related to 5-HT that has been released into the synapse and then transported back into the neurone (Joseph and Kennett, 1986; Kalén et al., 1988). With regard to the stressors used in the present work, an increase in the extracellular concentration of indoles, probably 5-HT, in response to restraint can be measured by in vivo voltammetry whilst restraint is ongoing (Joseph and Kennett, 1983). This would seem to indicate that there is increased release of 5-HT during restraint, even if restraint-induced increases in 5-HIAA concentration do not wholly arise from increased release of 5-HT.

It is also apparent that increased 5-HT release in response to restraint is dependent upon the synthesis of 5-HT from tryptophan, even in the presence of stored 5-HT (Kennett and Joseph, 1981). This was found to be so because it was possible to inhibit the increase in 5-HT release seen in response to restraint by pretreatment with the amino acid valine (Kennett and Joseph, 1981). This effect was assumed to arise from competition between tryptophan and valine for entry into the brain, with the consequence that reduced brain tryptophan uptake directly gave rise to reduced 5-HT synthesis and, subsequently, reduced 5-HT release. Stress-induced increases in plasma corticosterone concentration can be antagonized by the 5-HT_{1A}/ß-adrenoceptor antagonist pindolol and by spiperone (Haleem et al., 1988) suggesting

that release of 5-HT is stimulated during restraint.

The present experiments do not prove that there is increased 5-HT release during water deprivation. However, water deprivation did have the same effect on the 5-HIAA:5-HT ratio as restraint, which almost certainly does increase synaptic 5-HT concentrations.

2 - The control of 5-HT release by drugs and during stress.

There are many findings that 5-HT_{1A} receptor agonists can decrease the release of 5-HT in terminal regions (Hjörth and Sharp, 1991; Hutson et al., 1986; Marsden and Martin, 1986; Nomikos et al., 1992; Sharp et al., 1989a; Sharp et al., 1989b; Sharp et al., 1990). It is of interest that the maximal reduction in extracellular 5-HT concentration is of the order of half to three quarters the basal concentration (eg Hutson et al., 1989; Sharp et al., 1989; Wilkinson et al., 1991), a finding which does not sit well with electrophysiological reports which suggest that it is possible to completely silence Raphé cell firing with similar doses of 5-HT_{1A} receptor ligands (Jacobs and Azmitia, 1992; Sprouse and Aghajanian, 1986; Wilkinson et al., 1987). One possible explanation of these findings is that in the microdialysis studies, there is leakage of 5-HT from platelets, which clump round the implanted probe, into the dialysis fluid. In this case, a proportion of 5-HT in the dialysis fluid might be derived from platelets.

Interestingly, electrophysiological evidence from

experiments in the cat indicate that stressors capable of producing sympathetic activation and behavioural signs of distress, including restraint for 15 minutes, do not increase the rate of firing of serotonergic cells in the Raphé nuclei (Wilkinson and Jacobs, 1988). This evidence has been used to propose that increases seen in indices of 5-HT turnover do not reflect responses to stress, but are in fact responses to an increase in what is termed the 'behavioural state' of the animal (Wilkinson et al., 1991). According to this view, stress causes behavioural activation and so produces elevated 5-HT turnover, but this is a reflection of the heightened 'behavioural state' of the animal and not primarily a stress response. This view is supported by the findings that clicking sounds or an opening door produce increases in serotonergic activity but cannot be considered to be stressful (Jacobs et al., 1988). It has also been suggested that the release of 5-HT is controlled by 5-HT cell firing rate in most circumstances, but that when there is excessive serotonergic cell firing, occurring in response to behavioural activation, release is not able to keep pace with firing rate and consequently, this produces an increase in firing rates without an increase in 5-HT concentration (Jacobs and Fornal, 1991). The control of neurotransmitter release by heteroreceptor modulation might also be independent of effects on the firing rate of the cell (Wuttke et al.,1984). It might be possible that, under conditons of behavioural activation, the release of 5-HT into the synapse becomes disconnected

from the firing rate of the serotonergic cell (Wilkinson et al., 1991; Jacobs and Fornal, 1991; Jacobs and Azmitia, 1992). The 5-HT_{1A} receptor antagonist, spiperone, did not increase the firing rates of serotonergic cells in unstressed animals (Jacobs and Fornal, 1991), suggesting that there is no tonic inhibition of the firing of serotonergic cells. Evidence also suggests that a single injection of 8-OH-DPAT causes presynaptic 5-HT_{1A} receptor desensitisation when assessed 24 hours later (Beer et al., 1990) indicating that stimulation of these receptors causes desensitization. If stress increases 5-HT release, then one consequence might be that stressed rats could have desensitised presynaptic 5-HT_{1A} receptors, so leading to enhanced 5-HT tone. Antagonist activity at desensitised presynaptic 5-HT_{1A} receptors might promote a normalisation of the sensitivity and the function of these receptors.
This discussion must be viewed in the context of many experiments that suggest that 5-HT release and 5-HT neuronal activity are closely linked. Many studies have utilized the 5-HIAA:5-HT ratio as an indicator of 5-HT activity and have demonstrated its responsivity to manipulations that are thought to reduce 5-HT release (Hjörth and Magnusson, 1988; Larsson et al., 1990; Lima, 1990; Shannon et al., 1986).

3 - The standardization of the elevated X-maze.

The elevated X-maze was shown to detect the "anxiolytic"

effect of diazepam and the "anxiogenic" effects of picrotoxin. These results confirmed that it was possible to detect reponses to standard agents with both "anxiolytic" and "anxiogenic" properties. That RU 24969 was able to produce an "anxiogenic" profile, albeit not quite significant, indicated that modulation of the serotonergic system could cause changes in behaviour in the elevated X-maze and served to indicate that the initial failure to detect the effect of 8-OH-DPAT was not due to a general ability of the model at this stage to detect serotonergically mediated "anxiogenic" responses.

4 - Effects on "anxiety" of stress hormones.

The "anxiolytic" effect of corticosterone was not abolished by pretreatment with flumazenil at doses which were sufficient to antagonize the "anxiolytic" effect of diazepam. This suggests that the mechanism for the corticosterone effect is not mediated through the $GABA_A$ receptor complex, which is consistent with evidence from electrophysiological experiments that corticosterone does not influence the GABA mediated chloride flux (Gee et al., 1987). Many steroids have, however, been shown to potentiate GABA-ergic transmission in a manner similar to barbiturates (Lambert et al., 1989) and this is thought to be the mode of action of the steroid anaesthetic alphaxaolone (Gee et al., 1987; Majewska et al., 1986). The hypothesis that the "anxiolytic" effect of water

deprivation was due to the elevation of plasma corticosterone was supported by the appearance of an "anxiolytic" effect of administered corticosterone and a recent report has suggested that in the Vogel test, corticosterone produces an "anxiolytic"-like increase in the number of shocks accepted (Söderpalm and Engel, 1992). Doses chosen for the present experiments were based on evidence from published work showing that these doses produced plasma corticosterone concentrations similar to those found in response to stress (Dickinson et al., 1985; Hodges and Jones, 1963; Tizabi et al., 1989). However, the hypothesis was not supported by results of the experiments with restraint in the elevated X-maze. This stressor had the opposite effect on behaviour on the X-maze, despite producing similar changes in plasma corticosterone and brain 5-HT turnover. Additionally, water deprivation did not change the total number of entries made, whereas treatment with corticosterone did increase total entries at a dose that was not significantly "anxiolytic". Therefore, it does not seem likely that stress-induced corticosterone production was the cause of the "anxiolytic" effect of water deprivation or the "anxiogenic" effect of restraint in the elevated X-maze. It is possible that another steroid, THDOC, is responsible for the "anxiolytic" effect of water deprivation. This has been shown to have selective "anxiolytic" activity in the light/dark box test and in the Vogel conflict test (Crawley et al., 1986) and is released in response to stress (Schambelan and Biglieri, 1976).

5 - Effect of 5-HT_{1A} ligands in the elevated X-maze.

The failure to detect any "anxiety"-related effects of ipsapirone and buspirone in the elevated X-maze would suggest that this model is not able to detect the clinical anxiolytic potential of these drugs. However, as many authors have been able to detect effects of both these agents, albeit in both "anxiolytic" and "anxiogenic" directions, this still does not explain why there is such variability, nor why the present experiments failed to detect activity.

The reasons for this variation in response have been variously attributed to dose and route of drug, strain or species of animal, pre- or post- synaptic action of the drug, control baseline of the response and the type of model being used, although no systematic variation of these parameters has been shown to be sufficient to explain the degree of variability in response to these agents.

Buspirone, ipsapirone and gepirone all have a common metabolite. This is 1-PP and has considerable potency as an adrenergic α_2 antagonist (Bianchi et al., 1988; Giral et al., 1987) and is present in brain tissue in concentrations approximately 5 times that of the parent molecule after a 10 mg/kg dose (ibid.; Caccia et al., 1986). Plasma 1-PP concentrations have been reported as reaching 1.6 nmol/ml after a 10 mg/kg oral dose of buspirone (Caccia et al., 1986). Adrenergic α_2 antagonists such as yohimbine have "anxiogenic" activity in the elevated X-maze (Handley and

Mithani, 1984; Johnston et al., 1988) and "anxiolytic" activity in the Vogel test (Gower and Tricklebank, 1988). Therefore, any tendency towards either an "anxiolytic" nor an "anxiogenic" action of buspirone, ipsapirone and gepirone may well be competing with an "anxiogenic" action of a metabolite, at least in this test.

The additional weak activity of 1-PP at 5-HT_{1A} receptors (IC_{50} = 1.6 μM, Caccia et al., 1986) complicates the interpretation of these experiments. In the context of the experiments with 8-OH-DPAT using alterations in light intensity to modulate the response seen on the elevated X-maze, it is possible that the failure to observe consistent effects with buspirone and ipsapirone is because these experiments were conducted at a light intensity between that which would reveal an "anxiolytic" effect and that which would reveal an "anxiogenic" effect.

6 - Opposite effect of fluoxetine in two models of"anxiety"

There is little published information regarding the effects of acutely administered serotonin specific uptake inhibitors in animal models of "anxiety". Chronic studies in animal anxiety models have suggested that with repeated paroxetine, there is the appearance of "anxiolytic" effects in the social interaction test (Lightowler et al., 1992) and in the elevated X-maze test (Cadogan et al., 1992).

In the present study, it was apparent that fluoxetine had opposite effects in the elevated X-maze and Vogel tests.

The "anxiogenic" effect in the elevated X-maze is consistent with the general theory that elevated 5-HT function is "anxiogenic" (Iversen, 1984) because fluoxetine is known to elevate extraneuronal concentrations of 5-HT at the doses and time points used in these experiments (Schmidt et al., 1988). Acute "anxiogenic" effects of indalpine and paroxetine (Chopin and Briley, 1987) and of zimelidine (Njung'e, 1989) have been indicated previously, but the present study is the first to demonstrate a dose-related effect from fluoxetine. This "anxiogenic" profile would seem to be a property of this group of compounds.

A parallel may be drawn between the effects of buspirone in the X-maze and Vogel tests and the effects of fluoxetine in these two models. Buspirone produced "anxiolytic" activity in the Vogel test, but was found to be inactive in the elevated X-maze. Clinical experience with fluoxetine and buspirone indicates that initial effects are usually neutral or are anxiogenic (Newton et al., 1986; Nutt and Glue, 1989). The results sugested that the Vogel test is able to indicate the delayed anxiolytic effects of serotonergic agents, but after acute administration, although how such a short cut could be acheived is not indicated by these results. The elevated X-maze may detect the initial anxiogenic experience of these compounds.

There is evidence to suggest that 5-HT uptake inhibitors preferentially inhibit 5-HT uptake in the Raphé nucleus with respect to the cerebral cortex (Adell and Artigas, 1991; Bel and Artigas, 1992). One consequence of this is

that by reducing Raphé firing 5-HT uptake inhibitors actually reduce the extraneuronal 5-HT concentration in the cerebral cortex (Invernizzi et al., 1992). Fluoxetine has been shown to increase extracellular 5-HT concentrations in the striatum after the doses and route used here (Perry and Fuller, 1992). It is possible that regional differences in effect on synaptic 5-HT could underlie the differences in responses between the elevated X-maze and punished drinking observed here.

7 - Stress effects in models of anxiety and depression.

Recently, Falter et al (1992) have demonstrated that a number of stressful procedures do not influence behaviour on an elevated X-maze. The finding of the present study that a short period (15 minutes) of restraint did not influence behaviour was replicated (Falter et al., 1992), but longer time points were not investigated. It was also shown that the behaviour of animals on the elevated X-maze was not influenced by a prior forced swim test or prior electric shock (Falter et al., 1992). From these results, the authors' conclusions that the baseline of the elevated X-maze is fairly robust, seems quite logical. The stressors used in the present studies were, however, probably of greater intensity even than those reported in the study by Falter et al. (1992).

Restraint and water deprivation had similar effects on the ativity of brain 5-HT systems, but had opposing effects on

the behaviour of animals in the maze. As discussed in chapter 6 the possibility that the effects of these procudures on elevated X-maze behaviour were due to increased approach drive (water deprivation) or motor impairment (restraint) has not yet been resolved. The opposite behavioural effects of restraint and water deprivation suggest that there is not any simple relationship between 5-HT turnover and responses in the elevated X-maze and that there are other factors which have a major input in regulating biochemical and behavioural responses to these stressors. The two stressors clearly differ in their duration of impact upon the animal, with restraint being an acute procedure and water deprivation being sub-chronic. Noradrenaline and dopamine are also involved in the response to stress, but on prolonged challenge neurochemical stores of these amines become depleted (Anisman et al., 1981). This does not occur with serotonin (Adell et al., 1988) and perhaps this could be the source of the difference between the two stressors. However, the important measure is the turnover of these amines; measurements of noradrenaline and dopamine concentratins per se do not indicate the rate of turnover of the amine.

Previously, restraint has been found to have an "anxiogenic"-like effect in an open field (Kennett et al., 1985a) and in the light / dark model (Carli et al., 1989). This, together with the present effects of restraint in the elevated X-maze, is consistent with the postulated

association between increased serotonergic activity and anxiety (Iversen, 1984). However, in each case, these "anxiogenic" effects were still present 24 hours later, at a time when serotonin turnover has returned to normal according to the data of Dickinson et al. (1985). The reduction in exploration that occurs subsequent to restraint is attributed to an effect related to depression, rather than an "anxiogenic" effect. This claim is strengthened by evidence that, although antidepressants of different classes can reverse deficits in behaviour in response to restraint, acute treatment with benzodiazepines does not (Kennett et al., 1987a).

There was some similarity between the effect of a 1 hour restraint on behaviour on the elevated X-maze 24 hours later and a 1 hour exposure to the maze 24 hours before testing on the maze. In both cases, there was a tendency towards a reduction in the open : total entry ratio and open arm time and also a reduction in total activity. In view of the fact that both restraint and elevated X-maze exposure increase extracellular 5-HT concentration (Morgan et al., 1975; Rex et al., 1991), there may be some causal relationship between the neurochemical and behavioural changes.

8 -5-HT and anxiety - changing perspectives.

The theory linking 5-HT with anxiety mechanisms was developed from animal experiments which used the

reinstatement of responding suppressed by punishment to indicate "anxiolytic" effects (eg Robichaud and Sledge, 1969). According to some experimenters, pCPA (Robichaud and Sledge, 1969) and 5-HT antagonists (Graeff and Schoenfield, 1970) were able to produce an increase in punished responding similar to that of benzodiazepine anxiolytics (Geller and Seifter, 1960). Shortly after, it was discovered (Stein et al., 1973) that benzodiazepines decrease the activity of serotonergic neurones in the Raphé nuclei. Further evidence accumulated which was consistent with the postulated role for 5-HT in anxiety. For instance, 5,7-DHT lesions of the ventral tegmentum had a marked "anxiolytic" effect (Tye et al., 1977) which could be reversed by 5-HTP treatment (Tye et al., 1977).

This evidence was discussed in a seminal review (Iversen, 1984) which reiterated the main theme which had developed over the preceding 15 years, namely that a reduction in serotonergic activity lessened anxiety and that promoting serotonergic activity increased anxiety.

However, as was discussed in the introductory chapter, there are many findings which cannot be incorporated into this rather simplistic structure. An example is the finding of no "anxiolytic" activity of so-called classical 5-HT antagonists in animal models (eg Gardner and Guy, 1984). Another is the apparent "anxiolytic" activity of at least some doses of 5-HTP in both the elevated X-maze (Söderpalm et al., 1989) and the Vogel test (Hjörth et al., 1987b).

During the course of the work described in this thesis, a

new theory of how 5-HT systems are involved in anxiety states was proposed (Deakin and Graeff, 1991). The concept of Deakin and Graeff (1991) that brain 5-HT systems have multiple roles in the control of "anxiety" was presented in the first chapter. The key components of this theory can be summarised as follows. Two serotonergic systems were proposed to originate from the dorsal Raphé nucleus. One pathway innervates the dorsal periaqueductal gray (DPAG) whereas another pathway innervates many other structures, including the striatum. A reduction of 5-HT in the DPAG, which would follow from action on the somatodendritic 5-HT_{1A} receptor in the dorsal Raphé, promotes panic attacks in humans and in animals elicits the fight / flight response consistent with "anxiogenic" responses in animal models of anxiety. This has been termed the proximal avoidance reaction because it is presumed to be activated by proximal stimuli and functions to allow escape from the current situation. A reduction in the functional tone of 5-HT in the striatum, which would also arise from action at 5-HT_{1A} receptors on dorsal Raphé neurones, would enhance dopamine-mediated approach behaviour which in certain models would promote an "anxiolytic" effect. This has been termed the distal avoidance reaction because it has the function of allowing the animal to assess whether to approach or avoid a potentially threatening situation.

If the theory of Deakin and Graeff is correct, then the dorsal Raphé nucleus has a crucial role to play in determining the action of 5-HT_{1A} receptors in animal models

of anxiety, because agonist action at the same receptor could produce either an "anxiolytic" or an "anxiogenic" effect depending on the balance between the influence of the DPAG (mediating a proximal defence reaction) and the striatum (mediating a distal defence reaction) in controlling behaviour. Handley and M^{c}Blane (1991) suggested that the elevated X-maze should be sensitive to both the distal and the proximal defence reactions. Once on the open arm, the proximal defence reaction will promote escape from the open arm, but in the enclosed arm, the distal avoidance reaction will determine whether an animal enters an open arm. The suggestion that the elevated X-maze detects more than one anxiety mechanism has since been supported by the finding that, with one of the enclosed arms of the elevated X-maze closed off, acute diazepam treatment caused an "anxiolytic" decrease in emergence latency after placing rats in an enclosed arm, but had no effect on latency to leave an open arm, when rats were placed on an open arm (Viana et al., 1992). As the dorsal Raphé is thought to control both systems, a 5-HT_{1A} receptor ligand could in theory produce both "anxiolytic" and "anxiogenic" effects by suppressing dorsal Raphé firing.

Previous investigations from this laboratory suggested that the "anxiogenic" effect of 8-OH-DPAT in the elevated X-maze is derived from a presynaptic action in the dorsal Raphé nucleus (Critchley et al., 1992). This was an interesting finding because it had previously been ascertained that the "anxiolytic" effect of 8-OH-DPAT in the Vogel test was also

of presynaptic origin (Engel et al., 1984). These two pieces of evidence again suggest a dual role for 5-HT systems in modulating anxiety.

Many of the results in this thesis are consistent with the proposition that both "anxiolytic" and "anxiogenic" responses to 5-HT_{1A} ligands are presynaptically mediated. The response to 8-OH-DPAT in the X-maze was altered by restraint and by light intensity, although not by water deprivation.

The"anxiolytic" effect of 8-OH-DPAT in restrained rats may or may not derive from a presynaptic mechanism. It could conceivably be due to an antagonist effect at postsynaptic 5-HT_{1A} receptors, as discussed in chapter 6, or to desensitising presynaptic 5-HT_{1A} receptors by restraint. Kostowski et al. (1989) showed an "anxiolytic" effect of intrahippocampal buspirone, although it is not known whether agonist or antagonist action produced this effect. The effect of restraint on presynaptic 5-HT_{1A} receptor function has not been directly investigated. It may be that the loss of "anxiogenic" effect of 8-OH-DPAT in rats previously exposed to a single restraint episode reflects a reduction in the sensitivity of the presynaptic receptor. This conclusion is based on evidence that the "anxiogenic" effect of 8-OH-DPAT is presynaptic (Critchley et al., 1992) and that presynaptic 5-HT_{1A} receptors become subsensitive 24 hours after a single administration of 8-OH-DPAT (Beer et al., 1990). Perhaps increased 5-HT release in response to restraint stress causes stimulation of presynaptic 5-HT_{1A}

autoreceptors to produce desensitization in a similar manner to treatment with a large dose of 8-OH-DPAT.

The antidepressant effect of 5-HT_{1A} ligands in animal models of depression has been explained in the context of their ability to desensitize presynaptic 5-HT_{1A} receptors (Kennett et al., 1987). This group of ligands reverse restraint stress-induced deficits in locomotor activity in a manner similar to other antidepressants, an effect not possessed by anxiolytics (Kennett et al., 1987) and do so under conditions where they are known to produce desensitization to tests of presynaptic 5-HT_{1A} receptor function such as hypothermia (Goodwin et al., 1985). Presynaptic desentisation might conceivably underlie the "anxiolytic" effect of 5-HT_{1A} ligands in animal models.

In restrained rats, intrahippocampal 8-OH-DPAT has been shown to elicit an "anxiolytic" effect 24 hours later in the elevated X-maze (Guimaraes, 1993). These results are of relevance to the present discussion because in these experiments, the "anxiolytic" effect of intrahippocampal 8-OH-DPAT was only apparent in rats that had been restrained. Repeated restraint does sensitize postsynaptic 5-HT_{1A} receptors (Dickinson et al., 1985; Kennett et al., 1985a, b) but this effect is not observed after a single restraint (ibid.).

Although this evidence suggests postsynaptic hippocampal 5-HT_{1A} receptors are involved in "anxiety"-related effects of 5-HT_{1A} ligands, they do not disprove the supposition that both "anxiolytic" and "anxiogenic" effects can be derived

from presynaptic actions of 5-HT_{1A} ligands.

This discussion has concentrated heavily on the 5-HT_{1A} receptor and behavioural deficits induced by restraint. It is interesting to note that such deficits are also antagonised by antagonists of corticotrophin releasing factor (CRF) (Berridge and Dunn, 1989) and by the α_2 adrenoceptor agonist clonidine (ibid.) and that the anorexia induced by restraint can be prevented by antagonism of CRF (Krahn et al., 1986).

Light intensity was also found to modulate the action of 8-OH-DPAT in the elevated X-maze. The "anxiolytic" effect observed under high light intensity was shown to be abolished by pCPA pretreatment which might suggest that the effect was of presynaptic origin. This conclusion can only be tentative at present because the brain 5-HT content of the lesioned rats was not determined; however, this dose of pCPA is known to produce upwards of 70 % depletion of 5-HT concentration in rat brain (Engel et al., 1984; Critchley et al., 1992) with this regimen. Additionally, because pCPA itself had a large "anxiolytic" effect the possibility that a ceiling in the "anxiolytic" effect had been reached cannot be disregarded. However, with these reservations, the experiment suggested that the high light "anxiolytcic" effect was of presynaptic origin.

Results from both the restraint and the light intensity series of experiments indicated that it was possible to switch the action of a 5-HT_{1A} receptor agonist by modulation of the conditions of the experiment. Therefore, it was

shown that the elevated X-maze is capable of detecting opposite effects of the same drug under circumstances which are identical apart from a single manipulation, such as light intensity or previous restraint.

The ability of the elevated X-maze to detect two opposing mechanisms of action of 8-OH-DPAT on anxiety may be the cause of the reported variability of 5-HT_{1A}-related compounds in this test if the two mechanisms are in unstable balance. The experiments with fluoxetine in X-maze and Vogel tests also support the view that animal models can detect opposite actions of serotonin in "anxiety".

9 Concluding Comments.

Several interesting lines of investigation are suggested by the results obtained during the course of this work. Several exciting possibilities lie in further experiments into the Deakin and Graeff model of the dual role of the dorsal Raphé nucleus in anxiety. Do lesions of the DPAG remove the "anxiogenic" responses to 5-HT_{1A} ligands in general and of 8-OH-DPAT in particular in the elevated X-maze ? Similarly, do lesions of the striatun allow only "anxiogenic" responses to be detected? Selective 5-HT_{1A} antagonists such as WAY 100135 could be used to investigate "anxiolytic" effects on the X-maze after intrahippocampal injection. It would be predicted that such compounds would block the "anxiolytic" effect of buspirone in the Vogel test and block both the "anxiogenic" and "anxiolytic"

effects of 8-OH-DPAT in the elevated X-maze if these are due to agonist actions. Further investigations with 5-HT_{1A} ligands other than 8-OH-DPAT on the ability of light intensity and of restraint to modulate responses to these ligands in the elevated X-maze are warranted as are 5,7-DHT lesion studies to identify the source of the "anxiolytic" effect of 8-OH-DPAT. Finally, it would be of interest to discover whether water deprivation increased extracellular concentrations of 5-HT. The technique of microdialysis would be appropriate to answer this question. Clearly, more experiments are required to answer these questions.

Throughout this thesis, the words anxiolytic and anxiogenic have appeared in inverted commas when applied to an animal model. Animal models are only able to provide information regarding changes in the expressed behaviour in the presence of a perceived threat. These changes are adaptive and consitute some form of appropriate response for the animal in that situation. In contrast, human anxiety states are, by definition, maladaptive and a considerable amount of distress may be present without there being any overt changes in human behaviour. For these reasons, it is difficult to say that the results reported here have a direct relevance to the understanding of the clinical disorder of anxiety. Their importance lies in the ability of animal models to contribute to an understanding of mechanisms of action of drugs used to treat anxiety.

References.

Aarseth, P. and Klug, D. (1972) Acta Physiol. Scand. 85 277-282 Dehydration-induced reductions in blood volume and in pulmonary blood volume in rats.

Abbot, A. (1987) Trends Pharmacol. Sci. 8 93-94 can animal models really predict anxiety?

Adell, A. and Artigas, F. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. 343 237-244 Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex.

Adell, A., Garcia-Marquez, C., Armario, and Gelpí, E. (1988) J. Neurochem. 50 1678-1681 Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their response to a further acute stress.

Adham, N., Romanienko, P., Hartig, P., Weinshank, R.L. and Branchek, T. (1992) Mol. Pharmacol. 41 1-7 The rat 5-hydroxytryptamine$_{1B}$ receptor is the species homologue of the human 5-hydroxytryptamine$_{1D\beta}$ receptor.

Ahlenius, S. and Larsson, K. (1987) Evidence for a unique pharmacological profile of 8-OH-DPAT by evaluation of its effects on male rat sexual behaviour. in Brain 5-HT_{1A} receptors (Eds: C.T. Dourish, S.Ahlenius and P.H. Hutson) Ellis Horwood Ltd., Chichester, pp 185-198.

Ahlenius, S., Fernandez-Guasti, A., Hjörth, S. and Larsson, K. (1986) Eur. J. Pharmacol. 124 361-363 Suppression of lordosis behavior by the putative 5-HT receptor agonist 8-OH-DPAT in the rat.

Almeida, S.S., de Oliviera, I.M. and Graeff, F.G. (1991) Psychopharmacol. 103 513-518 Early life protein malnutrition changes exploration of the elevated plus-maze and reactivity to anxiolytics.

Amsterdam, J. D. (1992) Prog. Neuropsychopharmacol. Biol. Psychiat. 16 271-280 Gepirone, a selective serotonin (5-HT_{1A}) partial agonist in the treatment of major depression.

Anderson, I.M., Cowen, P.J. and Grahame-Smith, D.G. (1990) Psychopharmacol. 100 498-503 The effects of gepirone on neuroendocrine function and temperature in humans.

Andrade, R. and Nicoll, R.A. (1987a) Naunyn-Schmiedeberg's Arch. Pharmacol. 336 5-10 Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurones of the rat hippocampus.

Andrade, R. and Nicoll, R.A. (1987b) J. Physiol. 394 99-124 Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro.

Anisman, H., Pizzino, A. and Sklar, L.S. (1980) Brain Research 191 583-588 Coping with stress, norepinephrine depletion and escape performance.

Arnt, J. and Hyttel, J. (1989) Eur. J. Pharmacol. 161 45-51 Facilitation of 8-OH DPAT induced forepaw treading of rats by the 5-HT_2 agonist DOI.

Arvidsson, L.E., Hacksell, U., Lars, J., Nilsson, G., Hjörth, S., Carlsson, A., Lindeberg, P., Sanchez, D. and Wilkström, H. (1981) J. Med. Chem. 24 921-923 8-hydroxy-2-(di-n-propylamino) tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist.

Azmitia, E.C. and McEwen, B.S. (1969) Science 166 1274-1276 Corticosterone regulation of tryptophan hydroxylase in midbrain of the rat.

Backus, L.I., Sharp, T. and Grahame-Smith, D.G. (1990) Brit. J. Pharmacol. 100 793-799 Behavioural evidence for a functional interaction between central 5-HT_{1A} and 5-HT_2 receptors.

Bagdy, G., Calogero, A.E., Murphy, D.L. and Szemeredi, K. (1989) Endocrinol. 125 2664-2669 Serotonin agonists cause parallel activation of the sympathoadrenomedullary system and the hypothalamo-pituitary-adrenocortical axis in conscious rats.

Baldwin, H.A., Johnston, A.L. and File, S.E. (1989) Eur. J. Pharmacol. 159 211-215 Antagonistic effects of caffeine and yohimbine in animal tests of anxiety.

Barnes, J.M., Barnes, N.M., Costall, B., Ironside, J.W. and Naylor, R.J. (1989) J. Neurochem. 53 1787-1793 Identification and characterization of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue.

Barnes, J.M., Barnes, N.M., Costall, B., Naylor, I.L., Naylor, R.J. and Rudd, J.A. (1990) Naunyn-Schmiedeberg's Arch. Pharmacol. 342 17-21 Topographical distribution of 5-HT_3 receptor recognition sites in the ferret brain stem.

Barrett, J.E. and Gleeson, S. (1991) in 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines : their comparative pharmacology (Eds: R.J. Rodgers and S.J. Cooper) John Wiley and Sons pp 59-105 Anxiolytic effect of 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines : conflict and drug discrimination studies.

Barrett, J.E. and Witkin, J.M. (1991) in Buspirone : mechanisms and clinical aspects (Eds: G. Tunnicliff, A.S. Eison and D.P. Taylor) Academic Press Inc. pp 37-79 Buspirone in animal models of anxiety.

Barrett, J.E., Hoffman, S.M., Olmstead, S.N., Foust, M.J., Harrod, C. and Weissman, B.A. (1989) Psychopharmacol. 97 319-325 Behavioural and neurochemical effects of the serotonin (5-HT)$_{1A}$ receptor ligand spiroxatrine.

Baudrie, V. and Chaouloff, F. (1991) Eur. J. Pharmacol. 198 129-135 Repeated treatment with the 5-HT$_{1A}$ receptor agonist, ipsapirone, does not affect 8-OH-DPAT- and stress-induced increases in plasma adrenaline levels in the rat.

Bealer, S.L. and Schneider, E.G. (1985) Am. J. Physiol. 248 R161-165 Plasma corticosterone and volume after preoptic recess lesions and volume depletion.

Beckett, S.R.G., Lawrence, A.J., Marsden, C.A. and Marshall, P.W. (1992) Psychopharmacol. 108 110-114 Attenuation of chemically induced defence response by 5-HT$_1$ receptor agonists administered into the periaqueductal gray.

Bedard, P. and Pycock, C.J. (1977) Neuropharmacol. 16 663-670 "Wet-dog" shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity.

Beer, M., Kennett, G.A. and Curzon, G. (1990) Eur. J. Pharmacol. 178 179-187 A single dose of 8-OH-DPAT reduces raphe binding of [^{3}H]8-OH-DPAT and increases the effect of raphe stimulation on 5-HT metabolism.

Bel, N. and Artigas, F. (1992) Eur. J. Pharmacol. 229 101-103 Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei : an in vivo microdialysis study.

Bendotti, C. and Samanin, R. (1987) Life Sci. 41 635-642 The role of putative 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in the control of feeding in rats.

Benjamin, D., Lal, H. and Meyerson, L.R. (1990) Life Sci. 47 195-203 The effects of 5-HT$_{1B}$ characterizing agents in the mouse elevated plus-maze.

Berendson, H.H.G., Jenck, F. and Broekkamp, C.L.E. (1989) Pharmacol. Biochem. Behav. 33 821-827 Selective activation of 5-HT$_{1A}$ receptors induces lower lip retraction in the rat.

Berridge, C.W. and Dunn, A.J. (1989) J. Neurosci. 9 3513-3521 Restraint-induced changes in exploratory behaviour appear to be mediated by norepinephrine-stimulated release of CRF.

Bianchi, G., Caccia, S., Della Vedova, F. and Garatini, S. (1988) Eur. J. Pharmacol. 151 365-371 The α_2 antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl) piperazine (PmP).

Bill, D.J., Brown, G. and Fletcher, A. Brit. J. Pharmacol. (in press) Stereospecific blockade of 8-OH-DPAT-induced hypothermia in rodents by the selective 5-HT_{1A} receptor antagonist WAY 100135.

Bitar, M. and Weiner, M. (1984) Diabetes 33 37-44 Diabetes-induced metabolic alterations in heme synthesis and degradation and various heme-containing enzymes in female rats.

Björk, L., Cornfiled, L.J., Nelson, A., Hillver, S.E., Anden, N.E., Lewander, T and Hacksell, U. (1991) J. Pharmacol. Exp. Ther. 258 58-65 Pharmacology of the novel 5-hydroxytryptamine$_{1A}$ receptor antagonist (S)-5-fluoro-8-hydroxy-2-(di-n-propylamino) tetralin: inhibition of (R)-8-hydroxy-2-(di-n-propylamino) tetralin-induced effects.

Blackburn, T.P. (1992) in Central serotonin receptors and psychotropic drugs (Eds: C.A. Marsden and D.J. Heal) Blackwell Scientific Publications, London, pp 175-197 5-HT receptors and anxiolytic drugs.

Blakely, T.A. and Parker, L.F. (1973) Pharmacol. Biochem. Behav. 1 609-613 The effects of parachlorophenylalanine on experimentally induced conflict behaviour.

Blanchard, D.C., Rogers, R.J., Hendrie, C.A. and Hori, K. (1988) Pharmacol. Bioochem. Behav. 31 269-278 "Taming" of wild rats (*Rattus rattus*) by $5HT_{1A}$ agonists buspirone and gepirone.

Blier, P. and De Montigny, C. (1987) Synapse 1 470-480 Modification of 5-HT neurone properties by sustained administration of the 5-HT_{1A} agonist gepirone : electrophysiological studies in the rat brain.

Blier, P. and De Montigny, C. (1990) J. Cardiovasc. Pharmacol. 15 S42-S48 Electrophysiological investigation of the adaptive response of the 5-HT system to the administration of 5-HT_{1A} receptor agonists.

Bliss, E.L., Ailion, J. and Zwanziger, J. (1968) J. Pharmacol. Exp. Ther. 164 122-134 Metabolism of norepinephrine, serotonin and dopamine in rat brain with stress.

Bockeart, J., Dumuis, A., Bouhelal, R., Sebben, M. and Cory, R.N. (1987) Naunyn-Schmiedeberg's Arch. Pharmacol. 335 588-592 Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists at 5-HT_{1A} receptors negatively coupled with adenylate cyclase in hippocampal neurons.

Boddeke, H.W.G.M., Fargin, A., Raymond, J.R., Schoeffter, P. and Hoyer, D. (1992) Naunyn-Schmiedeberg's Arch. Pharmacol. 345 257-263 Agonist/antagonist interactions with cloned human 5-HT_{1A} receptors : variations in intrinsic activity studied in transfected HeLa cells.

Bohus, B., Koolhaas, J.M., Korte, S.M., Bouws, G.A.H., Eisenga, W. and Smit, J. (1990) Neurosci. Biobehav. Rev. 14 529-534 Behavioural physiology of serotonergic and steroid-like anxiolytics as antistress drugs.

Boissier, J.R., Simon, P. and Aron, C. (1968) Eur. J. Pharmacol. 4 145-151 A new method for rapid screening of minor tranquillizers in mice.

Bradley, P.B., Engel, G., Fenuik, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986) Neuropharmacol. 25 563-576 Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine.

Briley, M., Chopin, P. and Moret, C. (1990) Psychopharmacol. 101 187-189 Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat.

Britton, D.R. and Indyk, E. (1990) Psychopharmacol. 101 366-370 Central effects of corticotropin-releasing factor (CRF) : evidence for similar interactions with environmental novelty and with caffeine.

Britton, K.T., McLeod, S., Koob, G.F. and Haugher, R. (1992) Pharmacol. Biochem. Behav. 41 399-403 Pregnane steroid alphaxalone attenuates anxiogenic behavioral effects of corticotropin releasing factor and stress.

Brocco, M.J., Koek, W., Degryse, A.-D. and Colpeart, F.C. (1990) Behavioural Pharmacology 1 403-418 Comparative studies on the anti-punishment effects of chlordiazepoxide, buspirone and ritanserin in the pigeon, Geller-Seifter and Vogel conflict procedures.

Bush, I.E. (1953) J. Endocrinol. 9 95-100 Species differences in adrenocortical secretion.

Caccia, S., Conti, I., Vigano, E. and Garattini, S. (1986) Pharmacology 33 46-51 1-(2-pyrimidinyl)-piperazine as an active metabolite of buspirone in man and rat.

Cadogan, A.-K., Wright, I.K., Coombs, I., Kendall, D.A. and Tulloch, I. (1992) Neurosci. Lett. Suppl. 42 S8 Repeated paroxetine administration in the rat produces an anxiolytic profile in the elevated X-maze and a decreased ^{3}H-ketanserin binding.

Calogero, A.E., Bagdy, G., Szemeredi, K., Tartaglia, M.E., Gold, P.W. and Chirousos, G.P. (1990) Endocrinol. 126 1888-1894 Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic-pituitary-adrenal axis in the rat.

Cannon, W.B. (1929) Bodily changes in pain, hunger, fear and rage. Reprinted 1963 by Harper and Ross, New York.

Carli, M. and Samanin, R. (1982) Pharmacol. Biochem. Behav. 17 1-3 Evidence that agents increasing water consumption do not necessarily generate "false positives" in conflict procedures using water as a reinforcer.

Carli, M. and Samanin, R. (1988) Psychopharmacol. 94 84-91 Potential anxiolytic properties of 8-Hydroxy-2-(di-n-propylamino) tetralin, a selective serotonin$_{1A}$ receptor agonist.

Carli, M., Pronterra, C. and Samanin, R. (1989a) Brit. J,. Pharmacol. 96 829-836 Evidence that central 5-hydroxy-tryptaminergic neurones are involved in the anxiolytic activity of buspirone.

Carli, M., Pronterra, C. and Samanin, R. (1989b) Neuropharmacol. 28 471-476 Effect of 5-HT$_{1A}$ agonists on stress-induced deficit in open field locomotor activity of rats : evidence that this model identifies anxiolytic-like activity.

Carvalho, J.S. (1989) J. Cardiovasc. Pharmacol. 13 603-607 Heterologous serum albumin as tracer for plasma volume measurements evaluated in conscious nondehydrated and 48-hour water-deprived rats.

Ceulemans, D.L.S., Hoppenbrowers, M.L.J.A., Gelders, Y.G. and Reyntjens, A.J.M. (1985) Pharmacol. Psychiat. 18 303-305 The influence of ritanserin, a serotonin antagonist, in anxiety disorders: a double blind placebo-controlled study versus lorazepam.

Chaouloff, F. and Jeanrenaud, B. (1987) J. Pharmacol. Exp. Ther. 243 1159-1166 5-HT$_{1A}$ and alpha-2 adrenergic receptors mediate the hyperglycaemic and hypoinsulinaemic effects of 8-hydroxy-2-(di-n-propylamino) tetralin on the conscious rat.

Chaouloff, F., Baudrie, V. and Laude, D. (1990) Eur. J. Pharmacol. 177 107-110 Evidence that the 5-HT_{1A} receptor agonists buspirone and ipsapirone activate adrenaline release in the conscious rat.

Chaput, Y., De Montigny, C. and Blier, P. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 333 342-348 Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors : electrophysiological studies in the rat brain.

Charney, D.S., Woods, S.W., Goodman, W.K. and Heninger, G.R. (1987) Psychopharmacol. 92 14-24 Serotonin function in anxiety II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects.

Chignon, J.M. and Lepine, J.P. (1989) Lancet II 46-47 Panic and hypertension associated with single dose of buspirone.

Chojnacka-Wojcik, E. and Przegalanski, E. (1991) Neuropharmacol. 30 703-709 Evidence for the involvement of 5-HT_{1A} receptors in the anticonflict effect of ipsapirone in rats.

Chopin, P. and Briley, M. (1987) Trends Pharmacol. Sci. 8 383-388 Animal models of anxiety : the effect of compounds that modify 5-HT neurotransmission.

Claustre, Y., Rouquier, L., Serrano, A., Bénavidès, J. and Scatton, B. (1991) Eur. J. Pharmacol. 204 71-77 Effect of the putative 5-HT_{1A} receptor antagonist NAN-190 on rat brain serotonergic transmission.

Colino, A. and Halliwell, J.V. (1986) Eur. J. Pharmacol. 130 151-152 8-OH-DPAT is a strong antagonist of 5-HT action in rat hippocampus.

Commissaris, R.L. and Rech, R.H. (1982) Psychopharmacol. 76 282-285 Interactions of metergoline with diazepam, quipazine and hallucinogenic drugs on a conflict behaviour in the rat.

Conn, P.J., Sanders-Bush, E., Hoffman, B.J. and Hartig, P.R. (1986) Proc. Natl. Acad Sci. USA 83 4086-4088 A unique serotonin receptor in choroid plexus is linked to phosphatidyl inositol turnover.

Cook, L. and Davidson, A.B. (1973) in The Benzodiazepines (Eds : S. Garatini, E. Mussini and L.O. Randall) Raven Press N.Y. Effects of behaviourally active drugs in a conflict-punishment procedure in rats.

Cook, L. and Sepinwall, J. (1975) Adv. Biochem. Psychopharmacol. 14 1-28 Behavioural analysis of the effects and mechanisms of action of benzodiazepines.

Cooke, D.J. and Hole, D.J. (1983) Brit. J. Psychiat. 143 397-400 The aetiological importance of stressful life events.

Corne, S.J. and Pickering, R.W. (1967) Psychopharmacol. 11 65-78 A possible correlation between drug-induced hallucinations in man and a behavioural response in mice.

Costall, B. and Naylor, R.J. (1992) Pharmacol. Toxicol. 70 157-162 Anxiolytic potential of 5-HT_3 receptor antagonists.

Costall, B., Kelly, M.E., Tomkins, D.M. and Tyers, M.B. (1987) J. Psychopharmacol. 1 10P Profile of action of diazepam and 5-HT_3 receptor antagonists in the elevated X-maze.

Costall, B., Kelly, M.E., Naylor, R.J. and Onavi, E.S. (1988) J. Pharm. Pharmacol. 40 494-500 Actions of buspirone in a putative model of anxiety in the mouse.

Crawley, J.N. Pharmacol. Biochem. Behav. (1981) 15 695-699 Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines.

Crawley, J. and Goodwin, F.K. (1980) Pharmacol. Biochem. Behav. 13 167-170 Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines.

Crawley, J.N., Glowa, J.R., Majewska, M.D. and Paul, S.M. (1986) Brain Research 398 382-385 Anxiolytic activity of an endogenous adrenal steriod.

Crespi, F., Wright, I.K. and Möbius, C. (1992) Exp. Brain Research 88 495-501 Isolation rearing of rats alters release of 5-hydroxytryptamine and dopamine in the frontal cortex : an in vivo electrochemical study.

Critchley, M.A.E. and Handley, S.L. (1986) Psychopharmacol. 89 S56 (170) Anxiogenic effects of three 5-HT agonists.

Critchley, M.A.E. and Handley, S.L. (1987) Psychopharmacol. 93 502-506 Effects in the X-maze anxiety model of agents acting at 5-HT_1 and 5-HT_2 receptors.

Critchley, M.A.E., Njung'e, K. and Handley, S.L. (1987) Brit. J. Pharmacol. 90 659P 5-HT ligand effects in the social interaction test of anxiety.

Critchley, M.A.E., Njung'e, K. and Handley, S.L. (1992) Psychopharmacol. 106 484-490 Actions and some interactions of 5-HT_{1A} ligands in the elevated X-maze and effects of dorsal raphe lesions.

Cunningham, K.A., Callahan, P.M. and Appel, J.B. (1987) Eur. J. Pharmacol. 138 29-36 Discriminitive stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) : implications for understanding the actions of novel anxiolytics.

Curzon, G. and Marsden, C.A. (1975) J. Neurochem. 25 251-256 Metabolism of a tryptophan load in the hypothalamus and other brain regions.

Curzon, G., Joseph, M.J. and Knott, P.J. (1972) J. Neurochem. 19 1967-1974 Effects of immobilization and food deprivation on rat brain tryptophan metabolism.

Dahlströhm, A. and Fuxe, K. (1964) Acta Physiol. Scand. 62 1-55 Evidence for the existence of monoamine containing neurones in the central nervous system - 1 - demonstration of monoamines in the cell bodies of brain stem neurones.

Davis, M. (1979a) Psychopharmacol. 54 341-347 Morphine and naloxone : effects on conditioned fear as measured by the potentiated startle paradigm.

Davis, M. (1979b) Psychopharmacol. 62 1-7 Diazepam and flurazepam : effects on conditioned fear measured with the potentiated startle paradigm.

Davis, M. (1992) Trends Pharmacol. Sci. 13 35-41 The role of the amygdala in fear-potentiated startle : implications for animal models of anxiety.

Davis, M., Cassella, J.V. and Kehne, J.H. (1988) Psychopharmacol. 94 14-20 Serotonin does not mediate anxiolytic effects of buspirone in the fear-potentited startle paradigm : comparison with 8-OH-DPAT and ipsapirone.

Deakin, J.F.W. (1991a) in Serotonin, sleep and mental disorders (Eds: C. Idzikowski and P.J. Cowen) Wrightson Biomedical Publishing pp161-178 Serotonin subtypes and affective disorders.

Deakin, J.F.W. (1991b) in Behavioural models in pyschopharmacology: theoretical, industrial and clinical perspectives (Ed: P. Willner) Cambridge Univeristy Press, Cambridge, pp 157-174 The clinical relevance of animal models of depression.

Deakin, J.F.W. and Graeff, F.G. (1991) J. Psychopharmacol. 5 305-315 Critique : 5-HT and mechanisms of defence.

Deakin, J.F.W., Graeff, F.G. and Guimaraes, F.S. (1992) in Central serotonin receptors and psychotropic drugs (Eds: C.A. Marsden and D.J. Heal) Blackwell Scientific Publications, London, pp 147-174 5-HT receptor subtypes and the modulation of aversion.

De Montigny and Blier, P. (1992) in Central serotonin receptors and psychotropic drugs (Eds: C.A. Marsden and D.J. Heal) Blackwell Scientific Publications, London, pp 223-238 Effects of antidepressant treatments on 5-HT neurotransmission.

Derkach, V., Suprenant, A. and North, R.A. (1989) Nature 339 706-709 5-HT_3 receptors are membrane ion channels.

De Vivo, M. and Maayani, S. (1985) Eur. J. Pharmacol. 119 231-234 Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists.

De Vivo, M. and Maayani, S. (1986) J. Pharmacol. Exp. Ther. 238 248-253 Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea-pig and rat hippocampal membranes.

Dickinson, S.L., Kennett, G.A. and Curzon, G. (1985) Brain Research 345 10-18 Reduced 5-hydroxytryptamine-dependent behaviour in rats following chronic corticosterone treatment.

Di Nicolantonio, R. and Mendelsohn, F.A.O. (1986) Am. J. Physiol. 250 R898-901 Plasma renin and angiotensin in dehydrated and rehydrated rats.

Dourish, C.T., Hutson, P.H. and Curzon, G. (1986) Trends Pharmacol. Sci. 7 212-214 Putative anxiolytics 8-OH-DPAT, buspirone and TVX Q 7821 are agonists at 5-HT_{1A} autoreceptors in the raphe nuclei.

Dourish, C.T., Kennett, G.A. and Curzon, G. (1987) J. Psychopharmacol. 1 23-30 The 5-HT_{1A} agonists 8-OH-DPAT, buspirone and ipsapirone attenuate stress-induced anorexia in rats.

Dourish, C.T., Clark, M.L. and Iversen, S.D. (1988) Psychopharmacol 95 185-188 8-OH-DPAT elicits feeding and not chewing : evidence from liquid diet studies and a diet choice test.

Dourish, C.T., Gorka, Z., Williams, A.R. and Iversen, S.D. (1989) J. Psychopharmacol. 4 38P Potential influence of social support in a rodent model of depression.

Dowling, J.E. (1963) J. Gen. Physiol. 46 1287-1301 Neural and photochemical mechanisms of visual adaptation in the rat.

Dumuis, A., Sebben, M. and Bockeart, J. (1988) Mol. Pharmacol. 33 178-186 Pharmacology of 5-hydroxytryptamine-$_{1A}$ receptors which inhibit cAMP production in hippocampal and cortical neurons in primary culture.

Dumuis, A., Sebben, M. and Bockeart, J. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol. 340 403-410 The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT$_4$) positively coupled to adenylate cyclase in neurones.

Duncan, G.E., Oglesby, S.A., Greenwood, R.S., Meeker, R.B., Hayward, J.N. and Stumpf, W.E. (1989) Neuroendocrinol. 49 489-495 Metabolic mapping of functional activity in rat brain during water deprivation.

Dunn, R.W., Corbett, R. and Fielding, S. (1989) Eur. J. Pharmacol. 169 1-10 Effects of 5-HT$_{1A}$ receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze.

Dursun, S.M. and Handley, S.L. (1991) Brit. J. Pharmacol. 104 363P Behavioural evidence that 5-HT$_{1A}$ receptor modulation of DOI head-shake is presynaptic.

Eison, A.S., Eison, M.S., Stanley, M. and Riblet, L.A. (1986) Pharmacol. Biochem. Behav. 24 701-707 Serotonergic mechanisms in the behavioural effects of buspirone and gepirone.

Engel, G., Göthert, M., Hoyer, D., Schlicker, E. and Hillenbrand, K. (1986) Naunyn-Schiemdeberg's Arch. Pharmacol. 332 1-7 Identity of inhibitory presynaptic 5-Hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT$_{1B}$ binding sites.

Engel, J.A., Hjorth, S., Svensson, K., Carlsson, A. and Liljequist, S. (1984) Eur. J. Pharmacol. 105 365-368 Anticonflict effects of the putative serotonin receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT).

Engelman, K., Lovenburg, W. and Sjoerdsma, A. (1967) New Eng. J. Med. 227 1103-1108 Inhibition of serotonin synthesis by p-chlorophenylalanine in carcinoid syndrome.

Falk, J.L. and Burnidge, G.K. (1970) Physiol. Behav. 5 199-202 Fluid intake and punishment-attenuating drugs.

Falter, U., Gower, A.J. and Gobert, J. (1992) Behav. Pharmacol. 3 123-128 Resistance of baseline activity in the elevated plus-maze to exogenous influences.

Feighner, J.P. and Boyer, W.F. (1989) Psychopath. 22 S21-26 Serotonin$_{1A}$ anxiolytics : an overview.

Fernandez-Guasti, A. and Lopez-Rubalcava, C. (1990) Psychopharmacol. 101 354-358 Evidence for the involvement of the 5-HT$_{1A}$ receptor in the anxiolytic action of indorenate and ipsapirone.

File, S.E. (1978) Trends Neurosci. 1 9-11 Anxiety, ACTH and 5-HT.

File, S.E. (1990) Psychopharmacol. 100 281-282 One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze.

File, S.E. and Hyde, J.R.G. (1979) Brit. J. Pharmacol. 62 19-24 Can social interactions be used to measure anxiety?

File, S.E. and Johnstone, A.L. (1989) Psychopharmacol. 99 248-251 Lack of effect of 5-HT$_3$ receptor antagonists in the social interaction and elevated plus-maze tests of anxiety in the rat.

File, S.E. and Peet, L.A. (1980) Physiol. Behav. 25 753-758 The sensitivity of the rat corticosterone response to environmental manipulations and to chronic chlordiazepoxide treatment.

File, S.E. and Vellucci, S.V. (1978) J. Pharm. Pharmacol. 30 105-110 Studies on the role of ACTH and of 5-HT in anxiety, using an animal model.

File, S.E., Hyde, J.R.G. and MacLeod, N.K. (1979a) J. Aff. Disorders 1 115-122 5,7-dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test.

File, S.E., Vellucci, S.V. and Wendland, S. (1979b) J. Pharm. Pharmacol. 31 300-305 Corticosterone - an anxiogenic or an anxiolytic agent?

File, S.E., Johnston, A.L. and Baldwin, H.A. (1988) Stress Med. 4 221-230 Anxiolytic and anxiogenic drugs: changes in behaviour and endocrine responses.

Fletcher, P.J. and Davies, M. (1990) Psychopharmacol. 100 188-194 A pharmacological analysis of the eating response induced by 8-OH-DPAT injected into the dorsal raphe nucleus reveals the involvement of a dopaminergic mechanism.

Fozard, J.R. (1987) Trends Pharmacol. Sci. 8 501-506 5-HT : The enigma variations.

Francès, H., Khidichian, F. and Monier, C. (1990) Neuropharmacol. 29 103-107 Increase in the isolation-induced social behavioural deficit by agonists at 5-HT_{1A} receptors.

Frazer, A., Maayani, S and Wolfe, B.B. (1990) Ann. Rev. Pharmacol. Toxicol. 30 307-348 Subtypes of receptors for serotonin.

Friedman, E. and Dallob, A. (1979) Commun. Psychopharmacol. 3 89-92 Enhanced serotonin receptor activity after chronic treratment with imipramine or amitryptyline.

Gaddum, J.H. and Picarrelli, Z.P. (1957) Brit. J. Pharmacol. Chemother. 12 323-328 Two kinds of tryptamine receptor.

Gaillet, S., Lacheur, J., Malaval, F., Assenmacher, I. and Szafarczyk, A. (1991) Exp. Brain Research 87 173-180 The involvement of noradrenergic ascending pathways in the stress-induced activation of ACTH and corticosterone secretions is dependent on the nature of stressors.

Gardner, C.R. (1985) J. Pharmacol. Meth. 14 181-187 Distress vocalization in rat pups A simple screening method for anxiolytic drugs.

Gardner, C.R. (1986) Pharmacol. Biochem. Behav. 24 1479-1485 Recent developments in 5-HT-related pharmacology of animal models of anxiety.

Gardner, C.R. and Guy, A.P. (1984) Drug Dev. Res. 4 207 A social interaction model of anxiety sensitive to acutely administered benzodiazepines.

Gardner, C.R. and Piper, D.C. (1982) Eur. J. Pharmacol. 83 25-33 Effects of agents which enhance GABA-mediated neurotransmission on licking conflict in rats and exploration in mice.

Gee, K.W., Chang, W.C., Brinton, R.E. and M^{c}Ewen, B.S. (1987) Eur. J. Pharmacol. 136 419-423 GABA-dependent modulation of the Cl^- ionophore by steroids in rat brain.

Geller, I. and Hartman, R. (1982) J. Clin. Psychiat. 43 25-32 Effects of buspirone on operant behaviour of laboratory rats and cynomolgous monkeys.

Geller, I. and Seifter, J. (1960) Psychopharmacol. 1 488-491 The effects of meprobamate, barbiturates and d-amphetamine and promazine on experimentally induced conflict in the rat.

Geyer, M.A., Peurto, A., Menkes, D.B., Segal, D.S. and Mandell, A.J. (1976) Brain Research 106 257-270 Behavioural studies following lesions of the mesolimbic and mesostriatal serotonergic pathways.

Gilbert, F., Brazell, C., Tricklebank, M.D. and Stahl, S.M. (1988) Eur. J. Pharmacol. 147 431-437 Activation of the 5-HT_{1A} receptor subtype increases rat plasma ACTH concentration.

Giral, P., Soubrié, P. and Peuch, A.J. (1987) Eur. J. Pharmacol. 134 113-116 Pharmacological evidence for the involvement of 1-(2-pyridinyl)-piperazine (1-PmP) in the interaction of buspirone or gepirone with noradrenergic systems.

Glaser, T. and Traber, J. (1983) Eur. J. Pharmacol. 88 137-138 Buspirone action on serotonin receptors in calf hippocampus.

Gleeson, S. and Barrett, J.E. (1990) Pharmacol. Biochem. Behav. 36 335-337 5-HT_{1A} agonist effects on punished responding of squirrel monkeys.

Glick, S.D. and Carlson, J.N. (1989) Brain Research 504 231-237 Regional changes in brain dopamine and serotonin metabolism induced by conditioned circling in rats : effect of water deprivation, learning and individual differences in asymmetry.

Goa, K.L. and Ward, A. (1986) Drugs 32 114-129 Buspirone - a preliminary review of its pharmacological properties and therapeutic efficiency as an anxiolytic.

Goldberg, H.L. and Finnerty, R.J. (1979) Am. J. Psychiat. 136 1184-1187 The comparative efficacy of buspirone and diazepam in the treatment of anxiety.

Gonzalez-Heydrich, J. and Peroutka, S.J. (1990) J. Clin. Psychiat. 51 5-11 Serotonin receptor and reuptake sites : pharmacologic significance.

Goodwin, G.M. and Green, A.R. (1985) Brit. J. Pharmacol. 84 743-753 A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors.

Goodwin, G.M., De Souza, R. and Green, A.R. (1985a) Nature 317 531-533 Presynpatic serotonin receptor-mediated responses in mice attenuated by antidepressant drugs and electroconvulsive shock.

Goodwin, G.M., De Souza, R.J. and Green, A.R. (1985b) Neuropharmacol. 24 1187-1194 The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) : a model of presynaptic 5-HT_1 function.

Goodwin, G.M., de Souza, R.J. and Green, A.R. (1986) Psychopharmacol. 89 382-387 The effect of a 5-HT_1 receptor ligand isapirone (TVX Q 7821) on 5-HT synthesis and on the behavioural effects of 5-HT antagonists in mice and rats.

Goodwin, G.M., De Souza, R.J. and Green, A.R. (1987) Psychopharmacol. 91 500-505 Attenuation by electroconvulsive shock and antidepressant drugs of the 5-HT_{1A} receptor mediated hypothermia and serotonin syndrome produced by 8-OH-DPAT in the rat.

Gower, A.J. and Tricklebank, M.D. (1988) Eur. J. Pharmacol. 155 129-137 α_2 adrenoceptor antagonist activity may account for the effects of buspirone in an anticonflict test in the rat.

Graeff, F.G. (1970) J. Pharmacol. Exp. Ther. 180 344-350 Tryptamine antagonists and punished behaviour.

Graeff, F.G. (1990) in The neurobiology of anxiety. Vol 3 (Eds G.D. Burrows, M. Roth and R. Noyes Jr.), Elsevier, Amsterdam, pp 307-354 Brain defense systems and anxiety.

Graeff, F.C. and Schoenfield, R.I. (1970) J. Pharmacol. Exp. Ther. 173 277-283 Tryptaminergic mechanisms in punished and nonpunished behaviour.

Graeff, F.G., Zuardi, A.W., Cjimiglio, J.S., Filho, E.C. and Karniol, I.G. (1985) Psychopharmacol. 86 334-338 Effect of metergoline on human anxiety.

Grahame-Smith, D.G. (1971) J. Neurochem. 18 1053-1066 Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan.

Gray, J.A. (1982) The neuropsychology of anxiety : an enquiry into the functions of the septo-hippocampal system. Oxford University Press, Oxford.

Green A.R. and Curzon, G. (1968) Nature 220 1095-1097 Release of 5-hydroxytryptamine in the brain provoked by hydrocortisone and its prevention by allopurinol.

Green, A.R. and Grahame-Smith, D.G. (1976) Nature 262 594-596 (-) Propranolol inhibits the behavioural responses of rats to increased 5-hydroxytryptamine in the central nervous system.

Gruel, J.M. and Glaser, T. (1992) Eur. J. Pharmacol. 211 211-219 The putative 5-HT_{1A} receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphé nucleus in vitro.

Guimaraes, F.S. (1993) 5-HT receptors and consolidation of stressful memories. Behav Brain Res (in press).

Gurling, J., Ashworth-Preece, M.A., Hartley, J.E., Fletcher, A., Dourish, C.T. and Routledge, C. (1993) Brit. J. Pharmacol. (in press) Effects of the putative 5-HT_{1A} receptor antagonist SDZ 216,525 in two animal models of somatodendritic 5-HT_{1A} autoreceptor function.

Haleem, D.J., Kennett, G,.A., Whitton, P.S. and Curzon, G. (1989) Eur. J. Pharmacol. 164 435-443 8-OH-DPAT increases corticosterone but not other 5-HT_{1A} receptor dependent responses more in females.

Hamon, M., Bourgoin, S., Gozlan, H., Hall, M.D., Goetz, C., Artaud, F. and Horn, A.S. (1984) Eur. J. Pharmacol. 100 263-276 Biochemical evidence for the 5-HT agonist properties of PAT (8-hydroxy-(2-di-n-propylamino) tetralin in the rat brain.

Handley, S.L. (1991) in Serotonin, sleep and mental disorders (Eds: C. Idzikowski and P.J. Cowen) Wrightson Biomedical Publishing pp 89-115 Serotonin in animal models of anxiety - the importance of stimulus and response.

Handley, S.L. and Dursun, S.M. (1992) Brit. J. Pharmacol. 106 143P α_2 adrenoceptor antagonists prevent a 5-HT_{1A}/5-HT_2 receptor interaction.

Handley, S.L. and McBlane, J.W. (1991) J. Psychopharmacol. 5 322-326 5-HT - the disengaging transmitter?

Handley, S.L. and Mithani, S. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 327 1-5 Effects of alpha-adrenoceptor agonists and anatgonists in a maze exploration of "fear"-motivated behaviour.

Hård, E. and Engel, J. (1988) Neuropharmacol. 27 981-986 Effects of 8-OH-DPAT on ultrasonic vocalization and audiogenic immobility reaction in pre-weanling rats.

Heal, D.J., Luscombe, G.P. and Martin, K.F. (1992) in Central serotonin receptors and psychotropic drugs (Eds: C.A. Marsden and D.J. Heal) Blackwell Scientific Publications, London, pp 56-99 Pharmacological identification of 5-HT receptor subtypes using behavioural models

Heaton, J.C.P. and Handley, S.L. (1989) Brit. J. Pharmacol. 98 687P Comparison of DOI-induced scratching and head-twitch behaviours: differential action of 8-OH-DPAT.

Heilig, C.W., Stromski, M.E., Blumenfeld, J.D., Lee, J.P. and Gullans, S.R. (1989) Am. J. Physiol. 257 F1108-1116 Characterization of the major brain osmolytes that accumulate in salt-loaded rats.

Heuring, R.E. and Peroutka, S.J. (1987) J. Neurosci. 7 894-903 Characterization of a novel [^{3}H]-5-hydroxytryptamine binding site subtype in bovine brain membranes.

Heuring, R.E., Schlegel, J.R. and Peroutka, S.J. (1986) Eur. J. Pharmacol. 128 279-282 Species variation in RU 24969 interactions with non 5-HT_{1A} binding sites.

Higgins, G.A., Bradbury, A.J., Jones, B.J. and Oakley, N.R. (1988) Neuropharmacol. 27 993-1001 Behavioural and biochemical consequences following activation of 5-HT_1-like and GABA receptors in the dorsal raphé nucleus of the rat.

Hjörth, S. (1991) Eur. J. Pharmacol. 199 237-242 Single dose 8-OH-DPAT pretreatment does not induce tachphylaxis to the 5-HT release-reducing effect of 5-HT_{1A} autoreceptor agonists.

Hjörth, S. and Carlsson, A. (1982) Eur. J. Pharmacol. 83 299-303 Buspirone : effects on central monoaminergic transmission - possible relevance to animal experiments and clinical findings.

Hjörth, S. and Carlsson, A. (1986) Eur. J. Pharmacol. 129 131-133 Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors?

Hjörth, S. and Magnusson, T. (1988) Naunyn-Schmiedeberg's Arch. Pharmacol. 338 463-471 The 5-HT_{1A} receptor agonist 8-OH-DPAT preferentially activates cell body 5-HT autoreceptors in rat brain in vivo.

Hjörth, S. and Sharp, T. (1990) Life Sci. 46 955-963 Mixed agonist / antagonists properties of NAN-190 at 5-HT_{1A} receptors : behavioural and *in vivo* brain microdialysis studies.

Hjörth, S. and Sharp, T. (1991) Life Sci. 48 1779-1786 Effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on the release of 5-HT in dorsal and median raphe-innervated brain regions as measured by in vivo microdialysis.

Hjörth, S., Engel, J.A. and Carlsson, A. (1986) Pharmacol. Biochem. Behav. 24 237-240 Anticonflict effects of low doses of the dopamine agonist apomorphine in the rat.

Hjörth, S., Carlsson, A. and Engel, J.A. (1987a) Psychopharmacol. 92 371-375 Anxiolytic-like action of the 3-PP enantiomers in the Vogel conflict paradigm.

Hjörth, S., Söderpalm, B. and Engel, J.A. (1987b) Psychopharmacol. 92 96-99 Biphasic effects of L-5-HTP in the Vogel conflict model.

Hodges, J.R. and Jones, M.T. (1963) J. Physiol. 167 30-37 The effect of injected corticosterone on the release of adrenocorticotrophic hormone in rats exposed to acute stress.

Holmberg, G. and Gershon, S. (1961) Psychopharmacol 2 93-106 Autonomic and psychic effects of yohimbine hydrochloride.

Hoyer, D. (1988a) J. Receptor Res. 8 59-91 Functional correlates of serotonin 5-HT_1 recognition sites.

Hoyer, D. (1988b) Trends Pharmacol. Sci. 9 89-94 Molecular pharmacology and biology of 5-HT_{1c} receptors.

Hoyer, D. and Middlemiss, D.N. (1989) Trends Pharmacol. Sci. 9 130-132 Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain.

Hoyer, D. and Schoeffter, P. (1988) Eur. J. Pharmacol. 147 145-147 5-HT_{1D} receptor-mediated inhibition of forskolin-stimulated adenyl cyclase activity in calf substantia nigra.

Hoyer, D. and Schoeffter, P. (1991) J. Receptor Res. 11 197-214 5-HT receptors : subtypes and second messengers.

Hoyer, D., Engel, G. and Kalkman, H.O. (1985) Eur. J. Pharmacol. 118 13-23 Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes : radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (-) [^{125}I]iodo-cyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin.

Hoyer, D., Pazos, A., Probst, A. and Palacois, J.M. (1986a) Brain Research 376 85-96 Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites.

Hoyer, D., Pazos, A., Probst, A. and Palacois, J.M. (1986b) Brain Research 376 97-107 Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT_2 recognition sites.

Hoyer, D., Schoeffter, P., Palacois, J.M., Kalkman, H.O., Bruinvels, A.T., Fozard, J.R., Siegl, H., Seiler, M.P. and Stoll, A. (1991) Brit. J. Pharmacol. 105 29P SDZ 216,525: a selective, potent and silent 5-HT_{1A} receptor antagonist.

Hunkler, W., Möhler, H., Pieri, L., Pole, P., Bonnetti, E.P., Cumin, R., Schaffner, R. and Haefely, W. (1981) Nature 290 514-516 Selective antagonists of benzodiazepines.

Hutson, P.H., Sarna, G.S. and Curzon, G. (1984) J. Neurochem. 43 291-293 Determination of daily variations of brain 5-hydroxytryptamine and dopamine turnovers and of the clearance of their acidic metabolites in conscious rats by repeated sampling of cerebrospinal fluid.

Hutson, P.H., Dourish, C.T. and Curzon, G. (1986) Eur. J. Pharmacol. 129 347-352 Neurochemical and behavioral evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors.

Hutson, P.H., Sarna, G.S., Kantamaneni, B.D. and Curzon, G. (1985) J. Neurochem. 44 1266-1273 Monitoring the effect of a tryptophan load on brain indole metabolism in freely moving rats by similtaneous cerebrospinal fluid sampling and brain dialysis.

Hutson, P.H., Sarna, G.S., O'Connell, M.T. and Curzon, G. (1989) Neurosci. Lett. 100 276-280 Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OH-DPAT into the nucleus raphe dorsalis.

Hynes, M.D., Lochner, M.A., Bemis, K.G. and Hymson, D.L. (1985) Life Sci. 47 961-969 Effects of sytemically administered monoamine reuptake blocking agents on patterns of buspirone-induced analgesia in rats.

Imai, H., Stendler, D.A. and Kitai, S.T. (1986) J. Comp. Neurol. 243 363-380 The organization of divergent axonal projections from the midbrain raphe nucluie in the rat.

Insel, T., Roy, B.F., Cohen, R.M. and Murphy, D.L. (1982) Am. J. Psychiat. 139 954-955 Possible development of the serotonin syndrome in man.

Invernizzi, R., Belli, S. and Samanin, R. (1992) Brain Research 584 322-324 Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effects in the frontal cortex.

Iversen, S.D. Neuropharmacol. 23 1553-1560 5-HT and anxiety.

Jacobs, B. L. (1976) Life Sci. 19 777-786 An animal model for studying central serotonergic synapses.

Jacobs, B.L and Azmitia, E.C. (1992) Physiol. Rev. 72 165-229 Structure and function of the brain serotonin system.

Jacobs, B.L. and Fornal, C.A (1992) Pharmacol. Rev. 43 563-578 V. Activity of brain serotonergic neurons in the behaving animal.

Jacobs, B., Wilkinson, L.O. and Fornal, C.A. (1990) Neuropsychopharmacol. 3 473-479 The role of brain serotonin - a neurophysiologic perspective.

Jacobson, A.F., Dominguez, R.A., Goldstein, B.J. and Steinbook, R.M. (1985) Pharmacother. 5 290-296 Comparison of buspirone and diazepam in generalized anxiety disorder.

Jenck, F., Broekkamp, C.L.E. and Van Delft, A.M.L. (1989) Eur. J.Pharmacol. 161 219-221 Opposite control mediated by central 5-HT_{1A} and non-5-HT_{1A} (5-HT_{1B} or 5-HT_{1C}) receptors on periaqueductal gray aversion.

Jenck, F., Broekkamp, C.L.E. and Van Delft, A.M.L. (1990) Psychopharmacol. 100 372-376 5-HT_{1C} receptors in the serotonergic control of periaqueductal gray induced aversion in rats.

Johnston, A.J., Baldwin, H.A. and File, S.E. (1988) J. Psychopharmacol. 2 33-38 Measures of anxiety and stress in the rat following chronic treatment with yohimbine.

Jones, B.J., Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J., Oakley, N.R. and Tyers, M.B. (1988) Brit. J. Pharmacol. 93 985-993 The potential anxiolytic activity of GR 38032F, a 5-HT_3-receptor antagonist.

Joseph, M.J. and Kennett, G.A. (1983a) Brain Research 270 251-257 Stress-induced release of 5-HT in the hippocampus and its dependence on increased tryptophan availability : an in vivo electrochemical study.

Joseph, M.J. and Kennett, G.A. (1983b) Psychopharmacol. 79 79-81 Corticosteriod response to stress depends upon increased tryptophan availibility.

Joseph, M.J. and Kennett, G.A. (1986) Ann. N. Y. Acad. Sci. 473 256-266 Serotonin release in rat hippocampus examined by *in vivo* voltammetry : serotonergic function and tryptophan availibility.

Kabra, P.M., Tsai, L.-L. and Marton, L.J. (1979) Clin. Chem. 25 1293-1296 Improved liquid-chromatographic method for determination of serum cortisol.

Kalén, K.P., Strecker, R.E., Rosengren, E. and Björklund, A. (1988) J. Neurochem. 51 1422-1435 Endogenous release of neuronal serotonin and 5-HIAA in the caudate putamen of the rat as revealed by intracerebral dialysis coupled to high pressure liquid chromatography with fluorimetric detection.

Kalén, K.P., Strecker, R.E., Rosengren, E. and Björklund, A. (1989a) Brain Research 492 187-202 Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis : role of exitatory amino acids and GABA.

Kalén, K.P., R.E., Rosengren, E., Lindvall, O. and Björklund, A. (1989b) Eur. J. Neurosci. 1 181-188 Hippocampal noradrenaline and serotonin release over 24 hours as measured by the dialysis technique in freely moving rats : correlation to behavioural activity state, effect of handling and tail-pinch.

Kalkman, H.O. (1989) Eur. J. Pharmacol. 173 121-125 ß-adrenoceptor blockade in rats enhances the ambulation induced by 5-HT_{1A} receptor agonists.

Kataoka, Y., Shibata, K., Miyazaki, A., Inoue, Y., Tominaga, K., Koizumi, S., Ueki, S. and Niwa, M. (1991) Neuropharmacol. 30 475-480 Involvement of the dorsal hippocampus in mediation of the anti-anxiety action of tandospirone, a 5-hydroxytryptamine$_{1A}$ agonistic anxiolytic.

Kehne, J.H., Cassella, J.V. and Davis, M. (1988) Psychopharmacol. 94 8-13 Anxiolytic effects of buspirone and gepirone in the fear-potentiated startle paradigm.

Keim, K.L. and Sigg, E.B. (1977) Pharmacol. Biochem. Behav. 6 79-85 Plasma corticosterone and brain catecholamines in stress : effects of psychotropic drugs.

Kelder, D. and Ross, S.B. (1992) Naunyn-Schmiedeberg's Arch. Pharmacol. 346 121-126 Long lasting attenuation of 8-OH-DPAT-induced corticosterone secretion after a single injection of a 5-HT$_{1A}$ receptor agonist.

Kennett, G.A. (1992) Psychopharmacol. 107 379-384 5-HT$_{1c}$ receptor antagonists have anxiolytic-like actions in the rat social interaction model.

Kennett, G.A. and Curzon, G. (1988) Brit. J. Pharmacol. 94 137-147 Evidence that mCPP may have behavioural effects mediated by central 5-HT$_{1c}$ receptors.

Kennett, G.A. and Curzon, G. (1991) Brit. J. Pharmacol. 103 2016-2020 Potencies of antagonists indicate that 5-HT$_{1c}$ receptors mediate 1-3(chlorophenyl)piperazine-induced hypophagia.

Kennett, G.A. and Joseph, M.J. (1981) Neuropharmacol. 20 39-43 The functional importance of increased brain tryptophan in the serotonergic response to restraint stress.

Kennett, G.A. and Joseph, M.H. (1982) Brain Research 236 305-316 Does in vivo voltammetry in the hippocampus measure 5-HT release ?

Kennett, G.A., Dickinson, S.L. and Curzon, G. (1985a) Brain Research 330 253-263 Enhancement of some 5-HT dependent behavioural responses following repeated immobilization in rats.

Kennett, G.A., Dickinson, S.L. and Curzon, G. (1985b) Eur. J. Pharmacol. 119 143-152 Central serotonergic responses and behavioural adaptation to repeated immobilization : the effect of the corticosterone synthesis inhibitor metyrapone.

Kennett, G.A., Chaouloff, F., Marcou, M. and Curzon, G. (1986) Brain Research 382 416-421 Female rats are more vulnerable than males in an animal model of depression : the possible role of serotonin.

Kennett, G.A., Dourish, C.T. and Curzon, G. (1987a) Eur. J. Pharmacol. 134 265-274 Anti-depressant-like action of 5-HT_{1A} agonists and conventional antidepressants in an animal model of depression.

Kennett, G.A., Dourish, C.T. and Curzon, G. (1987b) Eur. J. Pharmacol. 141 429-435 5-HT_{1B} agonists induce anorexia at a postsynaptic site.

Kennett, G.A., Marcou, M., Dourish, C.T. and Curzon, G. (1987c) Eur. J. Pharmacol. 138 53-60 Single administration of 5-HT_{1A} agonists decreases 5-HT_{1A} presynaptic, but not postsynaptic receptor-mediated responses : relationship to antidepressant-like action.

Kennett, G.A., Whitton, P., Shah, K. and Curzon, G. (1989) Eur. J. Pharmacol. 164 445-454 Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists.

Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Nature 330 746-748 Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding.

Kilts, C.D., Commissaris, R.L. and Rech, R.H. (1981) Psychopharmacol. 74 290-296 Comparison of anticonflict drug effects in three experimental animal models of anxiety.

King, M.B. (1992) Brit. J. Gen. Pract. 42 202-205 Is there still a role for benzodiazepines in general practice?

King, B.H., Brazell, C., Dourish, C.T. and Middlemiss, D.N. (1989) Neurosci. Lett. 105 174-176 MK 212 increases rat plasma ACTH concentration by activation of the 5-HT_{1C} receptor subtype.

Klint, T. (1991) Behav. Pharmacol. 2 481-489 Effects of 8-OH-DPAT and buspirone in a passive avoidance test and in the elevated X-maze test in rats.

Knott, P.J. and Curzon, G. (1972) Nature 239 452-453 Free tryptophan in plasma and brain tryptophan metabolism.

Knott, P.J. and Curzon, G. (1974) J. Neurochem. 32 1065-1071 Effect of increased rat brain tryptophan on 5-hydroxytryptamine and 5-hydroxyindolyl acetic acid in the hypothalamus and other brain regions.

Knott, P.J., Joseph, M.H. and Curzon, G. (1973) J. Neurochem. 20 249-251 Effects of food deprivation and immobilization on tryptophan and other amino acids in rat brain.

Koenig, J.I., Gudelsky, G.A. and Meltzer, H.Y. (1989) Eur. J. Pharmacol. 137 1-8 Stimulation of corticosterone and ß-endorphin secretion in the rat by selective 5-HT receptor subtype activation.

Kosofsky, B.E. and Molliver, M.E. (1987) Synapse 1 153-168 The serotonergic innervation of cerebral cortex : different classes of axon terminals arise from dorsal and median raphe nuclei.

Kostowski, W., Platznik, A. and Stefanski, R. (1989) Eur. J. Pharmacol. 168 393-396 Intra-hippocampal buspirone in animal models of anxiety.

Krahn, D.D., Gosnell, B.A., Grace, M. and Levine, A.S. (1986) Brain Res. Bull. 17 285-289 CRF antagonist partially reverses CRF- and stress- induced effects on feeding.

Kshama, D., Hrishikeshaven, H.J., Shanbogue, R. and Munonyedi, U.S. (1990) Behav. Neural Biol. 54 234-253 Modulation of baseline behaviour in rats by putative serotonergic agents in three ethoexperimental paradigms.

Kuemmel, B., Beneke, M., Krol, G. Schoellnhammer, G. and Spechtmeyer, H. (1990) Psychopharmacol. 100 Suppl. 1 353 Ipsapirone in the treatment of generalized anxiety disorders.

Kumar, A.M., Kumar, M., Deepika, K., Fernandez, J.B. and Eisdorfer, C. (1990) Life Sci. 47 1751- 1759 A modified HPLC technique for similtaneous measurement of 5-hydroxytryptamine and 5-hydroxyindoleactetic acid in cerebrospinal fluid, platelet and plasma.

Lader, M.H. (1991a) Presented at the 5th World congress of biological psychiatry. Satellite symposium, The role of ondansetron, a novel 5-HT_3 receptor antagonist, in the treatment of psychiatric disorders. Florence, pp 17-19 Ondansetron in the treatment of anxiety.

Lader, M.H. (1991b) in 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: their comparative behavioural pharmacology (Eds. R.J. Rodgers and S.J. Cooper) John Wiley and Sons, pp 343-363 Benzodiazepines and novel anxiolytics: clinical pharmacology, dependence and withdrawal.

Lahti, R.A. and Barsuhn, C. (1974) Psychopharmacol. 35 215-220 The effect of minor tranquilizers on stress-induced increases in rat plasma coricosteroids.

Lambert, J.J., Peters, J.A. and Cottrell, G.A. (1987) Trends Pharmacol. Sci. 8 224-227 Actions of synthetic and endogenous steriods on the $GABA_A$ receptor.

Larsson, L.-G., Rényi, L., Ross, S.B., Svensson, B. and Ängeby-Möller, K. (1990) Neuropharmacol. 29 85-91 Different effects on the responses of functional pre- and postsynaptic 5-HT_{1A} receptors by repeated treatment of rats with the 5-HT_{1A} receptor agonist 8-OH-DPAT.

Lazosky, A.J. and Britton, D.R. (1991) Psychopharmacol. 104 132-136 Effects of 5-HT_{1A} receptor agonists on CRF-induced behaviour.

Leaf, R.C. and Muller, S.A. (1965) Psychol. Rep. 17 819-823 Effects of shock intensity, deprivation and morphine in a simple approach-avoidance situation.

Le Fur, G., Guilloux, F., Mitrani, N., Mizoule, J. and Uzan, A. (1979) J. Pharmacol. Exp. Ther. 211 305-308 Relationships between plasma corticosteroids and benzodiazepines in stress.

Lesch, K.P., Hoh, A., Disselkamp-Tietze, J., Weissman, M., Osterheiderr, M. and Schulte, H.M. (1991) Arch. Gen. Psychiat. 48 540-547 5-hydoxytryptamine$_{1A}$ receptor responsivity in obsessive-compulsive disorder.

Lesch, K.P., Wiesman, M., Hoh, A., Müller, T., Disselkamp-Tietze, J., Osterheider, M. and Schulte, H.M. (1992) Psychopharmacol. 106 111-117 5-HT_{1A} receptor-effector system responsivity in panic disorder.

Lightowler, S., Williamson, I.J.R., Hegarty, J., Kennett, G.A., Fears, R.B. and Tulloch, I.F. (1992) Brit. J. Pharmacol. 106 44P Anxiolytic effects of paroxetine in the rat social interaction model of anxiety.

Lima, L. (1991) Pharmacol. Biochem. Behav. 39 671-676 Region-selective reduction of brain serotonin turnover rate and serotonin agonist-induced behaviour in mice treated with clonazepam.

Liposits, Z., Phelix, C., Paull, W.K. (1987) Histochem. 86 541-549 Synaptic interaction of serotonergic axons and corticotrophin releasing factor (CRF) synthesising neurones in the hypothalamic paraventricular nucleus of the rat.

Lista, A., Blier, P. and de Montigny, C. (1989) Eur. J. Pharmacol. 171 229-231 In vivo presynaptic modulation of serotonergic neurotransmission in the rat hippocampus by diazepam.

Lister, R.G. (1990) Pharmac. Ther. 46 321-340 Ethologically-based animal models of anxiety disorders.

Lopez-Ibor, J.J. (1988) Brit. J. Psychiat. 153 Suppl. 3 26-39 The involvement of serotonin in psychiatric disorders and behaviour.

Luscombe, G.P., Mazurkiewicz, S.E. and Buckett, W.R. (1990) Brit. J. pharmacol. 100 356P Evaluation of tricyclic antidepressants in the elevated plus-maze in rats: anxiolytic effect of dothiepin and doxepin.

Luscombe, G.P., Mazurkiewicz, S.E. and Heal, D.J. (1992) Brit. J. Pharmacol. 106 130P The 5-HT_{1A} ligand BP 554 mimics the anxiolytic activity of buspirone, gepirone and ipsapirone in the elevated plus-maze.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L. and Paul, S.M. (1986) Science 232 1004-1006 Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor.

Markstein, R., Hoyer, D. and Engel, G. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 333 335-341 5-HT_{1A} receptors mediate stimulation of adenylate cyclase in the rat hippocampus.

Marsden, C.A. and Martin, K.F. (1986) Brit. J. Pharmacol. 89 277-286 Involvement of 5-HT_{1A} and α_2 receptors in the decreased 5-hydroxytryptamine release and metabolism in rat suprachiasmatic nucleus after intravenous 8-hydroxy-2-(n-dipropylamino) tetralin.

Martin, K.F. and Mason, R. (1987) Eur. J. Pharmacol. 147 479-483 Isapirone is a partial agonist at 5 hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors in the rat hippocampus : electrophysiological evidence.

M^{c}Blane, J.W., Critchley, M.A.E. and Handley, S.L. (1992) Brit. J. Pharmacol. 105 221P Light intensity influences the response to 8-OH-DPAT in the elevated X-maze.

M^{c}Closkey, T.C., Paul, B.K. and Commissaris, R.L. (1987) Pharmacol. Biochem. Behav. 27 171-175 Buspirone effects in an animal conflict procedure : comparison with diazepam and phenobarbital.

McKenna, D.J. and Peroutka, S.J. (1989) J. Neurosci. 9 3482-3490 Differentiation of 5-hydroxytryptamine$_2$ receptor subtypes using ^{125}I-R-(-)2,5-dimethoxy-4-iodo-phenylisopropylamine and ^{3}H-ketanserin.

McMillen, B.A., Scott, S.M., Williams, H.L. and Sanghera, M.K. (1987) Naunyn Schmiedeberg's Arch. Pharmacol. 335 454-464 Effects of gepirone, an arylpiperazine anxiolytic drug, on aggressive behaviour and brain monoaminergic neurotransmission.

Meert, T.F. and Colpeart, F.C. (1986a) Psychopharmacol. 88 445-450 The shock probe conflict procedure : a new assay responsive to benzodiazepines, barbiturates and related compounds.

Meert, T.F. and Colpaert, F.C. (1986b) Psychopharmacol. 89 S23 (69) The effects of agonists and antagonists at putative 5-HT receptor subtypes in the shock probe conflict procedure.

Meller, E., Goldstein, M. and Bohmaker, K. (1990) Mol. Pharmacol. 37 231-237 Receptor reserve for 5 hydroxytryptamine$_{1A}$ - mediated inhibition of serotonin synthesis : possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists.

Messing, R.B. (1972) Vision Res. 12 753-761 The sensitivity of albino rats to lights of different wavelength : a behavioural assessment.

Mezey, E., Reisine, T.D., Skirboll, L., Beinfeld, M. and Kiss, J.Z. (1986) Proc. Natl. Acad. Sci. 83 3510-3512 Role of cholecystokinin in corticotrophin release : coexistance with vasopressin and corticotrophin-releasing factor in cells of the rat hypothalamic paraventricular nucleus.

Millan, M.J. and Colpaert, F.C. (1991) J. Pharmacol. Exp. Ther. 256 993-1001 5-hydroxytryptamine (5-HT) $_{1A}$ receptos and tha tail-flick response. II. High efficacy 5-HT$_{1A}$ agonists attenuate morphine-induced antinociception in mice in a competitive-like manner.

Mitchell, S.N. and Thomas, P.J. (1988) Pharmacol. 37 105-113 Effect of restraint stress and anxiolytics on 5-HT turnover in rat brain.

Mithani, S. (1984) PhD Thesis Aston Univ. Aspects of monoamine involvement in some aversively motivated behaviours.

Molineaux, S.M., Jessel, T.M., Axel, R. and Julius, D. (1989) Proc. Natl. Acad. Sci. 86 6793-6797 5-HT_{1C} receptor is a prominent serotonin receptor subtype in the central nervous system.

Montgomery, K.C. (1955) J. Comp. Physiol. Psychol. 48 254-260 The relation between fear induced by novel stimulation and exploratory behaviour.

Morato, S. and Castrechini, P. (1989) Braz. J. Med. Biol. Res. 22 707-710 Effects of floor surface and environmental illumination on exploratory activity in the elevated plus-maze.

Morgan, W.W., Rudeen, K. and Pfell, K.A. (1975) Life Sci. 17 143-150 Effect of immobilization stress on serotonin content and turnover in regions of the rat brain.

Moser, P.C. (1989) Psychopharmacol. 99 48-53 An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone.

Moser, P.C. (1991) Eur. J. Pharmacol. 193 165-172 The effect of putative 5-HT_{1A} receptor antagonists on 8-OH-DPAT-induced hypothermia in rats and mice.

Moser, P.C., Tricklebank, M.D., Middlemiss, D.N., Mir, A.K., Hibert, M.F. and Fozard, J.R. (1990) Brit. J. Pharmacol. 99 343-349 Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety : comparison with buspirone, 8-OH-DPAT and diazepam.

Mosko, S.S. and Jacobs, B.L. (1974) Physiol. Behav. 13 589-593 Midbrain raphé neurons : spontaneous activity and response to light.

Motta, V., Maisonnette, S., Morato, S. , Castrechini, P. and Brandao, M.L. (1992) Psychopharmacol. 107 135-139 Effects of blockade of 5-HT_2 receptors and activation of 5-HT_{1A} receptors on the exploratory activity of rats in the elevated plus-maze.

Nahmod, V.E., Fimkielman, S., Benarroch, E.E. and Pirola, C.J. (1978) Science 202 1091-1093 Angiotensin regulates release and synthesis of serotonin in brain.

Nanry, K.P. and Tilson, H.A. (1989) Psychopharmacol. 97 507-513 The role of 5-HT_{1A} receptors in the modulation of the acoustic startle reflex in rats.

Napoliello, M.J. and Domantay, A.G. (1991) Brit. J. Psychiat. 159 Suppl. 12 40-44 Buspirone: a worldwide update.

Nash, J.F. and Meltzer, H.Y. (1989) J. Pharmacol. Exp. Ther. 249 236-241 Effect of gepirone and ipsapirone on the stimulated and unstimulated secretion of prolactin in the rat.

Nash, J.F., Meltzer, H.Y. and Gudelsky, G.A. (1989) Pharmacol. Biochem. Behav. 33 781-785 Selective cross-tolerance to 5-HT_{1A} and 5-HT_2 receptor-mediated temperature and corticosterone responses.

Nashold, B.S. Jr., Wilson, N.P. and Slaughter, G.S. (1969) J. Neurosurg. 30 14-24 Sensations evoked by stimulation in the midbrain of man.

Nausieda, P.A., Carvey, P.M. and Weiner, W.J. (1982) Eur. J. Pharmacol. 78 335-343 Modification of central serotonergic and dopaminergic behaviours in the course of chronic corticosterone administration.

Neale, R.F., Fallon, S.L., Boyar, W.C., Wasley, J.W.F., Martin, L.L., Stone, G.A., Glaeser, B.S., Sinton, C.M. and Williams, M. (1987) Eur. J. Pharmacol. 136 1-9 Biochemical and pharmacological characterization of CGS 12066B, a selective serotonin-1B agonist.

Neckers, L. and Sze, P.Y. (1975) Brain Research 93 123-132 Regulation of 5-hydroxytryptamine metabolism in mouse brain by adrenal glucocorticoids.

Neppe, V.M. (1989) Lancet II. 8677 1458 High-dose buspirone in case of tardive dyskinesia.

Newton, R.E., Maruncysz, J.D., Alderdice, M.T. and Napoliello, M.J. (1986) Am. J. Med. 80 Suppl. 3B 17-21 Review of the side effect profile of buspirone.

Njung'e, K. (1989) PhD Thesis Aston Univ. Effects of 5-hydroxytryptamine and related ligands in anxiety models.

Njung'e, K., Critchley, M.A.E. and Handley, S.L. (1992) J. Psychopharmacol. (in press) Effects of ß-adrenoceptor ligands in the elevated X-maze "anxiety" model and antagonism of the "anxiogenic" response to 8-OH-DPAT.

Nolan, N.A. and Parkes, M.W. (1973) Psychopharmacol. 29 277-288 The effects of benzodiazepines on the behaviour of mice on a hole-board.

Nomikos, G.G., Arborelius, L. and Svensson, T.H. (1992) Eur. J. Pharmacol. 216 373-378 The novel 5-HT_{1A} receptor antagonist (S)-UH-301 prevents (R)-8-OH-DPAT-induced decrease in interstitial concentrations of serotonin in the rat hippocampus.

Nutt, D.J. and George, D.T. (1990) in Handbook of Anxiety (Eds: G.D. Burrows, M. Roth and R. Noyes, Jr.) pp 189-221 Serotonin and anxiety.

Nutt, D.J. and Glue, P. (1989) Pharmac. Ther. 44 309-334 Clinical pharmacology of anxioltics and antidepressants: a psychopharmacological perspective.

Oakley, N.R. and Jones, B.J. (1983) Eur. J. Pharmacol. 87 499-500 Buspirone enhances [^{3}H]flunitrazepam binding in vivo.

O'Connell, M.T., Sarna, G.S. and Curzon, G. (1992) Brit. J. Pharmacol. 106 603-609 Evidence for postsynaptic mediation of the hypothermic effect of 5-HT_{1A} receptor activation.

O'Connor, J.J., Rowan, M.J. and Anwyl, R. (1990) Brit. J. Pharmacol. 101 171-177 Actions of 5-HT_1 ligands on excitatory transmission in the hippocampus of alert rats.

Owens, M.J., Edwards, E. and Nemeroff, C.B. (1990) Eur. J. Pharmacol. 190 113-122 Effects of 5-HT_{1A} receptor agonists on hypothalamo-pituitary-adrenal axis activity and corticotropin-releasing factor containing neurons in the rat brain.

Pandey, S.C., Isaac, L., Davis, J.M. and Pandey, G.N. (1991) Eur. J. Pharmacol. 202 221-225 Similar effects of treatment with desipramine and electroconvulsive shock on 5-hydroxytryptamine$_{1A}$ receptors in rat brain.

Parent, A., Descarrries, L. and Beaude, A. (1981) Neurosci. 6 115-138 Organization of ascending serotonergic systems in the rat brain. An autoradiographic study after intravenous administration of [^{3}H]-5-hydroxytryptamine.

Pazos, A. and Palacois, J.M. (1985) Brain Research 346 205-230 Quantitavive autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin$_1$ receptors.

Pazos, A., Hoyer, D. and Palacois, J.M. (1984) Eur. J. Pharmacol. 106 539-546 The binding of serotonergic ligands to the porcine choroid plexus : characterization of a new type of serotonin recognition site.

Pazos, A., Cortés, R. and Palacois, J.M. (1985) Brain Research 346 231-249 Quantative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin$_2$ receptors.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981) J. Neurochem. 36 220-226 Discrimination of multiple [^{3}H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain.

Pellow, S., Chopin, P., File, S.E. and Briley, M. (1985) J. Neurosci. Meth. 14 149-167 Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat.

Pellow, S. and File, S.E. (1986) Pharmacol. Biochem. Behav. 24 525-529 Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze : a novel test of anxiety in the rat.

Pellow, S., Johnston, A.L. and File, S.E. (1987) J. Pharm. Pharmacol. 39 917-928 Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG7142 using the elevated plus maze in the rat.

Peroutka, S.J. (1985) Biol. Psychiat. 20 971-979 Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_{1A}$ receptors.

Peroutka, S.J. (1989) J. Neurochem. 53 316-318 Serotonin 5-HT$_2$ receptors : two states versus two subtypes.

Peroutka, S.J. (1992) Neuropharmacol. 31 609-613 Phylogenetic tree analysis of G protein-coupled 5-HT receptors: implications for receptor nomenclature.

Peroutka, S.J. and Snyder, S.H. (1979) Mol. Pharmacol. 16 687-699 Multiple serotonin receptors : differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol.

Perry, K.W. and Fuller, R.W. (1989) Biochem. Pharmacol. 38 3169-3173 Determination of brain concentrations of 8-hydroxy-2-(di-n-propylamino) tetralin by liquid chromatography with electrochemical detection.

Perry, K.W. and Fuller, R.W. (1992) Life Sci. 22 1683-1690 Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum.

Petersen, E.N. and Lassen, J.B. (1981) Psychopharmacol. 75 236 A water lick conflict paradigm using drug experienced rats.

Petersen, E.N., Paschelke, G., Kehr, W., Nielsen, M. and Braestrup, C. (1982) Eur. J. Pharmacol. 82 217-221 Does the reversal of the anticonflict effect of phenobarbirtal by ß-CCE and FG 7412 indicate benzodiazepine receptor mediated anxiogenic properties?

Phillips, M.I. (1987) Ann. Rev. Physiol. 49 413-435 Functions of angiotensin in the central nervous system.

Piezzi, R.S. and Wurtman, R.J. (1970) Science 169 285-286 Pituitary serotonin content : effects of melatonin or deprivation of water.

Popova, J. and Petkov, V.V. (1990) Gen. Pharmacol. 21 223-225 Changes in 5-HT_1 receptors in different brain structures of rats with isolation syndrome.

Prezgalinski, E., Budziszewska, B., Warchol-Kania, A. and Blaszczynskna, E. (1989) Pharmacol. Biochem. Behav. 33 329-334 Stimulation of corticosterone secretion by the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the rat.

Price, W.A and DiMarzio, L.R. (1988) Am. J. Psychiat. 145 1178-1179 Bulimia, menstruation and PMS : treatment implications.

Reisne, T., Affolter, H.U., Rougon, G. and Barbet, J. (1986) Trends Neusosci. 9 574-579 New insights into the molecular mechanisms of stress.

Redmond, D.E. (1979) Psychopharmacol. Bull. 15 26-27 The effect of destruction of the locus coereleus on nonhuman primate behaviors.

Rex, A., Marsden, C.A. and Fink, H. (1991) in Monitoring molecules in neuroscience Proc. Vth International Conference on In Vivo Methods 250-252 Increased 5-HT release in the guinea-pig frontal cortex on exposure to the X-maze.

Richardson, B.P. amd Engel, G. (1986) Trends Neurosci. 9 424-428 The pharmacology and function of 5-HT_3 receptors.

Rickels, K., Schweizer, E. and Case, W.G. (1989) in The pyshopharmacology of anxiety (Ed: P Tyrer) Oxford University Press, Oxford, pp 283-293 Withdrawal problems with anti-anxiety drugs : nature and management.

Rickels, K., Amsterdam, J.D., Clary, C., Hassman, J. and London, J. (1991) Psychopharmacol. Bull. 26 219-222 Buspirone in depressed outpatients : a controlled study.

Rickels, K., Amsterdam, J.D., Clary, C., Puzzuoli, G. and Schwieizer, E. (1991) J. Clin. Psychiat. 52 34-38 Buspirone in major depression : a controlled study.

Robichaud, R.C. and Sledge, K.L. (1969) Life Sci. 8 965-969 The Effects of pCPA on experimentally induced conflict in the rat.

Robinson, D.S. (1991) in Buspirone mechanisms and clinical aspects (Eds: G. Tunnicliff, A.S. Eison and D.P. Taylor) Academic Press Inc., pp 3-17 Buspirone in the treatment of anxiety.

Rodgers, R.J. (1991) J. Psychopharmacol. 5 316-319 A step in the right direction : Comment on '5-HT and mechanisms of defence'.

Rogers, L.W. and Giordani, J. (1990) Life Sci. 47 961-969 Systemically administered monoamine reuptake blocking agents on patterns of buspirone-induced analgesia.

Sanger, D.J. (1990) J. Pharmacol. Exp. Ther. 254 420-426 Effects of buspirone and related compounds on suppressed operant responding in rats.

Sanger, D.J. (1992) J. Pharmacol. Exp. Ther. 261 513-517 Increased rates of punished responding produced by buspirone-like compounds in rats.

Sanger, D.J., Joly, D. and Zivkovic, B. (1985) J. Pharm. Exp. Ther. 232 831-837 Behavioural effects of nonbenzodiazepine anxiolytic drugs: a comparison of CGS 9896 and zopiclone with chlordiazepoxide.

Sapolsky, R.M., Krey, L.C. and McEwen, B.S. (1984) Proc. Natl. Acad. Sci. 81 6174-6177 Glucocorticoid-sensitive hippocampal neurones are involved in terminating adrenocortical stress response.

Sapolsky, R.M., Zola-Morgan, S. and Squire, L.R. (1991) J. Neurosci. 11 3695-3704 Inhibition of glucocorticoid secretion by the hippocampal formation.

Schefke, D.M., Fontana, D.J. and Commissaris, R.L. (1989) Psychopharmacol. 99 427-429 Anti-conflict efficacy of buspirone following acute versus chronic treatment.

Schmidt, M.J., Fuller, R.W. and Wong, D.T. (1989) Br. J. Psychiat. 153 Suppl 3 40-46 Fluoxetine, a highly selective serotonin reuptake inhibitor : a review of preclinical studies.

Schütz, M.T.B., De Aguiar, J.C. and Graeff, F.G. (1985) Psychopharmacol. 85 340-345 Antiaversive role of serotonin in the dorsal periaqueductal gray matter.

Schweitzer, E.E., Amsterdam, J.A., Rickels, K., Kaplan, M. and Droba, M. (1986) Psychopharmacol. Bull. 22 183-185 Open trial of buspirone in the treatment of major depressive disorders.

Segal, M., Azmitia, E.C. and Whittaker-Azmitia, P.M. (1989) Brain Research 502 67-74 Physiological effects of selective 5-HT_{1A} and 5-HT_{1B} ligands in rat hippocampus : comparison to 5-HT.

Selye, H. (1956) The stress of life M°Graw Hill.

Shambelan, M. and Biglieri, E.G. (1972) J. Clin. Endocrinol. Metab. 34 695-704 Deoxycorticosterone production and regulation in man.

Shannon, N.J., Gunnet, J.W. and Moore, K.E. (1986) J. Neurochem. 47 958-965 A comparison of biochemical indices of 5-hydroxytryptaminergic neuronal activity following electrical stimulation of the dorsal raphé nucleus.

Sharp, T. and Hjörth, S. (1990) J. Neurosci. Meth. 34 83-90 Application of brain microdialysis to study the pharmacology of the 5-HT_{1A} autoreceptor.

Sharp, T., Backus, L.I., Hjörth, S., Bramwell, S.R. and Grahane-Smith, D.G. (1990) Eur. J. Pharmacol. 176 331-340 Further investigation of the in vivo pharmacological properties of the putative 5-HT_{1A} antagonist, BMY 7378.

Sharp, T., Bramwell, S.R., Hjörth, S. and Grahame-Smith, D.G. (1989) Brit. J. Pharmacol. 98 989-997 Pharmacological characterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release in vivo as measured by microdialysis.

Sheehan, D.V., Raj, A.B., Sheehan, K. and Soto, S. (1988) Pharmacol. Biochem. Behav. 29 815-817 The relative efficacy of buspirone, imipramine and placebo in panic disorder : a preliminary report.

Sheehan, D.V., Raj, A.B., Sheehan, K.H. and Soto, S. (1990) J. Clin. Psychopharmacol. 10 3-11 Is buspirone effective for panic disorder?

Shibata, K. and Onodera, M. (1988) J. Chromatog. 430 381-387 Similtaneous micro-determination of serotonin and 5-hydroxyindole-3-acetic acid with 5-hydroxy-N-methyltryptamine as an internal standard, in biological materials by high performance liquid chromatography with electrochemical detection.

Shibata, S., Yamashita, K., Yamamoto, E., Ozaki, T. and Ueki, S. (1989) Psychopharmacol. 98 38-44 Effects of benzodiazepine and GABA antagonists on anticonflict effects of antianxiety drugs injected into the rat amygdala in a water-lick suppression test.

Shoemaker, H. and Langer, S.Z. (1986) Eur. J. Pharmacol. 124 371-373 [^{3}H]-8-OH-DPAT labels the serotonin transporter in the rat striatum.

Singh, L., Field, M.J., Hughes, J., Menzies, R., Cles, R.J., Vass, C.A. and Woodruff, G.N. (1991) Brit. J. Pharmacol. 104 239-245 The behavioural properties of CI-988, a selective cholecystokininB receptor antagonist.

Smith, L.M. and Peroutka, S.J. (1986) Pharmacol. Biochem. Behav. 24 1513-1519 Differential effects of 5-hydroxytryptamine$_{1A}$ selective drugs on the behavioural syndrome.

Söderpalm, B. and Engel, J.A. (1990) Eur. Neuropsychopharmacol. 1 7-13 Serotonergic involvement in conflict behaviour.

Söderpalm, B. and Engel, J.A. (1992) Life Sci. 51 315-326 The 5,7-DHT-induced anticonflict effect is dependent on intact adrenocortical function.

Söderpalm, B., Hjorth, S. and Engel, J.A. (1989) Pharmacol. Biochem. Behav. 32 259-265 Effects of 5-HT$_{1A}$ receptor agonists and L-5-HTP in Montgomery's conflict test.

Soubrié, P. (1988) in Neuronal serotonin (Eds: N.N. Osborne and M. Hamon) John Wiley & Sons Serotonin and behaviour, with special regard to animal models of anxiety, depression and waiting ability. pp255-270.

Spencer, D.G. and Traber, J. (1987) Psychopharmacol. 91 25-29 The interoceptive discrimintive stimuli induced by the novel anxiolytic TVX Q 7821 : behavioural evidence for the specific involvement of serotonin 5-HT$_{1A}$ receptors.

Sprouse, J.S. and Aghajanian, G.K. (1986) Eur. J. Pharmacol. 128 295-298 (-) Propranolol blocks the inhibition of serotonergic dorsal raphé cell firing by 5-HT_{1A} selective agonists.

Sprouse, J.S. and Aghajanian, G.K. (1988) Neuropharmacol. 27 707-715 Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists : a comparative study with dorsal raphe neurons.

Stein, L., Wise, C.D. and Berger, B.D. (1973) in The benzodiazepines Raven Press N.Y. 299-326 Antianxiety action of benzodiazepines decrease in activity of serotonin neurons in the punishment system.

Stein, L., Wise, C.D. and Beluzzi, J.D. (1975) in Mechanisms of action of benzodiazepines (Eds : E. Costa and P. Greengard) Raven Press N.Y. 29 Effects of benzodiazepines on central serotonergic mechanisms.

Stone, E.A. (1979) J. Neurochem. 32 1335-1337 Reduction by stress of cyclic AMP in rat cerebral cortex.

Stone, E.A. and Platt, J.E. (1982) Brain Research 237 405-414 Brain adrenergic receptors and resistance to stress.

Stutzmann, J.M., Eon, B., Darche, F., Lucas, M., Rataud, J., Piot, O., Blanchard, J.C. and Laudron, P.M. (1991) Neurosci. Lett. 128 4-8 Are 5-HT_2 antagonists endowed with anxiolytic properties in rodents?

Tanaka, M., Kohno, Y., Nakagawa, R., Ida, Y., Takeda, S. and Nagasaki, N. (1982) Pharmacol. Biochem. Behav. 16 315-319 Time-related differences in noradrenaline turnover in rat brain regions by stress.

Tanaka, T., Yokoo, H., Mizoguchi, K., Yoshida, M., Tsuda, A. and Tanaka, M. (1991) Brain Research 544 174-176 Noradrenaline release in the rat amygdala is increased by stress : studies with intracerebral microdialysis.

Tanaka, M., Tsuda, A., Yokoo, H., Yoshida, M., Mizoguchi, K. and Shimizu, T. (1991) Pharmacol. Biochem. Behav. 39 191-195 Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine.

Taylor, D.P. (1990) Ann. N. Y. Acad. Sci. 600 545-557 Serotonin agents in anxiety.

Thiébot, M.H., Hamon, M. and Soubrié, P. (1982) Neurosci. 7 2287-2294 Attenuation of induced anxiety in rats by chlordiazepoxide : role of the raphé dorsalis benzodiazepine binding sites and serotonergic neurons.

Thiébot, M.H., Soubrié, P., Hamon, M. and Simon, P. (1984) Psychopharmacol. 82 355-359 Evidence against the involvement of serotonergic neurones in the antidepressant action of diazepam in the rat.

Thierry, A.M., Fekete, M. and Glowinski, J. (1968) Eur. J. Pharmacol. 4 384-389 Effects of stress on the metabolism of noradrenaline, dopamine and serotonin (5-HT) in the central nervous system of the rat.(II) Modifications of serotonin metabolism.

Thierry, A. M., Tassin, J.P., Blanc, G. and Glowinski, J. (1976) Nature 263 242-244 Selective activation of the mesocortical dopaminergic system by stress.

Titeler, M. and Herrick-Davis, K.L. (1988) Soc. Neurosci. Abs. 221 13 Detection and characterization of the novel 5-HT_{1E} receptor in human and other mammalian brain tissue.

Tizabi, Y., Gilad, V.H. and Gilad, G.M. (1989) Neurosci. Lett. 105 177-183 Effects of chronic stressors or corticosterone treatment on the septohippocampal cholinergic system of the rat.

Tomkins, D.M., Costall, B. and Kelly, M.E. (1990) Psychopharmacol. 101 s57 Actions of ritanserin and DOI on the elevated X-maze.

Traber, J. and Glaser, T. (1987) Trends Pharmacol. Sci. 8 432-437 5-HT_{1A} receptor related anxiolytics.

Treit, D. (1990) Pharmacol. Biochem. Behav. 36 203-205 A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics.

Tricklebank, M.D., Neill, J., Kidd, E.J. and Fozard, J.R. (1987) Eur. J. Pharmacol. 133 47-56 Mediation of the discrimintaive stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) by the putative 5-HT_{1A} receptor.

Tricklebank, M.D. (1985) Trends Pharmacol. Sci. 6 403-405 The behavioural response to 5-HT receptor agonists and subtypes of the central 5-HT receptor.

Trulson, M.E. and Arasteh, K. (1986) J. Pharm. Pharmacol. 38 380-382 Buspirone decreases the activity of 5-hydroxytryptamine-containing dorsal raphe neurons in-vitro.

Trulson, M.E. and Henderson, L.J. (1985) Eur. J. Pharmacol. 106 195-197 Buspirone increases locus coereleus noradrenergic neuronal activity in vitro.

Trulson, M.E. and Trulson, T.J. (1986) Neuropharmacol. 25 1263-1266 Buspirone decreases the activity of serotonin-containing neurons in the dorsal raphe in freely-moving cats.

Twarog, B.M. and Page, I.H. (1953) Am. J. Physiol. 175 156-167 Serotonin content of some mammalian tissues and urine and a method for its determination.

Tye, N.C., Everitt, B.J. and Iversen, S.D. (1977) Nature 268 741-743 5-hydroxytryptamine and punishment.

Tye, N.C., Iversen, S.D. and Green, A.R. (1979) Neuropharmacol. 18 689-695 The effects of benzodiazepine and serotonergic manipulations on punished responding.

Tyrer, P. (1989) Choice of treatment in anxiety. in The psychopharmacology of anxiety Tyrer, P. (ed) Oxford University Press pp255-282.

Upton, N. and Blackburn, T.P. (1991) Brit. J. Pharmacol. 102 253P Anxiolytic-like activity of the selective 5-HT_3 receptor antagonist, BRL 46470A, in the rat elevated X-maze.

Urban, J.H., Vande Kar, L.D., Lorens, S.A. and Bethen, C.C. (1986) Pharmacol. Biochem. Behav. 25 457-462 Effect of the anxiolytic drug buspirone on prolactin and corticosterone secretion in stressed and unstressed rats.

Ushijima, I., Mizuki, Y., Hara, T., Obara, N., Minematsu, N. and Yamada, M. (1992) Pharmacol. Biochem. Behav. 42 431-435 Effects of lithium and purigenic compounds on the behavioural and physiological aspects of restraint stress in rats.

Vale, W., Speiss, J., Rivier, C. and Rivier, J. (1981) Science 213 1394-1397 Characterization of a 41-residue ovine hypothalamic peptide that stuimulates secretion of corticotrophin and ß-endorphin.

VanderMaelen, C.P., Matheson, G.K., Widerman, R.C. and Patterson, L.A. (1986) Eur. J. Pharmacol. 129 123-130 Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a nonbenzodiazepine anxiolytic drug.

VanderMaelen, C.P., Matheson, G.K., Wilderman, R.C. and Patterson, L.A. (1986) Eur. J. Pharmacol. 129 123-130 Inhibition of serotonergic dorsal raphe neurones by systemic and iontophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug.

Van Wijngaarden, IK., Tulp, M. Th. M. and Soudin, W. (1990) Eur. J. Pharmacol. 188 301-312 The concept of selectivity in 5-HT receptor research.

Varrault, A. and Bockeart, J. (1992) Naunyn-Schmiedeberg's Arch. Pharmacol. 346 367-374 Differential coupling of 5-HT_{1A} receptors occupied by 5-HT or 8-OH-DPAT to adenylyl cyclase.

Viana, M.B., Tomaz, C.A.B. and Graeff, F.G. (1992) In VII Annual Meeting of FESBE (Caxambu, Brazil) Abstr p49 Effect of diazepam in rats placed on the elevated T-maze, a possible experimental model of anxiety and memory.

Vogel, J.R., Beer, B. and Clody, D.E. (1971) Psychopharmacol. 21 1-7 A simple and reliable conflict procedure for testing antianxiety agents.

Wada, T. and Fukuda, N. (1991) Psychopharmacol. 104 444-450 Effects of DN-2327, a new anxiolytic, diazepam and buspirone on exploratory activity of the rat in an elevated plus-maze.

Waeber, C., Dietl, M.M., Hoyer, D., Probst, A. and Palacois, J.M. (1988) Neurosci. Lett. 88 11-16 Visualisation of a novel serotonin recognition site (5-HT_{1D}) in the human brain by autoradiography.

Weiss, S., Sebben, M., Kemp, D.E. and Bockeart, J. (1986) Eur. J. Pharmacol. 120 227-230 Serotonin 5-HT_{1A} receptors mediate inhibition of cyclic AMP production in neurons.

Weissman, D., Mach, E., Oberlander, C., Demassey, Y. and Pujol, J.-F (1986) Neurochem. Int. 9 191-200 Evidence for hyperdensity of 5-HT_{1B} binding sites in the substantia nigra of the rat after 5,7-dihydroxytryptamine intraventricular injection.

Whitton, P. and Curzon, G. (1990) Psychopharmacol. 100 138-140 Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus.

Wilkinson, L.O. and Jacobs, B.L. (1988) Exp. Neurol. 101 445-457 Lack of response of serotonergic neurons in the dorsal raphe nucleus of freely moving cats to stressful stimuli.

Wilkinson, L.O., Auerbach, S.B. and Jacobs, B.L. (1991) J. Neurosci. 11 2732-2741 Extracellular serotonin levels change with behavioural state but not with pyrogen-induced hyperthermia.

Wilkinson, L.O., Abercrombie, E.D., Rasmussen, K. and Jacobs, B.L. (1987) Eur. J. Pharmacol. 136 123-127 Effect of buspirone on single unit activity in locus coeruleus and dorsal raphe nucleus in behaving cats.

Winslow, J.T. and Insel., T.R. (1991) Psychopharmacol. 105 513-520 Serotonergic modulation of the rat pup ultrasonic isolation call : studies with 5-HT_1 and 5-HT_2 subtype selective agonists and antagonists.

Winter, J.C. (1988) Pharmacol. Biochem. Behav. 29 193-195 Generalization of the discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and ipsapirone to yohimbine.

Wise, C.D., Berger, B.D. and Stein, L. (1972) Science 177 180-183 Benzodiazepines: anxiety-reducing activity by reduction of serotonin turnover.

Witkin, J.M. and Barrett, J.E. (1986) Pharmacol. Biochem. Behav. 24 751-756 Interaction of buspirone and dopaminergic agents on punished behaviour of pigeons.

Witkin, J.M., Mansbach, R.S., Barrett, J.E., Bolger, G.T., Skolnick, P. and Weissman, B. (1987) J. Pharmacol. Exp. Ther. 243 970-977 Behavioural studies with anxiolytic drugs. IV. Serotonergic involvement in the effects of buspirone on punished behaviour of pigeons.

Wolf, W.A. and Kuhn, D.M. (1991) Neurochem. Int. 19 39-44 The 5-HT transporter as an additional site of action for the 5-HT agonists RU 24969 and TFMPP.

Wright, I.K., Upton, N. and Marsden, C.A. (1991) in Monitoring molecules in neuroscience Proc. Vth International Conference on In Vivo Methods 208-211 Effects of potential anxiolytics on extracellular 5-HT in the ventral hippocampus of rats observed on the elevated X-maze using in vivo microdialysis.

Wright, I.K., Ismail, H., Upton, N. and Marsden, C.A. (1991) Psychopharmacol. 105 259-264 Effects of isolation rearing on 5-HT agonist-induced responses in the rat.

Wright, I.K., Heaton, M., Upton, N. and Marsden, C.A. (1992) Psychopharmacol. 107 405-414 Comparison of acute and chronic treatment of various serotonergic agents with those of diazepam and idazoxan in the rat elevated X-maze.

Wuttke, W., Demling, J., Fuchs, E., Mansky, T., Keck, H., Lamberts, R. and Graf, M. (1984) in Catecholamines: neuropharmacology and central nervous system - theoretical aspects (Ed: Liss, A.R.) NY, pp 463-468 Norepinephrine-mediated effects of GABA on LH release.

Young, R., Urbancic, A., Emery, T.A., Hal, P.C. and Metcalf, G. (1987) Eur. J. Pharmacol. 143 361-371 Behavioural effects of several new anxiolytics and putative anxiolytics.