# 5-HT FUNCTION IN RODENT MODELS OF ANXIETY

by

*Ian Kevin Wright, B.Sc.*

Department of Physiology and Pharmacology,
Medical School ,
Queen's Medical Centre,
Nottingham.

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy, October, 1991.

*For Mum and Dad*

**CONTENTS**

**ACKNOWLEDGEMENTS**

I would like to thank Professor C.A. Marsden for his help, encouragement and support during my three years in Nottingham. In addition, I am very grateful to Dr. N. Upton at SmithKline Beecham for his constant help, advice and interest throughout the three years.

I would like to thank Professors A.T. Birmingham and P. Fentem for the use of the research facilities of the Department of Physiology and Pharmacology. I wish to thank Graham Errington and his staff in the Animal Unit for looking after my rats and for general assistance, particularly Ian, Magrit, Brenda and Sue. In addition I would like to thank Dr. K. Pittaway for her never-ending faith in the fear-potentiated acoustic startle paradigm during my time at SmithKline Beecham. I acknowledge the Science and Engineering Research Council and SmithKline Beecham for financial support.

I would also like to thank several people for their support and friendship over the past three years including Sue, Raj, Jeni, Emma, Simon, Chris and Chris, and especially Tony and Kerry.

**PUBLICATIONS**

Crespi, F., Wright, I.K., Mobius, C., Robbins, T.W. and Marsden, C.A. (1991). Isolation rearing of rats alters behaviour and release of 5-hydroxytryptamine and dopamine in the frontal cortex. J. Neurochem. (submitted).

Marsden, C.A., Kidd, E.J., Garratt, J.C. and Wright, I.K. (1990). *In vivo* regulation of 5-HT release - a microdialysis study. Neurosci. Lett. **Suppl. 38**, S11.

Marsden, C.A., Kidd, E.J., Garratt, J.C., Wright, I.K. and Crespi, F. (1990). The regulation of 5-HT release and neuronal activity *in vivo*. Acta Physiol. Polonica **41 (Suppl. 34(1))**, 51-52.

Marsden, C.A., Lawrence, A.J., Kidd, E.J., Garratt, J.C., Mundey, M.K. and Wright, I.K. (1991). *In vivo* assessment of pre-synaptic 5-HT receptor function in the CNS. Serotonin 1991 Meeting, Birmingham University, July 14-17$^{th}$, S9.

Marsden, C.A., Wright, I.K. and Crespi, F. (1991). *In vivo* neurochemical techniques in the analysis of behaviour. J. Psychopharmacol. (in press).

Wright, I.K., Crespi, F., Marsden, C.A., Jones, G.H. and Robbins, T.W. (1989). Effect of isolation-rearing on 5-HT release in the rat frontal cortex. J. Neurosci. Meth. **29**, 283.

Wright, I.K., Heaton, M., Upton, N. and Marsden, C.A. (1991). Comparison of acute and chronic treatment of diazepam and ritanserin in the elevated plus maze model of anxiety. In: "New Concepts in Anxiety" (Eds. M. Briley and S.E. File), Macmillan Press, London, pp. 277-287.

Wright, I.K., Heaton, M., Upton, N. and Marsden, C.A. (1991). Comparison of acute and chronic treatment of various serotonergic agents with those of diazepam and ritanserin in the elevated X-maze model of anxiety. Psychopharmacology (submitted).

Wright, I.K., Ismail, H., Upton, N. and Marsden, C.A. (1990). Isolation-rearing affects the 5-HT behavioural syndrome induced by 5-HT agonists. 5-HT Satellite meeting, Basel. P110.

Wright, I.K., Ismail, H., Upton, N. and Marsden, C.A. (1990). Effect of isolation-rearing on performance in the elevated plus-maze and open field behaviour. Br. J. Pharmacol. **100**, 357P.

Wright, I.K., Ismail, H., Upton, N. and Marsden, C.A. (1991). Effect of isolation-rearing on 5-HT agonist induced responses in the rat. Psychopharmacology (in press).

Wright, I.K., Upton, N. and Marsden, C.A. (1991). Effect of diazepam on extracellular 5-HT in the ventral hippocampus observed in rats on the X-maze using in vivo microdialysis. Serotonin 1991 Meeting, Birmingham University, July 14-17$^{th}$, P118.

Wright, I.K., Upton, N. and Marsden, C.A. (1991). Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. Physiol. Behav. (in press).

Wright, I.K., Upton, N. and Marsden, C.A. (1991). Effect of diazepam on extracellular 5-HT in the ventral hippocampus observed in rats on the elevated X-maze model of anxiety using *in vivo* microdialysis. Br. J. Pharmacol. (in press).

**ABSTRACT**

This thesis attempts to determine the role of 5-hydroxytryptamine (5-HT) in rodent models of anxiety. Using the elevated X-maze it was possible to detect dose-dependent anxiolytic effects of diazepam and dose-dependent anxiogenic effects of FG 7142 (a ß-carboline derivative) and idazoxan. The fear-potentiated acoustic startle paradigm also detected the effects of these compounds but the results were neither dose-dependent nor very reproducible. Using the X-maze the 5-$HT_{1A}$ receptor partial agonist ipsapirone had no effect after either acute or chronic treatment, while both the 5-$HT_2$ receptor antagonist, ritanserin, and the 5-$HT_3$ receptor antagonist, ondansetron, had anxiolytic activity after chronic treatment.

Using *in vivo* mirodialysis it was demonstrated that extracellular levels of 5-HT in the ventral hippocampus increase when the animal is on the X-maze, and both diazepam and F 2692 (1-(3'-trifluoro-methylphenyl) 1,4-dihydro 3-amino 4-oxo 6-methyl pyridazine) reduced the increased 5-HT levels and produced an anxiolytic behavioural profile in the same animal. Thus, the inhibition of increased 5-HT release may be important for anxiolytic activity. However, ipsapirone also reduced the increased 5-HT but did not produce an anxiolytic profile. The lack of an anxiolytic effect may be the result of postsynaptic 5-$HT_{1A}$ receptor stimulation.

Rats reared in isolation immediately post-weaning (21 days of age) displayed enhanced locomotor activity and an anxiogenic profile on the X-maze. This anxiogenic profile was neither reversed by resocialisation of the isolation-reared rats nor produced by isolation of adult socially housed animals, indicating a permanent developmental change. Isolation-reared rats had reduced stimulated release of 5-HT measured in the frontal cortex, indicating reduced presynaptic function, and enhanced responsiveness to agonists acting at postsynaptic 5-$HT_{1A}$ and 5-$HT_2$ receptors, suggesting supersensitivity at these sites.

Overall, 5-HT appears to be involved in anxiety, with 'anxious' behaviour either on the X-maze or by isolation-rearing causing changes in 5-HT function. Thus one of the mechanisms of action of established and putative anxiolytic and anxiogenic compounds may be via modulation of the ascending dorsal raphe serotonergic neuronal pathways.

All work in this thesis was undertaken under Home Office Personal Licence PIL 70/02103 and Project Licences PPL 40/0380 (Nottingham University) and PPL 70/00349 (SmithKline Beecham).

# CHAPTER 1

# GENERAL INTRODUCTION

**Identification of 5-Hydroxytryptamine**

The compound now known as 5-hydroxytryptamine (5-HT) was first isolated from bovine serum and given the name "serotonin" by Rapport (1949). The name was derived from its source (serum) and its activity (vasoconstriction). In 1951, the biogenic amine, serotonin, was definitely identified as 3-(2-aminoethyl)-5-hydroxyindole or 5-HT (Hamlin and Fischer, 1951). About this time a substance called "enteramine", found in high concentrations in chromaffin cells of the intestinal mucosa, also seen to constrict smooth muscular elements, was shown to be identical to 5-HT (Erspamer and Asero, 1952). In 1953, Twarog and Page identified 5-HT in the brain of rats and rabbits; and interest in 5-HT as a possible central nervous system (CNS) transmitter dates from the same year, when Fingl and Gaddum (1953) found that minute quantities of lysergic acid diethylamide (LSD) could antagonize the effect of 5-HT on the rat uterus, and suggested that its central effects (it was already known to be a potent psychotomimetic substance) might also be related to this action. Even though CNS 5-HT accounts for only about 1% of the total body content, it occupies a central position in the neurochemical hegemony (Jacobs and Gelperin,1981; Osborne,1982).

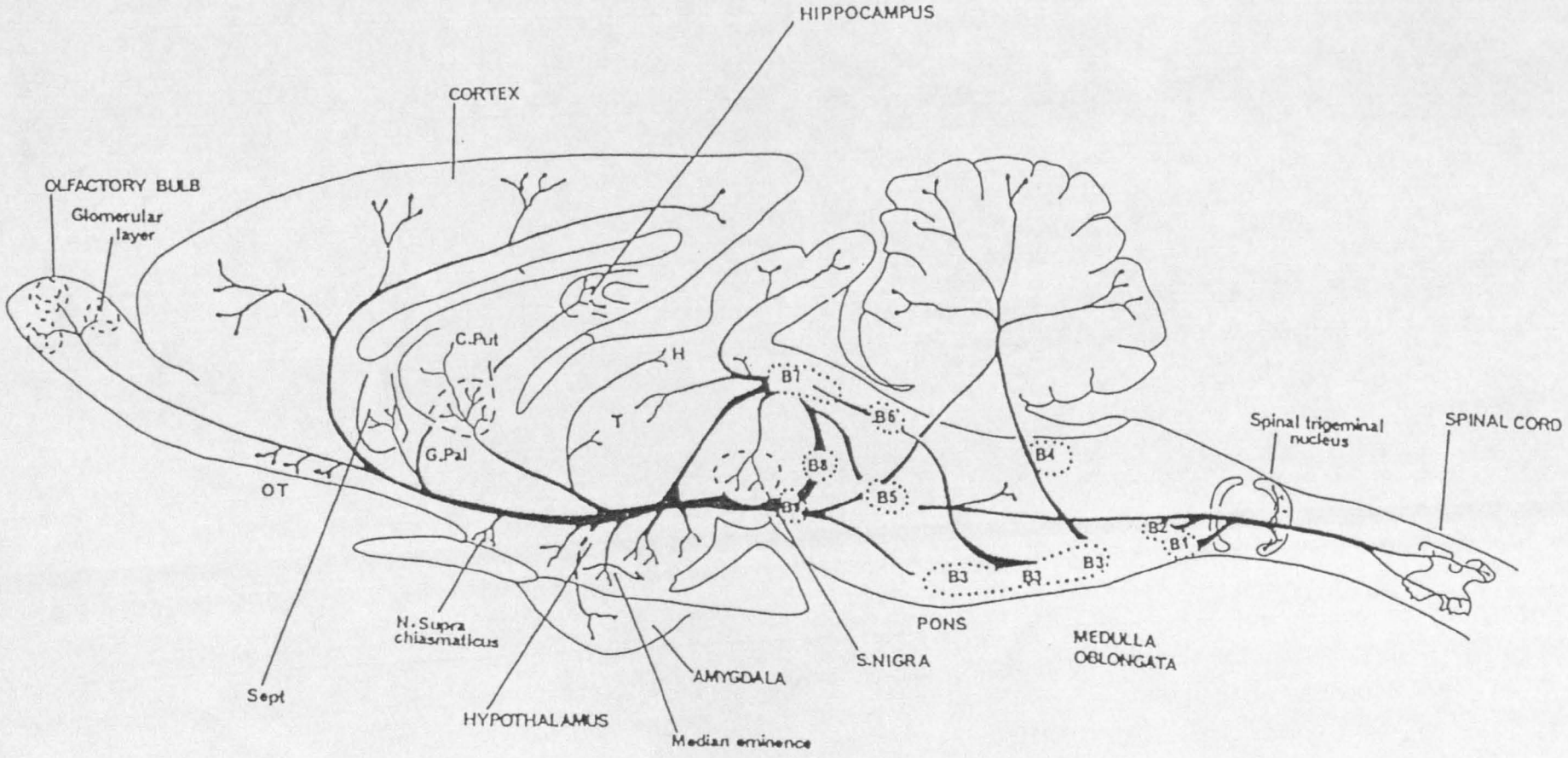
**Distribution of 5-HT**

Estimation of the amount of 5-HT in dissected brain areas by Amin et al. (1954) showed that the highest concentration was found in the hypothalamus, area postrema and midbrain. Using the technique of fluorescence histochemistry, developed by Falck et al. (1962), Dahlstrom and Fuxe (1964) showed that there were nine groups of cells (named B1-B9) containing 5-HT in the brainstem and midbrain, located mainly in the raphe nuclei. These cells send projections to the spinal cord, brain stem nuclei, and the forebrain (Dahlstrom and Fuxe, 1964). A sagital section showing these projections is shown in Figure 1. The dorsal (B7) and median (B8) raphe nuclei were shown to contain most of the 5-HT-producing neurones of the midbrain. From numerous studies it has emerged that there is good agreement in the overall distribution of the 5-HT-containing cell bodies in different mammalian species. Anden et al. (1966) demonstrated a loss of fluorescent 5-HT axons in forebrain following lesions of the anterior raphe nuclei or of the hypothalamus. These results, combined with the inability to find 5-HT-containing cell bodies in the forebrain, lead to the

**Figure 1**

Diagrammatic representation of a sagittal section through a rat brain showing the serotonergic cell groups, B1 - B9 and their major neuronal pathways which provide the serotonergic innervation of the brain. The drawing was taken from Consolazine and Cuello (1982).

Key: OT = olfactory tuberculum, sept = septum, C.Put = caudate putamen, G.Pal = globus pallidus, T = thalamus, H = habenula, S.Nigra = substantia nigra.


HIPPOCAMPUS
CORTEX
OLFACTORY BULB
Glomerular layer
C.Put
H
T
G.Pal
OT
Sept
N. Supra chiasmaticus
HYPOTHALAMUS
Median eminence
AMYGDALA
S.NIGRA
PONS
MEDULLA OBLONGATA
Spinal trigeminal nucleus
SPINAL CORD
B1
B6
B8
B7
B5
B4
B2
B3

proposal that most of the ascending 5-HT projections arise from cell bodies situated in the raphe nuclei of the midbrain. Using anterograde transport of [$^3$H]-proline it was shown that the projections from both the dorsal (B7) and median (B8) raphe nuclei ascended in 6 tracts and innervated different areas of the brain (Azmitia and Segal, 1978). Using intracerebroventricular administration of [$^3$H]-5-HT (Parent et al., 1981) two major ascending systems of fibres, the transtegmental and periventricular which met in the medial forebrain bundle in the hypothalamus, were found. Parent et al. (1981) also described the distribution of varicosities and terminals in various areas of the brain, including a uniform innnervation of all cortical layers, obtaining clearer results than those seen with fluorescence (Fuxe, 1965) or [$^3$H]-proline (Azmitia and Segal, 1978). Using an antibody to 5-HT to examine the distribution of 5-HT cell bodies and terminals Steinbush (1981) found that besides labelling cell groups B1-B9, cell bodies were also seen in the area postrema, locus coeruleus and interpeduncular nucleus.

**Synthesis, Release and Metabolism of 5-HT**

5-HT is formed by a two-step process from the essential amino acid, L-tryptophan, which enters the brain from the blood by both diffusion and carrier-mediated transport (Young and Sourkes, 1977; Pratt, 1979). In the cytoplasm of serotonergic neurones, L-tryptophan is converted to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase and then decarboxylated to 5-HT by aromatic L-amino acid decarboxylase (Boadle-Biber, 1982). Administration of tryptophan to rats causes an elevation in brain 5-HT levels (Fernstrom and Hirsh, 1974); however, the carrier-mediated transport depends both on the amount of tryptophan and the relative proportions of other amino acids in the diet (Partridge, 1979).

Newly synthesized 5-HT either remains in the cytoplasm or is taken up into synaptic vesicles. As most of the 5-HT in the brain is stored in vesicles it has been suggested that 5-HT is released by exocytosis, a process which is calcium-dependent (for review see Sanders-Bush and Martin, 1982). However, there is evidence which shows that 5-HT is not necessarily released by exocytosis and that it is newly synthesized 5-HT which is preferentially released (Kuhn et al., 1986). There appears to be two pools of 5-HT, one in the cytoplasm and one in the vesicles, the latter being a stable store which is

only released after prolonged stimulation (Grahame-Smith, 1971, 1974; Elks et al., 1979). The release mechanism of 5-HT is unclear and all of the putative mechanisms put forward still require calcium for normal functioning (Unvas and Aborg, 1984; Kuhn et al., 1986).

After release, 5-HT is taken up into neurones by a carrier which is dependent upon $Na^+/K^+$ ATPase (for review see Ross, 1982). After reuptake, 5-HT either enters the vesicles or is rapidly metabolised to either melatonin by the enzymes N-acetyltransferase and hydroxindole-O-methyltransferase, or 5-hydroxyindole acetic acid (5-HIAA) by monoamine oxidase A and aldehyde dehydrogenase (Kuhn et al., 1986). There is evidence which suggests that excessive 5-HT in the nerve terminal is also metabolised to 5-HIAA and thus 5-HIAA levels are not a good marker of 5-HT release (Grahame-Smith, 1971, 1974; Kuhn et al., 1986).

The actions of 5-HT are profound and it is thought to play a modulatory role in many physiological functions, such as feeding (Blundell and Latham, 1982; Samanin, 1983), locomotor activity (Jacobs et al., 1984), and social and sexual behaviour (Ahlenius et al., 1981; Hillegaart et al., 1989). The diverse actions of 5-HT suggest that it is interacting with many different subtypes of the 5-HT receptor.

**5-HT Receptor Classification**

In 1957 Gaddum and Picarelli presented evidence for the existence of two pharmacologically distinct types of receptor for 5-HT in guinea-pig ileum. One type of receptor mediated the contraction of the smooth muscle and was antagonised by phenoxybenzamine (dibenzyline), and was named the 'D' receptor subtype; the other type of receptor mediated depolarisation of the cholinergic nerves and was antagonised by morphine and named the 'M' receptor subtype (Gaddum and Picarelli, 1957). This hypothesis remained until the late 1970s when radioligand binding studies became available. Initially two binding sites were identified in the CNS using [$^3$H]-5-HT and [$^3$H]-spiperone (Peroutka and Snyder, 1979). Drugs such as 5-HT displayed different potencies for displacing [$^3$H]-5-HT and [$^3$H]-spiperone binding and the Hill coefficient for [$^3$H]-lysergic acid diethylamide (LSD) binding in the presence of 5-HT was less than one, indicating the existence of more than

one binding site. These two sites were called 5-$HT_1$ and 5-$HT_2$ respectively (Peroutka and Snyder, 1979); the 5-$HT_1$ sites were labelled with nanomolar concentrations of [$^3$H]-5-HT, and the 5-$HT_2$ sites were labelled with micromolar concentrations of [$^3$H]-5-HT and also showed high affinity for spiperone (Peroutka and Snyder, 1979). This discovery aroused interest and efforts were made to develop more selective compounds. Based on the atypical displacement curves of spiperone on [$^3$H]-5-HT the 5-$HT_1$ receptor was found to be divided into at least two subtypes, one with high affinity and the other with low affinity (Pedigo et al., 1981). The high affinity site was called the 5-$HT_{1A}$ site (Deshmukh et al., 1982) and characterised using the selective tetralin derivative, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, Gozlan et al., 1983; Middlemiss and Fozard, 1983). The low affinity site was named the 5-$HT_{1B}$ site and characterised with RU24969 (Sills et al., 1984; Middlemiss, 1985) and cyanopindolol (Sills et al., 1984; Hoyer et al., 1985). The 5-$HT_{1B}$ receptor is present only in some rodent brains and has not been found in guinea-pig, cow, chicken, turtle, frog, or human brain membranes (Hoyer et al., 1986a; Heuring et al., 1986). A novel 5-HT binding site was found in porcine choroid plexus which was distinct from 5-$HT_{1A}$, 5-$HT_{1B}$ and 5-$HT_2$ binding sites (Pazos et al., 1984). This site has been named the 5-$HT_{1C}$ site because it has high affinity for 5-HT and relatively low affinity for RU24969 and spiperone (Pazos et al., 1984). In bovine caudate a fourth type was found and called 5-$HT_{1D}$ (Heuring and Peroutka, 1987). This receptor subtype was initially not thought to exist in the rodent brain but has subsequently been found there (Herrick-Davis and Titeler, 1988). Recent evidence suggests two subtypes of the 5-$HT_{1D}$ binding site in guinea-pig, rabbit, dog and human cortex (Middlemiss et al., 1990; Elliott et al., 1991). It is possible that the 5-$HT_{1D}$ receptor in guinea-pig and non-rodent brains is equivalent to the 5-$HT_{1B}$ receptor in the rat and mouse (Hoyer and Middlemiss, 1989). Recently, other 5-$HT_1$ binding sites which have been described are the 5-$HT_{1E}$ receptor in human cortex (Leonhardt et al., 1989), the 5-$HT_{1P}$ receptor in guinea-pig enteric neurones (Mawe et al., 1986), and the 5-$HT_{1R}$ receptor in rabbit caudate (Xiong and Nelson, 1989). Given the known species variations in 5-$HT_{1D}$/5-$HT_{1B}$ sites (Hoyer and Middlemiss, 1989), it is possible that the 5-$HT_{1R}$ binding site may represent the rabbit version of the 5-$HT_{1D}$ receptor binding site. To date, little evidence exists to demonstrate that these binding sites are functional receptors. Bradley et al. (1986a) proposed that the subtypes of the 5-$HT_1$ receptor be referred to as '5-$HT_1$-like' receptors until functional correlates

have been found for each subtype. The D receptor in the periphery has been shown to be the functional correlate of the central 5-$HT_2$ receptor (Engel et al., 1985; Maayani et al., 1984; Bradley et al., 1986a). It has been suggested that the 5-$HT_2$ receptor has subtypes (McKenna and Peroutka, 1989) but there is much controversy surrounding this theory (Strange, 1990) with recent findings suggesting that apparent heterogeneity in functional 5-$HT_2$ receptors is species-dependent (Mylecharane and Martin, 1991). The M receptor was designated the 5-$HT_3$ receptor (Richardson and Engel, 1986; Bradley et al., 1986a) and was thought only to exist in the periphery (Bradley et al., 1986a) but has subsequently been found in the CNS (Kilpatrick et al., 1987). Work suggested that this receptor may have at least 3 distinct subtypes (Richardson et al., 1985; Richardson and Engel, 1986; Bradley et al., 1986a) and although this was thought not to be the case (Mylecharane, 1989) recent evidence suggests heterogeneity at this site (Butler et al., 1990; Eglen et al., 1991), and that the 5-$HT_3$ receptors of the rat, mouse and guinea-pig cervical ganglion may be different (Newberry et al., 1991). Recently, a fourth 5-HT receptor subtype has been found in mouse embryo colliculi neurons in primary culture (Dumuis et al., 1988). This has been named the 5-$HT_4$ receptor, and more recently has been found in rodent brain (Dumuis et al., 1989), guinea-pig ileum (Eglen et al., 1990), piglet left atrium (Kaumann et al., 1991) and guinea-pig ascending colon (Bunce et al., 1991; Elswood et al., 1991).

**5-HT Receptor Subtypes**

**5-$HT_{1A}$ Receptors**

5-$HT_{1A}$ receptors are distributed in a number of brain regions with the highest densities in the cortex, hippocampus, and raphe nuclei, especially the dorsal raphe nucleus (DRN) (Pazos and Palacios, 1985; Hoyer et al., 1985; Verge et al., 1986). The receptors are located both pre- and post-synaptically. The pre-synaptic sites are found in the DRN on the cell bodies and dendrites (Pazos and Palacios, 1985; Hoyer et al., 1985; Sotelo et al., 1991; Verge et al., 1986; Waeber et al., 1989) and the receptors in the hippocampus and frontal cortex appear to be located post-synaptically (Pazos and Palacios, 1985; Hoyer et al., 1985; Verge et al., 1986; Waeber et al., 1989).

Recently, a genomic clone (G-21), when expressed in monkey kidney cells, has the appearance of a 5-$HT_{1A}$ receptor (Fargin et al., 1988). The binding sites expressed by the cells have high affinity for [$^3$H]-8-OH-DPAT and appeared to have two affinity states. High affinity binding was converted to low affinity binding by the addition of guanyl nucleotides. The G-21 clone has 7 transmembrane domains (a sign of a G-protein coupled receptor) and a 45% homology with adrenergic receptors (Fargin et al., 1988).

5-$HT_{1A}$ receptors are coupled to adenylate cyclase as their second messenger transduction system. Stimulation of adenylate cyclase by 5-$HT_{1A}$ receptor activation has been demonstrated in guinea-pig hippocampus (Shenker et al., 1987) and rat cortex and hippocampus (Markstein et al., 1986; Mork and Geisler, 1990). 5-$HT_{1A}$ receptor activation also inhibits forskolin- or $Ca^{2+}$-stimulated adenylate cyclase activity in guinea-pig and rat hippocampus (de Vivo and Maayani, 1986; Bockaert et al., 1987). The exact roles of the two different couplings still remains unclear.

Electrophysiological data have demonstrated that 5-$HT_{1A}$ agonists inhibit both the spontaneous firing rate of 5-HT neurones in the DRN (Fallon et al., 1983; VanderMaelen and Wilderman, 1984; Sprouse and Aghajanian, 1986) and the population spike generated by hippocampal CA1 cells (Beck and Goldfarb, 1985).

Measurement of 5-HT *in vivo* using either microdialysis or differential pulse voltammetry, has found that 5-$HT_{1A}$ agonists decrease 5-HT release in terminal regions such as the frontal cortex and hippocampus (Crespi et al., 1990; Hjorth and Magnusson; 1988; Hutson et al., 1989; Sharp et al., 1989a). This effect is thought to be predominantly as a result of action at the somatodendritic autoreceptor in the dorsal raphe nucleus (Hjorth and Magnusson, 1988; Hutson et al., 1989; Sharp et al., 1989c).

Administration of 5-HT precursors such as L-tryptophan or 5-hydroxytryptophan (5-HTP) in the presence of a peripheral decarboxylase inhibitor results in a series of behaviours known as the '5-HT behavioural syndrome' (Grahame-Smith, 1971; Green et al., 1983b). The syndrome is also observed after 8-OH-DPAT (Hjorth et al., 1982; Tricklebank et al., 1985a; Goodwin and Green, 1985) and consists of hyperlocomotion, hindlimb abduction, head weaving, flat body posture and reciprocal forepaw treading

following prazosin or haloperidol pretreatment, hyperlocomotion and head weaving are lost, suggesting a catecholaminergic component (Tricklebank et al., 1985a). It is thought that the flat body posture and reciprocal forepaw treading are mediated by post-synaptic 5-$HT_{1A}$ receptors, and this theory is supported by the fact that these behaviours can be blocked by spiperone, pindolol, methiothepin, BMY 7378 and NAN-190 (Hjorth et al., 1982; Tricklebank et al., 1985a; Hjorth and Sharp, 1990; Sharp et al., 1990).

Administration of the selective 5-$HT_{1A}$ agonist 8-OH-DPAT (Arvidsson et al., 1981; Middlemiss and Fozard, 1983) results in a decrease in rectal temperature in rats and mice maintained at normal ambient temperature (Goodwin et al., 1985; Hutson et al., 1987). 8-OH-DPAT also affects food intake, inducing hyperphagia in freely feeding (satiated) rats (Dourish et al., 1985), and hypophagia in food deprived or restricted feeding rats or rats on a reversed light-dark cycle (Dourish et al., 1985; Marsden et al., 1989). 5-$HT_{1A}$ receptors may be involved in contractile vascular responses to 5-HT and in blood pressure control. The 5-$HT_{1A}$ receptor has been found to mediate contraction in the canine basilar artery and saphenous vein (Fenuik et al., 1985; Peroutka et al., 1986; Frenken, 1989) and the rabbit basilar artery (Bradley et al., 1986b).

**5-$HT_{1B}$ Receptors**

These receptors are found in many areas of rodent brain but appear to be absent in brains of higher mammals (Heuring et al., 1986). The greatest densities of 5-$HT_{1B}$ sites are in the dorsal subiculum, caudate putamen, globus pallidus and substantia nigra (Pazos and Palacios, 1985; Verge et al., 1986).

Synaptosomal release of 5-HT can be reduced by stimulation of the 5-$HT_{1B}$ receptor in rat brain (Raiteri et al., 1986). Other studies have shown that in the rat brain the terminal 5-HT autoreceptor in the frontal cortex is of the 5-$HT_{1B}$ subtype (Brazell et al., 1985; Hoyer et al., 1985; Middlemiss, 1985, 1986; Engel et al., 1986).

At present there are no behavioural tests of 5-$HT_{1B}$ receptor function but recent evidence suggests that RU24969-induced hyperlocomotion in mice is the result of 5-$HT_{1B}$ receptor activation and involves catecholamine neurones in its expression (Cheetham et al., 1991).

**5-$HT_{1C}$ Receptors**

5-$HT_{1C}$ receptor sites were initially only thought to exist in the choroid plexus, where they appear to account for all the 5-$HT_1$ sites (Waeber et al., 1989b), but using *in situ* hybridization histochemistry these sites have been found in lower concentrations in other brain regions such as the cortex, amygdala, hippocampus, thalamus, substantia nigra and brain stem (Hoyer et al., 1986b, c; Hoffman and Mezey, 1989; Molineaux et al., 1989).

The 5-$HT_{1C}$ receptor has been cloned and sequenced (Julius et al., 1988) and consists of a 460 amino acid sequence which contains 7 transmembrane domains (a sign of a G-protein coupled receptor) (Julius et al., 1988). The 5-$HT_{1C}$ and 5-$HT_2$ receptors show remarkable similarity in their amino acid sequence (Lubbert et al., 1987; Julius et al., 1988), and physiologically, activation of the 5-$HT_{1C}$, or 5-$HT_2$, site stimulates phosphoinositol (PI) hydrolysis (Conn et al., 1986). Physiologically, the 5-$HT_{1C}$ site appears to modulate a $Cl^-$ conductance channel via a pertussis toxin sensitive G-protein. However, selective antagonists are required for full characterization of the 5-$HT_{1C}$ receptor (Bradley et al., 1986a).

**5-$HT_{1D}$ Receptors**

5-$HT_{1D}$ receptors are found in the guinea-pig and man and are located in similar areas to 5-$HT_{1B}$ receptors in species such as the rat (Herrick-Davis and Titeler, 1988; Waeber et al., 1989), being located throughout the brain with high concentrations in the basal ganglia (Pazos and Palacios, 1985; Waeber et al., 1988a).

5-$HT_{1D}$ sites, like the 5-$HT_{1B}$ sites, are negatively coupled to adenylate cyclase in bovine substantia nigra (Hoyer and Shoeffer, 1988) and unilateral activation of the 5-$HT_{1D}$ receptors in the substantia nigra of the guinea-pig elicits contralateral rotation (Higgins et al., 1991). It is also thought to be involved with kidney perfusion and contraction of the rat stomach fundus (Heuring and Peroutka, 1987).

**5-$HT_2$ Receptors**

5-$HT_2$ receptors are located post-synaptically in the cortex, claustrum and brain stem with lower levels in the caudate putamen and substantia nigra (Blackshear et al., 1981; Leysen et al., 1982; Pazos et al., 1985; Hoyer et al., 1986b; McKenna et al., 1989).

The 5-$HT_2$ receptor has been cloned (Pritchett et al., 1988) and found to have 7 transmembrane domains, indicating possible coupling to G-proteins, and 51% sequence homology with the cloned 5-$HT_{1C}$ receptor (Pritchett et al., 1988). Xenopus oocytes injected with the RNA encoding for the nucleotide sequences produced 5-$HT_2$ receptors which controlled $Ca^{2+}$-sensitive chloride channels (Pritchett et al., 1988). Another group has cloned the 5-$HT_2$ receptor and found it coupled with PI hydrolysis (Julius et al., 1990). The same group found the 5-$HT_2$ and 5-$HT_{1C}$ receptor cDNAs distributed in different regions of the CNS and concluded they were not coding for the same receptor (Julius et al., 1990).

5-$HT_2$ receptors are coupled to PI metabolism as their second messenger transduction system. Stimulation of PI by 5-$HT_2$ receptor activation has been demonstrated in platelets (de Chaffoy de Courcelles et al., 1985), smooth muscle cells (Doyle et al., 1986) and rat cerebral cortex (Conn and Sanders-Bush, 1984, 1986; Kendall and Nahorski, 1985). 5-$HT_2$ receptor stimulated PI hydrolysis is not a large response, usually less than a 2-fold increase (Conn and Sanders-Bush, 1984, 1986; Kendall and Nahorski, 1985) which is biphasic, with selective 5-$HT_2$ receptor antagonists only capable of inhibiting the high affinity component (Kendall and Nahorski, 1985).

The main behavioural response seen after 5-$HT_2$ receptor agonists is the head-twitch response in mice (Corne et al., 1963; Corne and Pickering, 1967; Goodwin and Green, 1985; Heaton et al., 1988) and the 'wet-dog shake' (WDS) response in rats (Bedard and Pycock, 1977; Mathews and Smith, 1980; Goodwin and Green, 1985). Intrathecal administration of the selective 5-$HT_2$ agonists 2,5-dimethoxy-α,4-dimethyl-2-aminopropane (DOM) and 1-(2,5-dimethoxy-4-iodomethyl)-2-aminopropane (DOI) produces WDS and also back muscle contractions; both behaviours can be antagonised by ketanserin, a 5-$HT_2$ antagonist (Fone et al., 1989, 1991). Both $\alpha_1$-adrenoceptor antagonists and $\alpha_2$-adrenoceptor agonists inhibit the WDS response generated by 5-$HT_2$ agonists in rats and mice (Green and Heal, 1985; Handley and Brown, 1982), whereas ß-adrenoceptor agonists and $\alpha_2$-adrenoceptor antagonists potentiate the WDS response generated by 5-$HT_2$ agonists (Handley and Brown, 1982; Handley and Singh, 1986), indicating a catecholamine component. In addition, benzodiazepines also potentiate the WDS response (Handley and Singh, 1986). Finally, other compounds apart

from 5-$HT_2$ receptor agonists have been seen to produce the WDS response, such as TRH and its analogues (Drust and Connor, 1983; Fone et al., 1989) and enkephalins (Drust and Connor, 1983), suggesting that this is a complex response involving several neurochemical systems.

**5-$HT_3$ receptors**

5-$HT_3$ receptors are found in the cortex, amygdala, hippocampus, area postrema and the highest density is found in the area subpostrema (Kilpatrick et al., 1987; Pratt et al., 1990). 5-$HT_3$ sites have also been identified in the human brain (Barnes et al., 1988, 1989a) being predominantly located in the amygdala and hippocampus (Barnes et al., 1989a).

The second messenger transduction system of centrally located 5-$HT_3$ receptors has as yet not been elucidated and it is now thought that the receptor may be directly coupled to the ion channel (Clarke et al., 1989). However, a recent study has shown 5-$HT_3$ receptor agonists to stimulate PI hydrolysis in the fronto-cingulate and entorhinal cortices (Edwards et al., 1991). Using *in vivo* microdialysis it has been demonstrated that 5-$HT_3$ receptor stimulation in the ventral hippocampus increases 5-HT release in this region, suggesting presynaptic facilitatory 5-$HT_3$ receptors are involved in the control of 5-HT release (Martin et al., 1991).

Behavioural studies have shown that ondansetron (GR38032F), a selective 5-$HT_3$ antagonist, reduces dopamine mediated behaviour (produced by either amphetamine administration or chronic intracerebral dopamine infusion) back to basal levels and does not produce the catalepsy observed with dopamine antagonists (Hagan et al., 1987; Costall et al., 1987a), suggesting possible use in the treatment of schizophrenia. 5-$HT_3$ antagonists have also been found to increase acetylcholine release in cortical tissue but only in the presence of ritanserin (Barnes et al., 1989b) and to enhance cognitive performance in the marmoset (Domeney et al., 1991). 5-$HT_3$ antagonists produce no direct observable behavioural effects but are active in several models of anxiety (Jones et al., 1987, 1988; Costall et al., 1988, 1989a). There is evidence to suggest that 5-$HT_3$ antagonists reduce the withdrawal effects observed with drugs of abuse (ethanol, nicotine and cocaine) (Costall et al., 1990a, b). Finally, 5-$HT_3$ antagonists are very good anti-emetics especially

for cancer chemotherapy and radiation induced vomiting both in animal studies (Costall et al., 1987b) and the clinic (Carmicheal et al., 1988).

**5-$HT_4$ Receptors**

The 5-$HT_4$ receptor is the most recently described subtype and was first found in mouse embryo colliculi neurones in primary culture where it is positively coupled to adenylate cyclase (Dumuis et al., 1988). Since then these receptors have been found peripherally in guinea-pig ileum (Craig and Clarke, 1989; Eglen et al., 1990) where stimulation evokes peristalsis (Craig and Clarke, 1991) and centrally in guinea-pig hippocampus (Shenker et al., 1987). The hippocampal 5-$HT_4$ receptor is also positively coupled to adenylate cyclase (Shenker et al., 1987).

## ANXIETY

Anxiety exists as a normal state in all of us, however it is possible for it to develop into a full-blown pathological condition. The distinction between a pathological, as opposed to a normal, state of anxiety, is hard to draw, but in spite of (or perhaps because of) this diagnostic vagueness, anxiolytic drugs are among the most frequently prescribed substances. A recent study in Western European countries showed that the proportion of the total population using anxiolytic drugs regularly was 17% in Belgium and France, 14% in the U.K. and 10% in Spain.

A person suffering from anxiety experiences an unpleasant emotional state characterized by tension, nervousness, heart palpitations, tremor, nausea, dizziness, inability to think clearly and sometimes an inability to speak; other symptoms may include backache, headache and diarrhoea.

The Diagnostic and Statistical Manual of the American Psychiatric Association (American Psychiatric Association, 1987) has classified anxiety disorders as shown in Table 1.

These are essentially human manifestations, and except for certain components of the somatic and autonomic changes, have no obvious measurable counterpart in experimental animals. To develop new anxiolytic drugs it is essential to have animal tests that give a good guide to

**Table 1**

DSM-III-R. Classification of Anxiety Disorders. Taken from the Diagnostic and Statistical Manual of Mental Disorders (1987), 3rd Ed. revised, p. 7. American Psychiatric Association: Washington D.C..

DSM-III-R. Classification of Anxiety Disorders

Anxiety disorders (or anxiety and phobic neuroses)

Panic disorder

300.21 with agrophobia

Specify current severity of agrophobic avoidance

Specify current severity of panic attacks

300.01 without agrophobia

Specify current severity of panic attacks

300.22 Agrophobia without history of panic attacks

Specify with or without limited symptom attacks

300.23 Social phobia

Specify if generalized type

300.29 Simple phobia

300.30 Obsessive compulsive disorder (or obsessive compulsive neurosis)

309.89 Post-traumatic stress disorder

Specify if delayed onset

300.02 Generalized anxiety disorder

300.00 Anxiety disorder NOS

activity in man, and considerable effort has gone into developing and validating such tests.

## BEHAVIOURAL MODELS OF ANXIETY

Animal models of anxiety are needed both for use in research into the neural basis of anxiety and in screening new drugs for possible anxiolytic activity. For certain clinical conditions, such as depression or schizophrenia, it is unlikely that animal models will ever reflect the essential features of the condition. However, there is no obvious reason why animals should not experience anxiety.

The conditions most likely to elicit anxiety - unpredictability/uncertainty (e.g. triggered by a novel situation), or anticipation of aversive events are also able to produce profound behavioural and physiological responses in animals. It is then up to the pharmacologist to determine whether or not it is justifiable to infer from these changes that an animal is feeling anxious.

Animal models of anxiety can be broadly divided into three main groups:

1. those based on conflict or conditioned fear;
2. those exploiting the uncertainty and anxiety generated by novel situations;
3. those in which anxiety or aversion is chemically induced.

The following section will briefly review the various animal models, giving an indication of the reliability and extent of validation. The most extensively used method of validation is by studying the effects of known anxiolytic and anxiogenic compounds. This has meant relying heavily on the benzodiazepines (BDZ). Hence most of the tests have proved reliable in detecting behavioural changes after BDZ treatment but do not appear to be as sensitive to many of the newer anxiolytics such as buspirone. Thus, it is apt to ask whether the models are actually models of anxiety or models which detect compounds with BDZ-like activity. It is important to note that the BDZ also cause sedation, ataxia, are muscle relaxants and anticonvulsants, and these effects may overlap with the anxiolytic effects we are trying to study.

**CONFLICT TESTS**

**a) The Geller-Seifter test**

This is a widely used model of anxiety (Geller and Seifer, 1962, Geller et al., 1962). In this test the subject, usually a rat, is trained to lever-press on a schedule of food reward that has two components. In one component responses are reinforced with food, typically after a variable interval (VI) of time (i.e. the 'non-conflict' period). In the second component, which is signalled, responses are both reinforced with food and punished with electric shock (i.e. the 'conflict' period). Following fairly extensive training, rats reliably confine most of their responding to the VI, or 'non-conflict', component of the schedule, responding less during the 'conflict' periods. BDZs increase the responding during the 'conflict' component (punished responding) with a less marked effect on the 'non-conflict' component (unpunished responding). An assumption in the test that an increase in punished responding reflects a reduction in anxiety.

**b) Vogel punished drinking test**

A second test based on the conflict between reward and punishment introduced by Vogel et al. (1971). In this test, a water-deprived rat is punished with an electric shock when it drinks from a water spout (the shock being administered either through the spout or the feet). Again, it is important to include a measure of unpunished responding, in order that compounds which are dipsogenic are not detected as possible anxiolytic agents. This test has proved good at detecting anxiolytic drugs, but has a low reliability in that it has a high rate of false positives. It is possible to lower the shock intensity so that the control animals make a higher number of licks, allowing anxiogenic or pro-conflict drugs to be detected (Corda et al., 1983).

The first problem with the conflict tests is individual variability. Although it is an advantage that each animal acts as its own control, individual animals' baseline response rates during conflict periods may be quite variable and require careful titrations of shock intensities to maintain levels of response suppression that are sensitive to the 'disinhibitory' effects of anxiolytic agents (Howard and Pollard, 1977). A second problem occurs in that the two schedules are unlikely to be equally sensitive to drug effects and it is therefore possible that the punished schedule is more sensitive to rate-

enhancing effects. Hence false positives could be observed with drugs such as amphetamine (Lehmann and Ban, 1971; McKearney and Barrett, 1975; Miczek, 1973). A further problem with these conflict tests, is that by definition, they involve "crossed" motivations (e.g. the attainment of food and the avoidance of shock). It has been shown that many anxiolytics, including the BDZs, have powerful stimulatory effects on appetitive and consummatory behaviours (Wise and Dawson, 1974) and this effect could be misinterpreted as possible anxiolytic activity. The period of unpunished responding is meant to control for this but often the rate of responding is almost maximal during this period and thus increases due to hyperphagic or dipsogenic effects of the compound can easily be missed. The waiting capacity of a rat has been shown to be reduced by BDZs (Thiebot, 1986), thus it is possible that increased responding during the punished responding period is as a result of reduced waiting capacity and not anxiolytic activity.

## TESTS BASED ON CLASSICAL CONDITIONING

### Conditioned emotional responding

Conditioned emotional responding (CER) can be produced by presenting the subject with an unavoidable electric shock that is reliably preceded by a signal, i.e. a 'conditioned stimulus' (CS). The CS can be superimposed upon an ongoing behaviour that is being positively reinforced (i.e. an operant), either in the same situation as CS-shock pairings ('on baseline'), or in a different situation ('off-baseline'). The suppression of operant responding that occurs in the presence of the CS is called a conditioned emotional response (Brady and Hunt, 1955), or alternatively, conditioned suppression (Estes and Skinner, 1941).

The results of studies of anxiolytic agents on CER have not been particularly consistent. For example, anxiolytic agents have been reported to attenuate (Edwards and Eckermann, 1979; Methot and Deutsch, 1984) , facilitate (Stein and Berger, 1969), or have no effect on CER (Ray, 1964; Scobie and Garske, 1970). Non-anxiolytic agents have also been found to produce inconsistent results, with compounds such as amphetamine either attenuating CER (Capell et al., 1972) or having no effect (Tenen, 1967).

In spite of these problems, one variation of CER called the 'potentiated startle' paradigm may warrant further investigation (Davis, 1979a). In this procedure rats are first trained to associate a stimulus (e.g. light) with the onset of aversive footshock. When this conditioned 'fear' stimulus is presented in the context of a sudden, loud noise, the 'startle' reflex that occurs in response to the loud noise is 'potentiated'. Anxiolytics selectively block the 'potentiated' startle reflex without affecting the 'unconditioned' startle reflex that occurs when the loud noise is presented alone (Davis, 1979a). This paradigm is one which is studied in this thesis and a more detailed description of the model and its validation to date will be described later.

## TESTS BASED ON UNCERTAINTY/NOVELTY
## TESTS OF EXPLORATION

In low doses, BDZs can enhance exploratory behaviour of rodents in a novel environment. File (1985) reviewed the effects of BDZs on exploratory behaviour and concluded that, whilst undoubtedly anxiety is a factor influencing exploration, there are always several others also operating in any given test situation and it is important that these be remembered in the analysis of the results. The effect of BDZs appears to be biphasic, with a facilitatory effect occurring at low doses and an inhibitory effect occurring at high doses due to the emergence of concurrent sedative effects (File, 1985). The effect is time dependent: facilitation of exploratory behaviour typically occurs during the initial phase of the test session, and may reverse during later phases (Iwahara and Sakama, 1972).

### a) "Four-plate" test

In this test, usually with mice, locomotor activity around a small arena, consisting of four metal plates, is punished by administering electric shock through the metal plates every time the animal crosses from one plate to another (Boisser et al., 1968). Boisser et al. (1968) found that the reduction in crossings could be reversed by a number of known anxiolytics, whereas neuroleptics, antidepressants and analgesics were ineffective. However, some stimulants (e.g. amphetamine) did increase the number of crossings (Boisser et al., 1968). This test needs considerably more validation before its usefulness can be properly assessed.

**b) Two-Compartment Chamber (Black and White Box)**

This test, introduced by Crawley and her associates (Crawley and Goodwin, 1980; Blumstein and Crawley, 1983), utilizes the natural tendency of rodents to avoid brightly lit areas (File, 1980). Mice are placed on the brightly illuminated side of a two-compartment chamber, and time in each compartment, the number of transitions between the light and dark sides are recorded, along with total activity. The total amount of time spent in each of the two compartments is not reliably affected by anxiolytic compounds, indicating that the preference for the dark remained, but the balance between exploration of the novel two-compartment chamber and dark preference was shifted by anxiolytics (Crawley and Goodwin, 1980; Blumstein and Crawley, 1983). Further validation of this model using BDZ and non-BDZ compounds such as buspirone and 5-$HT_3$ antagonists has found all to increase the number of rears, line crossings, the time spent in the brightly illuminated side, the number of transitions from one side of the chamber to the other, and the latency of the first transit from the brightly illuminated side to the dark side (Costall et al., 1989a).

**c) Elevated plus-maze test**

The test is based on a procedure used by Montgomery (1955) who showed that exposure to an elevated (open) maze alley evoked an approach-avoidance conflict that was considerably stronger than that evoked by exposure to an enclosed maze alley. Montgomery (1955) then showed, using a Y-maze with a varying ratio of open:enclosed arms, that animals clearly preferred (i.e. spent more time on) the enclosed arms. Handley and Mithani (1984) described a procedure based on that of Montgomery (1955), which consisted of a plus-maze with two open and two enclosed arms, elevated to a height of 50cm. Pellow et al. (1985) described the validation of such a test by confining rats to either the two open arms or the two closed arms of the maze, and taking a series of measurements. There was a significant decrease in the number of entries, and a significant increase in the time spent motionless or freezing and in the number of defaecations in the rats confined to the open arms. Also, although rats confined to the open and enclosed arms had significantly elevated plasma corticosterone levels compared to home cage controls the elevation in corticosterone was significantly greater in the rats

confined to the open arms (Pellow et al., 1985). It was suggested that a drug which reduced anxiety should increase the overall proportion of entries into the open arms, i.e. increase the open-arm entries as a percentage of the total entries. Also, the percentage of the total time spent on the open arms could also provide a valid measure of anxiety. The total number of arm entries would provide a measure of general activity.

Drugs that are active in the clinic as anxiolytics, diazepam and chlordiazepoxide, were found to increase those measures on the plus-maze which are thought to reflect 'anxiety' (i.e. time on open arms, and percentage open:total entries and time) (Pellow et al., 1985). Other studies have shown that most compounds that are agonists at the benzodiazepine-$GABA_A$ receptor complex are active in the plus-maze, e.g. alprazolam, adinazolam, U-43,465 (File and Pellow, 1985), CGS 9896 (File and Pellow, 1986) and CL 218,872 (Pellow and File, 1986). Work using either inverse agonists at the benzodiazepine-$GABA_A$ receptor complex, such as the ß-carboline derivative FG 7142 (Pellow and File, 1986), or $\alpha_2$-adrenoceptor antagonists such as yohimbine (Handley and Mithani, 1984; Pellow et al., 1985), both of which are anxiogenic in the clinic (Dorow et al., 1983; Charney et al., 1984), found that these compounds significantly reduced the measures thought to reflect 'anxiety', indicating that the X-maze can also detect compounds with anxiogenic activity. Caffeine, an anxiogenic compound in man (Uhde et al., 1984), also had significant anxiogenic effects in the plus-maze (Pellow et al., 1985). The effects of various 5-HT compounds in this model is addressed in this thesis and information available in the literature on this subject is discussed later in the thesis.

It is important that this test is not confounded by sedation or arousal, since these could produce false positives or negatives, as is the case with the majority of animal tests of anxiety.

**d) Social Interaction**

Another test of anxiolytic agents which exploits the rodents natural proclivity to avoid brightly illuminated and unfamiliar surroundings is the 'social interaction' test. In this test the dependent variable is the time that pairs of male rats spend in active social interaction. The conditions of the test arena are manipulated so that it is either familiar or unfamiliar to the

rats and is illuminated by high or low light. A familiar arena with low illumination will cause the highest level of active social interaction in undrugged rats and this declines if the area is either brightly illuminated or unfamiliar or both; this decline is prevented by anxiolytic drugs (File and Hyde, 1978; File, 1980). By testing the animals in several test conditions and by simultaneously measuring locomotor activity, non-specific drug effects can also be detected. Extensive pharmacological validation of this model has taken place using BDZs (File and Hyde, 1978) and lesions of the serotonergic system (File et al., 1979; File and Deakin, 1980; File et al., 1981). More recent work has found ondansetron, a 5-$HT_3$ receptor antagonist, active in this test of anxiety, suggesting that it can detect anxiolytic activity of non-BDZ compounds (Jones et al., 1987).

However, changing variables such as the weight of the animal or the time of day can cause substantial shifts in 'baseline' social activity (File and Hyde, 1978). It is important to remember that social interaction is a composite measure of many behaviours, some of which, for example aggression, are not related in a simple way to the construct of 'anxiety'.

**e) Others**

There are many other uncertainty/novelty models which have yet to be fully validated as models of anxiety, such as the stretched attend posture (SAP) (Kaesermann and Dixon, 1986), the 'staircase test' in mice (Simiand et al. 1984; Emmanouil and Quock, 1990), shock-probe defensive burying in rats (Meert and Colpaert, 1986; Korte and Bohus, 1990) and marble-burying behaviour in rats (Njung'e and Handley, 1991).

## TESTS IN WHICH ANXIETY/AVERSION IS CHEMICALLY INDUCED

### Drug discrimination

Lal and Shearman (1982) described a drug discrimination procedure which they use as a model of chemically induced anxiety. In this model rats are trained to respond on one lever when injected with saline and on another when injected with pentylenetetrazole (PTZ - a drug which causes anxiety in humans); the discrimination is reinforced with a food pellet reward for pressing the correct lever. Test trials are then introduced and it has been

found that if rats are injected with an anxiolytic as well as PTZ there is a dose-dependent suppression of PTZ discrimination, i.e. they select the saline lever (Shearman and Lal, 1979, 1980; Lal et al., 1982). Also, if solely injected with an anxiogenic compound they selected the PTZ lever (Shearman and Lal, 1979, 1980; Lal et al., 1982). It is important to remember that the interoceptive cue provided by a drug will be as a result of all of its effects and will not just reflect one of them, i.e. the anxiolytic one.

This technique provides one of the best ways of comparing the subjective experience of drug effects in animals, but it is important to remember that it does not reflect the properties of a drug solely as they affect anxiety. By training animals to discriminate between saline and a BDZ the effects of other putative anxiolytics can be compared. Hence it is possible to determine if the subjective effects of a novel anxiolytic are similar to those of a BDZ.

Convulsant agents, such as picrotoxin and strychnine, can substitute for PTZ in this test, suggesting that the effective discriminative stimulus in the PTZ test might be some form of sub-clinical seizure activity (c.f. Lal and Shearman, 1980). Thus, it is important to remember that this anti-PTZ-discrimination effect of anxiolytics may be a result of their ability to suppress PTZ-induced seizures.

**ANXIETY TESTING ON HUMANS**

Various 'anxiety scale' tests have been devised (e.g. Hamilton Scale), in which a patient's responses to a standard list of questions are scored, and these are widely used to assay the clinical effectiveness of anxiolytic drugs. In these tests the effects of anxiolytics have been clearly demonstrated but what has also been found is that placebo produces significant responses, sometimes comparable to that produced by active drugs.

There are other tests which rely on measurement of the somatic and autonomic effects associated with anxiety. One of these is the galvanic skin response (GSR) in which the electrical conductivity of the skin is used as a measure of sweat production. Any novel stimulus, whether pleasant or unpleasant, causes a brief reduction in resistance. If an innocuous stimulus is repeated at intervals, habituation of the response occurs. The rate of

habituation is less in anxious patients than in normal subjects, and is increased by anxiolytic drugs (Lader and Wing, 1966). GSR habituation seems to be a particularly sensitive measure, as many tests of autonomic function in anxious patients (e.g. heart rate changes) show no clear cut change in response to anxiolytic drugs.

## INVOLVEMENT OF 5-HT IN ANXIETY

Early behavioural studies suggested that 5-HT was involved as a central neurotransmitter in the modulation of anxiety as a mediator in a central punishment system (Stein et al., 1975, 1977; Crow and Deakin, 1981), and that the anxiolytic effects of drugs such as BDZs resulted from a reduction in the activity of these serotonin systems (Wise et al., 1972). The involvement of 5-HT was supported by some observed activity of non-selective 5-HT antagonists in conflict models of anxiety (Cook and Sepinwall, 1975). However, more recent pharmacological studies have produced results which are not consistent with these hypotheses (Shephard et al, 1982) indicating the need for a reappraisal of the role of 5-HT in behavioural models of anxiety and the mechanism of action of anxiolytic agents.

### Effect of Lesions of 5-HT Pathways on Animal Models of Anxiety

Using the 'conflict' model of anxiety developed by Geller and Seifter (1960) it was found that depletion of 5-HT with parachlorophenylalanine (PCPA), the tryptophan hydroxylase inhibitor, increases food motivated punished behaviour (Geller and Blum, 1970; Tye et al., 1979; Shephard et al., 1982). The ability of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), to reverse the PCPA-induced increase in punished behaviour suggests that it is having its effect via depletion of 5-HT (Geller and Blum, 1970, Tye et al., 1979). Lesioning with the 5-HT neurotoxins 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) has been shown to release punished responding in this animal model after either intraventricular injection (Stein et al., 1975; Tye et al., 1977) or injection into the ascending 5-HT pathways at the medial forebrain bundle level (Tye et al., 1977). However, in another 'conflict' model, the Vogel punished drinking test (Vogel et al., 1971), lesions of central 5-HT pathways have produced inconsistent results (Petersen and Lassen, 1981; Commissaris et al., 1981). The situation is made

more uncertain by 8-OH-DPAT reversing the increase in punished responding obtained with PCPA in the Vogel punished drinking test but 8-OH-DPAT on its own resulting in increased punished responding, thus acting this time as an agonist (Engel et al., 1984).

As stated earlier, it is important to have a period of unpunished responding when studying these models, as depletion of central 5-HT has been shown to cause hyperphagia (Blundell, 1977) and polydipsia (Barofsky et al., 1980). During the period of unpunished responding it is hoped that any hyperphagic or polydipsic effects of treatment will be detected as an increase in the rate of feeding or drinking respectively.

Using the social interaction model (File and Hyde, 1978), File and Hyde (1977) showed that PCPA pre-treatment produced a behavioural profile similar to that seen in rats treated chronically with chlordiazepoxide. This was also observed with 5,7-DHT lesions of the dorsal raphe (File et al., 1979) and the lateral septum (Clarke and File, 1981). In contrast, 6-hydroxy-dopamine lesions in the lateral septum did not have the same effect. However, not all 5-HT pathway lesions have this effect on social behaviour. 5,7-DHT injections into the medial raphe or both the dorsal and medial raphe nuclei or into the amygdala decreased social interaction (File and Deakin, 1980; File et al., 1981). More recently, using the elevated plus-maze, 5,7-DHT lesions of the hippocampus produced an anxiolytic-like profile similar to that of the BDZs (Briley et al., 1990).

**Effect of Reducing 5-HT Function**

The 5-HT synthesis inhibitor PCPA has been seen to produce a release of response suppression in several behavioural models. Thus, it would be expected that lowering serotonergic transmission with 5-HT antagonists would also release response suppression; however, again the results are conflicting. An anxiolytic action for the non-selective 5-HT antagonists methysergide and cinanserin has been demonstrated in rat conflict procedures (Stein et al., 1973; Cook and Sepinwall, 1975), and methysergide and D-2-bromolysergic acid diethylamide have been reported to increase punished response rates in the pigeon (Graeff and Schoenfeld, 1970). However, others have failed to find any anti-conflict effects with these antagonists (Commissaris and Rech, 1982; Petersen and Lassen, 1981).

Microiontophoretic injections of 5-HT into the dorsal raphe were found to release punished responding (Thiebot et al., 1982). 5-HT is thought to be acting via inhibition of the somatodendritic autoreceptors, thus depressing the neuronal firing rate and supporting the hypothesis that decreased 5-HT activity results in attenuation of behavioural suppression.

**Effect of Enhancing 5-HT Function**

The effects of intracerebroventricular (i.c.v.) injection of 5-HT, whilst complex, were in general in the direction of a proconflict effect in the rat (Stein et al., 1973). The 5-HT agonists α-methyltryptamine and m-chlorophenylpiperazine (mCPP) suppress both the punished and unpunished responding in the rat (Stein et al., 1973; Graeff and Schoenfeld, 1970; Kilts et al., 1982). The fact that the results obtained with intraventricular administration of 5-HT are somewhat confusing is not that surprising since it is clear that 5-HT pathways in the brain and spinal cord are able to increase and decrease motor function independently (Gerson and Baldessarini, 1980) from their action on brain systems more specifically involved in the expression of behavioural inhibition induced by conflict. Wise et al. (1972) demonstrated that i.c.v. injection of 5-HT antagonized the punishment releasing effect of oxazepam. These authors also carried out biochemical studies which showed acute administration of oxazepam decreased turnover of both NA and 5-HT, but chronic treatment with the BDZ only decreased 5-HT turnover. In the CER paradigm, 5-HT applied directly in the vicinity of the dorsal raphe in the rat caused a marked release of suppressed responding (Thiebot et al., 1984). It is also known that 5-HT applied iontophoretically at this site and in amygdala will inhibit neuronal firing (Wang and Aghajanian, 1977), thus giving support to the theory that decreased 5-HT activity results in an attenuation of behavioural suppression.

Fenfluramine, which releases 5-HT, had no effect on punished responding, whereas 5-hydroxtryptophan, the 5-HT percursor, had a proconflict effect (Kilts et al., 1982). Amitriptyline, a 5-HT uptake inhibitor, depressed both punished and unpunished responding in a conflict task (Kilts et al., 1982). This non-specific depressant effect of amitriptyline, and of other 5-HT uptake inhibitors, has also been observed in the social interaction test (File, 1985). In a conditioned suppression of drinking conflict paradigm acute

amitriptyline or desipramine had no effect but chronic treatment was seen to increased punished responding (Fontana et al., 1989).

Up until recently, the range of agonists and antagonists of 5-HT available for such studies had not been great, with doubts about their specificity for 5-HT, since many of them influence other neurotransmitter systems. Also, as yet the contribution of their peripheral effects to the behavioural changes has not been fully evaluated. It is for these reasons that it is not surprising that there are almost as many negative findings regarding manipulations of 5-HT and punishment as there are positive ones. However, the recent development of selective agonists and antagonists has allowed further investigation of the involvement of 5-HT in anxiety, and this is discussed below.

**5-$HT_{1A}$ Agonists**

With the development of the non-BDZ anxiolytic buspirone, which has been found to have activity in the clinic (Goldberg and Finnerty, 1979), and the close analogues of buspirone, namely gepirone and ipsapirone, it is possible to further elucidate the involvement of the 5-$HT_{1A}$ receptor in anxiety. However, it is important to consider other actions (especially activity at other receptors) of these drugs when attempting to determine their effects and tie them in with the actions of 5-$HT_{1A}$ receptors. Buspirone, although it has high affinity for 5-$HT_{1A}$ receptors, also affects dopaminergic and noradrenergic systems (Taylor et al., 1982). Gepirone, previously known as BMY 13805, is more selective for 5-$HT_{1A}$ sites having only weak effects on dopamine systems (Yevich et al., 1983), and ipsapirone, previously known as TVXQ 7821, appears to be selective for the 5-$HT_{1A}$ site (Dompert et al., 1985; Glaser and Traber, 1985; Glaser et al., 1985). It is important to remember that with the 5-$HT_{1A}$ partial agonists the overall behavioural effect observed is a result of a balance between pre- and postsynaptic receptor stimulation (at the somatodentritic autoreceptor and receptors in the terminal regions respectively).

Buspirone, gepirone and ipsapirone all inhibit dorsal raphe neurone firing, mimicking the effect of 5-HT (Dourish et al., 1986; Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Wilkinson et al., 1987). In the hippocampus all three compounds produced only the inhibitory component of the action of 5-HT (Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Martin and Mason,

1987). The lack of specific antagonists prevents the direct confirmation that this inhibitory component is the result of 5-$HT_{1A}$ receptor stimulation; however, when the response to 5-HT was not present in the preparation, the response to buspirone was also absent (Van der Maelen et al., 1986). The ability of these compounds to activate the 5-$HT_{1A}$ site is further demonstrated in the drug discrimination test where buspirone, gepirone and ipsapirone will all substitute for the selective 5-$HT_{1A}$ ligand 8-OH-DPAT, and gepirone will substitute for buspirone (Mansbach and Barrett, 1987; Cunningham et al., 1985; Schuurman et al., 1986).

In animal models of anxiety (which have been mainly developed studying the effects of BDZ receptor ligands) the non-BDZ anxiolytics are not consistently active. Buspirone has shown activity in licking conflict paradigms (Elson et al., 1986; Merlo Pich and Samanin, 1986) and food-motivated operant conflict procedures (Geller and Hartmann, 1982; Weissmann et al., 1984) in rat and monkey but this has not always been able to be reproduced in other similar procedures (Goldberg et al., 1983; Gardner, 1986; Gleeson and Barrett, 1990). Both gepirone and ipsapirone showed activity in a licking conflict paradigm (Elson et al., 1986; Traber et al., 1984) and ipsapirone also showed activity in a conditioned avoidance paradigm (Traber et al., 1984; Schuurman et al., 1986). However in the social interaction model of anxiety using the protocol of File (File, 1985) buspirone had very little effect but using a modified method (Guy and Gardner, 1985) it showed an anxiolytic-like increase in social interaction of rats. Higgins et al. (1987) demonstrated an anxiolytic profile in social interaction with 8-OH-DPAT, buspirone and ipsapirone when administered locally into the dorsal raphe nucleus. Ipsapirone showed activity in the social interaction model (Boaventura et al., 1986) although an increase in social behaviour in a relatively non-anxious condition (Schuurman et al., 1986) suggests that this might not be a specific anxiolytic effect. No anxiolytic-like activity was seen with either buspirone or ipsapirone in both the shock probe conflict model (Meert and Colpaert, 1986) and the elevated plus maze (Pellow, 1986; File et al., 1987). Both acute and chronic buspirone treatment was found to have no effect in the elevated X-maze (Moser, 1989b). However, buspirone, gepirone and ipsapirone have been reported to be active in the elevated plus-maze but only over a narrow dose range (Soderpalm et al., 1989), and intrahippocampal buspirone has also produced an anxiolytic profile in this model (Kostowski et al., 1989). There are also reports in the literature of

these compounds having an anxiogenic profile in this model (Pellow et al., 1987; Redfern and Williams, 1989). In a recently developed model of anxiety both buspirone and ipsapirone inhibited stress-induced ultrasounds in rat pups (like BDZ anxiolytics), but this effect was associated with the induction of tremor and such disruption of behaviour can lead to the inhibition of ultrasounds so this may not be a specific anxiolytic effect (Gardner, 1985). It is now thought that the ultrasounds emitted by rat pups are not a result of separation from the mother but a result of hypothermia (File, personal communication), this further questions the validity of this model. In the fear-potentiated acoustic startle paradigm buspirone and ipsapirone have been shown to have an anxiolytic profile (Davis et al., 1988) but the doses found to be active were about 100-fold greater than those used by Soderpalm and co-workers (1989) on the X-maze. A further complication with buspirone, gepirone and ipsapirone is that their main metabolite, 1-pyrimidinylpiperazine (1-PP), is a potent $\alpha_2$-adrenoceptor antagonist (Bianchi et al., 1988) which, from the results of Handley and Mithani (1984) where another $\alpha_2$-adrenoceptor antagonist, yohimbine, produced an anxiogenic profile, would suggest that 1-PP would produce an anxiogenic profile, offsetting any anxiolytic effects of the parent compounds. However, it has been reported that 1-PP does not produce an anxiogenic profile on the X-maze (Moser, 1989b).

Thus, from the behavioural data available it is still unclear what the exact involvement of 5-$HT_{1A}$ sites in anxiety, as the results are not clear cut, with 5-$HT_{1A}$ agonists being active in certain behavioural models but not others. Both direct injections into the dorsal raphe nucleus and systemic administration of low doses of the 5-$HT_{1A}$ partial agonists, thought to predominantly act at the 5-$HT_{1A}$ somatodendritic autoreceptor, are generally active in some models of anxiety. Thus, by increasing the systemic dose this may offset any presynaptic anxiolytic activity by postsynaptic 5-$HT_{1A}$ receptor stimulation. It is now known that buspirone is active in the clinic (Goldberg and Finnerty, 1979; Kudoh, 1986; Saxenda et al., 1986) with a potency comparable to diazepam but with less sedation, so this leads us to question the ability of the behavioural models of anxiety to detect novel, non-BDZ anxiolytics. However, the clinical activity of buspirone indicates that there is an involvement of 5-HT, and 5-$HT_{1A}$ sites, in anxiety. Certain clinical features of buspirone may help to explain some of the diverse reports about its clinical efficacy (and may help to explain some of the behavioural

data). First, compared to BDZs, buspirone has a delayed onset of action of two or more weeks and is more effective in patients who have not previously been treated with BDZs (Goa and Ward, 1986; Lader and Olajide, 1987). Second, tolerance has not yet been seen to develop to buspirone and it also does not interact with ethanol (Traber and Glaser, 1987). Gepirone and ipsapirone have been seen to produce similar results with the added observation that the efficacy of ipsapirone as a clinical anxiolytic is not dependent on the patient's prior history of BDZ treatment (Traber and Glaser, 1987). Thus, clinical data indicate that 5-$HT_{1A}$ agonists have anxiolytic activity in man.

**5-$HT_2$ Receptor Antagonists**

Many 5-$HT_2$ antagonists are selective for these sites over other 5-HT receptor subtypes but may also affect noradrenaline and dopamine receptors (e.g. ketanserin has affinity for $\alpha_1$-adrenoceptors). Ritanserin is reasonably selective for 5-HT receptors (Van Neuten et al., 1986), and thus a large amount of the recent behavioural work has been carried out on this compound. As well as high affinity for the 5-$HT_2$ receptor ritanserin also binds to the 5-$HT_{1C}$ receptor (Leysen et al., 1985). Ritanserin acts as a pure antagonist in an LSD-saline drug discrimination test in the rat, blocking the effects of LSD, resulting in the rats generalizing to the saline lever (Colpaert et al., 1985). Also, ritanserin has been found to be more active than the BDZ chlordiazepoxide in a neophobia-based open field exploration model (Colpaert et al., 1985). However, ritanserin has shown little anxiolytic-like activity in the classical conflict models (licking conflict, food-motivated operant conflict or shock probe conflict) (Deacon and Gardner, 1986; Gardner, 1986; Meert and Colpaert, 1986; Ketelaars and Bruinvels, 1989) but these results need to be interpreted with caution as brain 5-HT neurons are also involved in the control of food and liquid intake, and ritanserin increases food intake in satiated rats (Fletcher, 1988). In the elevated plus-maze both anxiolytic- (Critchley and Handley, 1986) and anxiogenic-like effects (Pellow, 1986; File et al., 1987) have been reported. No specific effect was observed on stress-induced ultrasounds (Gardner, 1985).

A property which all 5-$HT_2$ antagonists possess is their ability to produce down-regulation of 5-$HT_2$ sites in the rat after chronic treatment rather than

the conventional supersensitivity seen with chronic antagonism of dopamine receptors (Blackshear et al., 1983; Leysen et al., 1986). It will be interesting to see if this property can be used to protect against tolerance and withdrawal effects associated with long-term clinical treatment. Clinical studies with ritanserin suggest that it is effective in generalized anxiety disorder (Ceulemans et al., 1985; Arriaga et al., 1986), which may be related to its ability to increase slow wave sleep (Arriaga et al., 1986). Initial studies have also shown ritanserin has therapeutic potential in patients with phobic anxiety (Humble et al., 1986).

**5-$HT_3$ Receptor Antagonists**

Recent behavioural studies have shown that ondansetron (originally known as GR38032F), a potent 5-$HT_3$ receptor antagonist, is active in both the social interaction test (Jones et al., 1987, 1988) and exploration of a light-dark two-chambered open field (Costall et al., 1987a, 1989). Ondansetron also decreases aggressive behaviour of marmosets and cynomolgus monkeys (Costall et al., 1987a; Jones et al., 1987). However, File and Johnston (1989) found that acute administration of ondansetron and two other 5-$HT_3$ antagonists (BRL 43694 and zacopride) had no significant effect on social interaction. In the elevated X-maze, ondansetron and another 5-$HT_3$ antagonist, BRL 46470A, have been shown to produce an anxiolytic profile (Costall et al., 1990a; Upton and Blackburn, 1991). There is evidence to suggest that ondansetron reduces the anxiogenic withdrawal effects observed with chronic BDZ treatment and drugs of abuse (Costall et al., 1990a). However, it has very little or no effect on other symptoms of withdrawal such as weight loss and reduced food intake (Leathley et al., 1990).

Ondansetron has also been found to reduce dopamine mediated behaviour (produced by either amphetamine administration or chronic intracerebral dopamine infusion) (Hagan et al., 1987; Costall at al., 1987a) and this reduction is only seen when the behaviour is enhanced, suggesting that 5-$HT_3$ antagonists have potential anti-psychotic as well as anti-anxiety activity. Finally, ondansetron has also been found to increase acetylcholine release in cortical tissue but only in the presence of ritanserin (Barnes et al., 1989b) and to enhance cognitive performance in the marmoset (Domeney et al., 1991), indicating involvement in memory and cognition.

In summary, the existing data indicate that reduced activity of 5-HT neuronal systems projecting to the cortical and limbic brain regions may be important in anxiolytic activity.

**Involvement of 5-HT Systems in the Anxiolytic Action of Benzodiazepines**

Since manipulation of 5-HT can to an extent mimic, the effect of BDZs in various behavioural models of anxiety, it has been suggested that the anxiolytic action of these drugs is mediated through their action on serotonergic pathways (Kahn et al., 1988; Iversen, 1984; Soubrie, 1986; Stein et al., 1975; Thiebot, 1986). Thus, the most important question is whether changes in serotonergic function can be related to the anxiolytic action of the BDZs. Wise et al. (1972) reported a reduction in 5-HT turnover after both acute and chronic oxazepam treatment, but a reduction in NA turnover only after acute treatment. This supported other work which showed that BDZs increased brain 5-HT concentrations (by decreasing 5-HT turnover) (Chase et al., 1970; Lidbrink et al., 1973). Wise et al (1972) also found an antagonism of the anxiolytic effect of oxazepam by the intraventricular administration of 5-HT. It has been suggested that the decrease in 5-HT turnover reflects reduced activity of 5-HT-containing neurones and thus decreased release of 5-HT. However, other studies failed to replicate these results. Cook and Sepinwall (1975) found a reduction in 5-HT turnover only after chronic treatment with chlordiazepoxide, and that the maximum anti-conflict effect was not accompanied by the maximum reduction in 5-HT turnover. Lister and File (1983) found 5 or 10 mg/kg CDP increased the levels of 5-HT, but if repeated dosing was administered then no change in 5-HT levels were found after 5-10 days of treatment. The results of Lister and File (1983) suggest that tolerance may have developed to the reduction in 5-HT turnover and Cook and Sepinwall (1975) also found tolerance to the decrease in 5-HT turnover, occurring between days 2 and 3 of treatment. A more recent study using *in vivo* microdialysis showed that both systemic and local administration of BDZ agonists (diazepam and flurazepam) inhibited the release of 5-HT from the ventral hippocampus, and this effect could be reversed by administration of the BDZ antagonist flumazenil (Ro 15-1788) (Pei et al., 1989b).

It is possible that BDZs reduce 5-HT turnover with very little effect on the activity of 5-HT cell bodies, depending on the relative strengths of the feedback systems. Potassium-evoked [$^3$H]-5-HT release from hippocampal synaptosomes is inhibited by diazepam with spontaneous 5-HT release being enhanced (Balfour, 1980), suggesting that BDZs can reduce 5-HT release via an action at the nerve terminals. Collinge and Pycock (1982) reported reductions in both spontaneous and stimulated release of [$^3$H]-5-HT from cerebal cortical slices.

Electrophysiological findings support an interaction between BDZs and 5-HT. In the rat, clonazepam depressed the firing of 5-HT neurones (Pratt et al., 1979). Laurent et al. (1983), recording multiunit activity in the *encephale isole* rat found that intravenous chlordiazepoxide and midazolam depressed neuronal firing in the dorsal raphe nucleus at doses which are known to be anxiolytic. Further, these effects were blocked by the BDZ antagonist flumazenil (Ro 15-1788) but the depressent effects of pentobarbitone on raphe neurones was not affected. It is now known that the BDZ receptor forms part of a receptor complex including a $GABA_A$ receptor and a $Cl^-$ ion channel and is found in all areas of the brain (Unnerstall et al., 1981). Furthermore, BDZs interact at the receptor complex and enhance transmission by GABA. In the CNS, the sites which contain 5-HT and are implicated in anxiety also have high densities of GABA-containing neurones. Iontophoretic application of GABA in the dorsal raphe, amygdala and hippocampus results in the inhibition of firing of 5-HT neurones, an effect enhanced by BDZs (Gallager, 1978). Local injections of BDZs or GABA into the dorsal raphe release punished responding on a conditioned emotional response paradigm (Thiebot et al., 1980; Thiebot and Soubrie, 1983) and GABA injected into the cortical/basolateral amygdala was active in a punished drinking test (Petersen and Scheel-Kruger, 1982). Peterson and Scheel-Kruger (1982) also showed muscimol, a $GABA_A$ agonist, to release punished drinking and that this effect was reversed by the $GABA_A$ antagonist bicuculline.

Thus, a body of evidence exists implicating benzodiazepine/$GABA_A$ interactions with a 5-HT pathway of origin in the raphe, innervating limbic structures, in the punishment-releasing effects of BDZs.

**The Involvement of Central Noradrenergic Pathways in Anxiety**

It can be seen from biochemical and electrophysiological studies (Laurent et al., 1983) that the BDZs influence catecholamine as well as indoleamine neurones, and hence other pathways may be involved in response suppression to aversive stimuli and that these pathways are sensitive to anxiolytic agents.

Noradrenergic innervation of the limbic system arises from the locus coeruleus, and stimulation of the locus coeruleus induces behavioural symptoms of fear in monkeys (Redmond and Huang, 1979). Drugs which enhance the function of NA have a similar effect in animals (for review, see Redmond and Huang, 1979) and in man subjective experience of fear and panic is reported after administration of yohimbine and piperoxan (Charney et al., 1984), two $\alpha_2$-adrenoceptor antagonists thought to be anxiogenic by blocking presynaptic $\alpha_2$-adrenoceptors. In contrast, drugs reducing NA function (such as propranolol and clonidine) are reported to be anxiolytic in both animals and man (Hoehn-Saric et al., 1981). Clonidine, an $\alpha_2$-adrenoceptor agonist, alleviates the intense fear and anxiety associated with opiate withdrawal (Gold et al., 1979) and this has been correlated with activity in the locus coeruleus. Clonidine has also been shown to inhibit behavioural arousal induced by locus coeruleus neurones (De Sarro et al., 1987).

Both the elevated X-maze and the fear-potentiated startle behavioural paradigms respond not only to manipulations of 5-HT but equally to adrenergic agonists and antagonists, which relieve or enhance anxiety respectively (Handley and Mithani, 1984; Davis et al., 1979), providing further evidence of an involvement of NA in anxiety.

It is possible that there are interactions between the NA and 5-HT systems. It is known that $\alpha_2$-adrenoceptors also regulate 5-HT release both *in vitro* (Gothert and Huth, 1980) and *in vivo* (Marsden and Martin, 1986; Routledge and Marsden, 1987), with agonists decreasing 5-HT release and antagonists increasing it. Furthermore, clonidine attenuates 5-$HT_2$ receptor-mediated behaviour in the rat (Heal et al., 1986), suggesting that there are $\alpha_2$-adrenoceptors situated on 5-HT nerve cells and/or terminals regulating 5-HT neuronal firing and release, and it has been reported that idazoxan, an $\alpha_2$-

adrenoceptor antagonist, increases 5-HT neuronal firing in the dorsal raphe nucleus (Garratt et al., 1991). Thus, any effects of noradrenergic compounds may be as result of a combined effect upon both the NA and 5-HT systems.

**SOCIAL ISOLATION**

The environment to which an animal is exposed (social environment) can have significant effects upon its subsequent behaviour. Thus there has been considerable interest in the consequences of different environmental conditions, and particularly social isolation (Morgan, 1973; Valzelli, 1977), which has been considered to have potential as an animal model of some human psychopathologies (Kraemer et al., 1984; Willner, 1984). Obtaining a clear picture of social isolation has been difficult largely due to the variable nature of the parameters employed. It is difficult to find two studies that have used the same periods of isolation, imposed at the same time point and using the same sex and species.

Many of the effects of social isolation are dependent upon the age at which isolated conditions are introduced (Einon and Morgan, 1977), and it has been suggested that the effects of stimulus deprivation (isolation) are inversely correlated with the age at which isolation was introduced (Bronfenbrenner, 1968). Differences between isolation at weaning age and isolation when mature would be expected given the relative plasticity of the brain at birth, which decreases with age. For example, the monoamine innervation of the CNS, often implicated in the effects of social isolation (Sahakian et al., 1975; Robbins et al., 1989), largely develops post-natally. Only approximately 20% of NA, 25% of DA, and 50% of 5-HT content of the adult rat brain is present at birth and much of the production of these monoamines occurs around weaning age. Around weaning, not only do monoamine concentrations increase rapidly but so do the associated enzymes such as tyrosine hydroxylase and dopamine ß-hydroxylase (for review see Mabry and Campbell, 1977). Thus, the prevailing environmental conditions would seem most likely to be able to influence neurochemical and behavioural processes and development earlier in life, rather than later.

**Locomotor activity and exploratory behaviour.**

Increased locomotor activity is one of the most prominent and reproducible findings following social isolation in many species including rats (Syme, 1973; Morley and Worsham, 1978), mice (Essman, 1966; Wilmot et al., 1986), monkeys (Mason et al., 1968), cats (Seitz, 1959) and dogs (Fox and Stelzner, 1966). Rats reared in isolation from weaning consistently produce locomotor hyperactivity on exposure to a novel environment, when measured in either an open field (Syme, 1973; Morgan, 1973; Gentsch et al., 1981a, b, 1982), or in photocell cages (Sahakian et al., 1977; 1982) but not on an elevated activity platform (Syme, 1973) or runway (Moyer and Korn, 1965). This effect is relatively independent of the length of isolation period, as anything from seven days (Sahakian et al., 1982) to twelve months (Garzon et al., 1979) of isolation, can elicit this increased response.

Gentsch et al. (1981a) describe locomotor activity in a novel environment as 'reactive activity', and isolation-reared rats show increases in this reactive activity, but not spontaneous activity (when habituated to the test environment), and may even show decreases (Gentsch et al., 1981a; 1982). However, Garzon et al., (1979) found that if the isolation period is of a sufficiently long time it may produce hyperactivity even after repeated exposures to an open field.

Thus, the increased locomotor activity in isolates is a change in the response to novel stimuli and may therefore reflect increases in exploratory behaviour. This is supported by evidence that isolates show an increased incidence of investigative bouts on exposure to novel stimuli (Einon and Morgan, 1977), and also show increased preference for novel environments (Sahakian et al., 1977). Not all studies have demonstrated this enhanced exploratory behaviour in isolation-reared rats (File, 1978; Hughes and Pither, 1987) and even reduced exploratory behaviour has been observed (Parker and Morinan, 1986).

Another interpretation of increased locomotor activity is that it represents "a response to heightened motivational factors" (Essman, 1966). Isolates have been found to eat more food and gain weight faster than socially-reared rats (Morgan and Einon, 1975; Fiala et al., 1977). Also, if isolated rats are re-housed in groups one month prior to testing not only are the body weight differences abolished but also the increases in exploratory behaviour are not

observed (File, 1978). This suggests that the increased weight gain and exploratory behaviour in isolates may be under the influence of a common mechanism - increased incentive-motivation for food, but other factors such as no competition for food in the isolated animals need to be considered.

**Response to psychomotor stimulant drugs**

Isolation-rearing in rats produces an increase in the intensity of the stereotyped forms of behaviour elicited by the indirect dopamine agonist amphetamine (Sahakian et al., 1975; Einon and Sahakian, 1979). Further, using intracerebral mircodialysis it has been shown that d-amphetamine produces enhanced DA release in isolation-reared rats compared to socially-reared controls (Jones et al., 1988; 1990). Apomorphine, a direct DA agonist, has also been reported to elicit more intense stereotyped behaviours in isolation-reared rats (Sahakian et al., 1975), although this effect does not seem as reliable as that induced by amphetamine. This suggests that social isolation alters pre-synaptic dopaminergic mechanisms more consistently than post-synaptic mechanisms, although both may be implicated (Sahakian et al., 1975; Weinstock et al., 1978; Wilmot et al., 1986)

**Conditioned Behaviour**

Isolation-reared rats are impaired in a number of tasks compared with socially-reared rats. For example, maze learning abilities are consistently impaired by rearing rats in a restricted environment (Greenough, 1976). Deficits have also been observed in response transfer (Morgan, 1973), learning a two-lever alternation schedule (Morgan et al., 1975) and in spatial memory tasks (Einon, 1980; Juraska et al., 1984). Jones et al. (1989) showed isolation-rearing to retard the acquisition of schedule-induced polydipsia in rats only when isolation was introduced at weaning.

**Other Alterations**

Chronic exposure to conventional stressors (e.g. immobilisation) is widely regarded to decrease central NA levels and to increase 5-HT utilisation (for review see Anisman, 1978), whereas the opposite effects are seen in socially isolated (either immature or mature) rats (Segal et al., 1973; Thoa et al., 1977; Morinan and Parker, 1985). Isolated rats have higher pain thresholds, either following short (Kehoe and Blass, 1986), or long term isolation (Gentsch et al., 1988). Similar decreases in pain sensitivity are also evident following acute exposure to a conventional stressor such as footshock (Akil

et al., 1976) or immobilisation (Amir and Amit, 1978). These alterations in pain sensitivity following social isolation are thought to reflect changes in opioid mechanisms, with higher pain thresholds in isolates being attenuated with naloxone (Puglisi-Allegra and Oliverio, 1983). Also, reductions in opioid receptor binding in whole brain homogenates (Schenk et al., 1982) and in the frontal cortex, striatum and hippocampus (Petkov et al., 1985) have been reported. However, isolation-reared rats show reduced sensitivity to the analgesic properties of morphine (Kostowski and Czlondowski, 1973), which is the reverse of what the previous data indicate. It is possible that this is a result of morphine being relatively selective for the μ receptor, and studying the effects of other opioid receptor agonists would help clarify this observation.

**Reversal of Effects of Isolation**

The reversal of the effects of social isolation can be brought about either by social factors or by pharmacological treatment. Resocialisation reverses the hyperactivity, greater tail-flick latencies (Gentsch et al., 1988), and maze learning deficits (Wood and Greenough, 1974) in isolation-reared rats. However, other studies have failed to detect recovery from isolation-induced impairments, particularly if isolation was introduced at weaning age (Einon and Morgan, 1977; File, 1978; Kraemer et al., 1984). This might be expected since the brain would be more susceptible to permanent change at an early age. Neurochemical changes (Blanc et al., 1980) and hypertension (Naranjo and Fuentes, 1985) induced by isolation of mature rats is completely reversed by resocialisation. The increased body weight in rats isolated immediately post-weaning can be reduced by rehousing in groups (File, 1978).

Benzodiazepines (Garzon et al., 1979; Hock and Scheich, 1986), monoamine oxidase inhibitors (Crawley, 1985), opiate antagonists (Naranjo and Fuentes, 1985), NA antagonists (Garzon and Del Rio, 1981), psychomotor stimulants (Weinstock et al., 1978) and DA antagonists (Garzon et al., 1979) have all demonstrated an ability to alleviate some, but not all, of the effects following social isolation in rodents. Because of this reversal, isolation has been considered an animal model for a range of psychopathological disorders including depression (Kraemer et al., 1984; Willner, 1984; Crawley, 1985) and anxiety (Crawley, 1985; Parker and Morinan, 1986).

## MEASUREMENT OF NEUROTRANSMITTER RELEASE *IN VIVO*

Neurotransmitter release has been measured *in vivo* for many years now using a variety of techniques. The first method developed was the cortical cup (MacIntosh and Oborin, 1953) which consisted of a small container filled with artificial CSF held against the surface of the cortex. Either the cup was replaced periodically or the fluid changed by a superfusion system and subsequently analysed. The main advantage of this system being lack of tissue penetration, however, only a limited brain area could be studied and there is direct contact between the perfusion fluid and the brain. In 1961 Gaddum described a 'push-pull cannulae' system where artificial CSF was delivered to the brain by one tube ('push') and collected by another ('pull'). With this technique all brain regions could be studied. However, as with the cortical cup there is direct contact between the tissue and the perfusate. It is possible to sample CSF directly by inserting a catheter into the cisternal space and collecting and analysing the samples (Sarna et al., 1983). This technique has no pumps pushing or pulling fluid, but only provides information about levels of compounds in the CSF which are not necessarily the same as those in brain tissue. Also, it is unknown how the removal of small quantities of CSF alters the dynamics of the turnover system.

One of the main collective drawbacks with all of the above techniques is that it involves an open system in which the brain comes in direct contact with the perfusion medium. Two methods which overcome these problems to some extent are intracerebral microdialysis and voltammetry. In voltammetric studies carbon fibre electrodes are used to oxidise neurotransmitters and the oxidation is measured by the generation of a small current (for review see Stamford, 1989). The advantages of this method are that the microelectrodes are very small (e.g. 12μm) and so all brain regions, including small nuclei can be accessed, rapid and repeated sampling means that changes occurring over seconds can be studied, and there is no direct contact of a perfusion medium with the brain. The main drawback is that it is difficult to positively identify the electroactive chemical being measured.

### Intracerebral Microdialysis

The principle of microdialysis is very simple. The probes consist of semi-permeable membrane which is filled with an artificial CSF solution, as close

to endogenous CSF as possible. Once the probe is in place compounds in the tissue diffuse down their concentration gradient across the membrane into the perfusate and continuous removal of the perfusate maintains this concentration gradient. The pore size in the membrane is limited to introduce a cut-off weight ensuring that large molecules such as proteins cannot cross the membrane.

The first description of the dialysis technique was by Bito et al. in 1966 where they measured amino acids using sacs made of dialysis tubing filled with 6% dextran/saline solution, which were implanted into the cortex of dogs and left in place for 10 weeks. Histological studies showed the sacs to be surrounded by collagen and capillary proliferation and glial cells in the surrounding area. The collagen capsule would have limited diffusion of compounds into the fluid but Bito et al. were still able to detect amino acids. An improved dialysis system was published in 1972 by Delgado and co-workers using what they called a 'dialytrode'. This was similar to both a push-pull cannula and the dialysis probes in use today, inside a bag made of dialysis membrane. Ringer solution was pumped through the dialysis sac and this allowed repeated sampling. Delgado and co-workers demonstrated that compounds travel in both directions across the membrane so substances can be introduced into the local environment of the probe. In 1982 Ungerstedt and co-workers described a dialysis probe consisting of a loop of dialysis tubing attached to to two stainless steel cannulae, which was implanted vertically into the brain. They also described a horizontal piece of dialysis tubing passing horizontally through the brain with glue over parts of the tubing so diffusion was only possible over a limited area (the regions of interest in the particular experiment). These two types of probe are still used today and have been joined by another design consisting of an outer and inner tube with the perfusate being passed down one and up the other (Ungerstedt, 1984). This last design is sold commercially and is the design on which the dialysis probes in this thesis are based.

A number of assays are available to measure various neurotransmitters, their precursors and their metabolites. One of the advantages of dialysis is that it can be used to measure any chemical substance provided that the molecule can pass through the membrane and that there is a suitable assay for that molecule. The development of high performance liquid chromatography with electrochemical detection (HPLC-ECD) has made the

study of neurotransmitters easier, with catechols and indoles being well detected (for review see Mefford, 1981). Ungerstedt et al. (1982) used HPLC-ECD to detect dopamine and 5-HT was first detected from dialysates *in vivo* by HPLC-ECD in 1985 by Brazell and co-workers. HPLC-ECD assays have now been developed for acetylcholine (Damsma et al., 1987) and noradrenaline (Routledge and Marsden, 1985).

**Recovery of Compounds by Dialysis**

The amount of a particular compound measured by dialysis depends upon its 'relative' recovery. The 'relative' recovery is "the relationship between the concentration of a particular substance in the perfusate compared to the concentration in the medium outside the tube" (Ungerstedt et al., 1982). The 'absolute' recovery is the amount of a substance collected in the perfusate per unit time (Ungerstedt, 1984). In all future discussion the term recovery is taken to mean relative recovery.

The recovery of a dialysis probe depends upon a number of factors (Ungerstedt, 1984; Benveniste, 1989; Benveniste and Huttemeier, 1990) including: (a) the physico-chemical properties of the membrane, (b) the size and design of probe used as the area of the membrane will influence the recovery as will variations in internal pressure, (c) the flow rate which is inversely related to recovery, (d) the tonicity and composition of the perfusion medium, (e) the nature of the compounds being dialysed as the diffusion coefficient, which reflects molecular weight, varies, (f) the temperature as recovery *in vitro* is often measured at room temperature, while the temperature *in vivo* will be 37°C and (g) interactions of the substances being dialysed with the dialysis membrane itself. Probes are calibrated *in vitro* by measuring the relative recovery. Calculations to correct for the fact that *in vivo* dialysis is carried out in tissue while recovery is measured in solutions are complex (Jacobson et al., 1985; Benveniste, 1989; Benveniste and Huttemeier, 1990; Bungay et al., 1990) and it is not clear whether true extracellular concentrations can be accurately determined.

**Limitations of the Dialysis Technique**

Although dialysis has advantages over many of the other methods described it has several limitations. The long time required to collect enough sample to assay (10 - 20 minutes) means that rapid changes may be missed. The

probes are quite large, limiting the size of the brain areas which can be dialysed. It is possible that the continuous removal of neurotransmitters will deplete the existing stores and create an artificial situation. Finally, cellular reactions to the implantation of the dialysis fibre limit the length of time the dialysis probe can be used *in vivo*. It has been shown that local cerebral glucose metabolism and blood flow returned to near normal after 24 hours (Benveniste et al., 1987). An experiment done to study the long term applications of dialysis for measuring 5-HT concluded that the method was most useful between 24 and 72 hours after implantation (Pei et al., 1989a). None of these techniques strictly measure release but rather overflow of neurotransmitter from the synaptic cleft into extracellular fluid.

**Aims of the Thesis**

The overall aim of the thesis is to study 5-HT neuronal function, using *in vivo* microdialysis to record 5-HT release, and to correlate this with ongoing behaviour thought to reflect anxiety. Initially several different behavioural models of anxiety are studied, namely the elevated X-maze, the fear-potentiated acoustic startle paradigm and social isolation, for their suitability to produce 'anxious' behaviour and to be combined with microdialysis for monitoring neurochemical changes. From this the elevated X-maze was chosen as it is possible to combine this paradigm with microdialysis and hereby monitor neurochemical and behavioural changes in the same animal, and then to study the effects of established (BDZ) and putative (5-HT-modulated) anxiolytics on these changes. Further, to determine whether the 'anxious' behaviour displayed by isolation-reared rats correlates with alterations in both pre- and postsynaptic 5-HT function using *in vivo* techniques.

# CHAPTER 2

## PHARMACOLOGICAL VALIDATION OF THE ELEVATED X-MAZE AND FEAR-POTENTIATED ACOUSTIC STARTLE MODELS OF ANXIETY

## INTRODUCTION

### 1. ELEVATED X-MAZE

The test is based on a procedure used by Montgomery (1955) who showed that exposure of rats to an elevated (open) maze alley evoked an approach-avoidance conflict that was considerably stronger than that evoked by exposure to an enclosed maze alley. Rats that were allowed access to an enclosed alley from their home cage explored considerably more than those allowed access to an open alley, and in the latter condition there were more retreats to the end of the cage. Montgomery (1955) then showed, using a Y-maze with a varying ratio of open:enclosed arms, that animals clearly preferred (i.e. spent more time on) the enclosed arms. Montgomery interpreted this behaviour by proposing that exposure to novel stimuli (in this case in the form of maze alleys) can evoke both exploratory drive and fear drive, thus generating approach-avoidance conflict behaviour, and that elevated (open) maze alleys evoke a greater fear, and hence more avoidance behaviour, than enclosed alleys.

Handley and Mithani (1984) described a procedure based on that of Montgomery (1955), which consisted of a plus-maze with two open and two enclosed arms, elevated to a height of 50cm. The X-maze and plus-maze are essentially the same, and throughout this Introduction the description used will be the same as that used by the particular authors. The apparatus used in this thesis will be referred to as an elevated X-maze. Pellow et al. (1985) described the validation of such a test by confining rats to either the two open arms or the two closed arms of the maze, and taking a series of measurements. There was a significant decrease in the number of entries, and a significant increase in the time spent motionless or freezing and in the number of defaecations in the rats confined to the open arms. Also, although rats confined to the open or enclosed arms had significantly elevated plasma corticosterone levels compared to home cage controls the elevation in corticosterone was significantly greater in the rats confined to the open arms (Pellow et al., 1985). It was suggested that a drug which reduced anxiety should increase the overall proportion of entries into the open arms, i.e. increase the open arm entries as a percentage of the total entries. Also, the percentage of the total time spent on the open arms could provide a valid

measure of anxiety. The total number of arm entries would provide a measure of general activity.

Pellow et al. (1985) then went on to pharmacologically validate the elevated plus-maze. Drugs that are active in the clinic as anxiolytics, diazepam and chlordiazepoxide, were found to increase those measures on the plus-maze which are thought to reflect reduced 'anxiety' (i.e. time on open arms, and percentage open:total entries and time). Other studies have shown that most compounds that are agonists at the benzodiazepine-GABA receptor complex are active in the plus-maze, e.g. alprazolam, adinazolam, U-43,465 (File and Pellow, 1985), CGS 9896 (File and Pellow, 1986) and CL 218,872 (Pellow and File, 1986). Having ascertained that the elevated plus-maze can detect compounds with anxiolytic activity, further studies by Pellow and File (1986) using inverse agonists at the benzodiazepine-GABA receptor complex, such as the ß-carboline derivative FG 7142, found that these compounds significantly enhance the measures thought to reflect 'anxiety'. Yohimbine, an $\alpha_2$-adrenoceptor antagonist has been shown to have anxiogenic activity in man (Charney et al., 1984) and is found to reduce the percentage open:total entries and time on the plus-maze (Handley and Mithani, 1984; Pellow et al., 1985). Caffeine, a psychostimulant compound in man (Uhde et al., 1984), also had significant anxiogenic effects in the plus-maze (Pellow et al., 1985). It is important when using the X-maze to attempt to have a measure of general locomotor activity in order that changes in open arm exploration observed on the X-maze are not a result of changes in general activity.

## 2. FEAR-POTENTIATED STARTLE PARADIGM

Anecdotal evidence indicates that people startle more when they are afraid; Brown et al. (1951) demonstrated that the amplitude of the acoustic startle reflex in the rat can be augmented by presenting the eliciting auditory startle stimulus in the presence of a cue (e.g. a light) that has previously been paired with a shock. This phenomenon has been termed the 'fear-potentiated startle effect' and has been replicated using either an auditory or a visual conditioned stimulus and when startle has been elicited by either a loud sound or an airpuff (e.g. Anderson et al., 1969 a, b; Davis, 1979 a, b; Davis and Astrachan, 1978; Davis et al., 1979; Galvani, 1970).

In this paradigm a central state of fear is considered to be the conditioned response (McAllister and McAllister, 1971). Conditioned fear is operationally defined by increased startle amplitude in the presence of a cue previously paired with a shock (see Fig. 1). Thus, the conditioned stimulus in this test does not elicit startle and also the startle-eliciting stimulus is never paired with a shock. In this model the conditioned stimulus is paired with shock and the startle is elicied by another stimulus either in the presence or absence of the conditioned stimulus. If the startle is greater in the presence of the conditioned stimulus the fear-potentiated startle is said to have occurred. Potentiated startle occurs using either foot-shocks or back-shocks (Davis and Astrachan, 1978), indicating that augmented startle probably results from increased fear and not because the animal alters its posture (e.g. crouching) which results in a greater startle response (Kurtz and Siegel, 1966). Also, the potentiation of startle measured electromyographically in neck muscles occurs without there being any obvious postural adjustment (Cassella et al., 1986). Additionally, the magnitude of potentiated startle correlates well with the degree of freezing, a common measure of fear (Leaton and Borszcz, 1985). This information, taken together, indicates that potentiated startle is a valid measure of classical fear conditioning.

**The acoustic startle pathway**

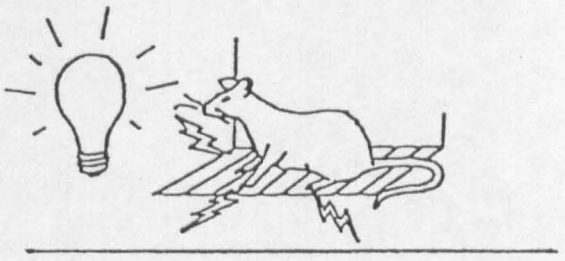
In the rat, the latency of acoustic startle is 6ms recorded electromyographically in the foreleg and 8ms in the hindleg (Ison et al., 1973). This very short latency indicates that only a few synapses are involved in mediating acoustic startle. Davis et al. (1982) determined the acoustic startle pathway in the rat (Fig. 2). The posteroventral cochlear nucleus (VCN) is the first synapse in the acoustic startle circuit. The next synapse occurs in the ventral nucleus of the lateral lemniscus (VLL), and the pathway then goes to the nucleus reticularis pontis caudalis (RPC). Cell bodies in the RPC send their axons to all levels of the spinal cord by way of the reticulo-spinal tract. This tract courses near or through the medial longitudinal fasciculus (MLF) on the midline and then bifurcates to form the ventral funiculi in the spinal cord (SC). These fibres synapse in the SC to form the final synapse before the neuromuscular junction. It has not yet been determined whether spinal interneurons are involved.

In order to determine the area within the startle pathway where fear alters neural transmission Berg and Davis (1985) implanted electrodes at various

**Figure 1**

Cartoon depicting the fear-potentiated startle paradigm. During training a neutral stimulus (conditioned stimulus) such as a light is consistently paired with a foot-shock. During testing startle is elicited by an auditory stimulus (e.g. a 100dB burst of white noise) in the presence (light-tone trial) or absence (tone alone trial) of the conditioned stimulus. The positions and postures pictured may not mimic the actual behaviour of the animals. Taken from Davis, 1988.

Training: light and shock paired

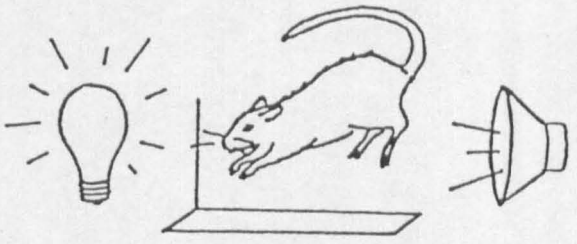


Testing

Noise-alone trials

Normal startle (in dark)

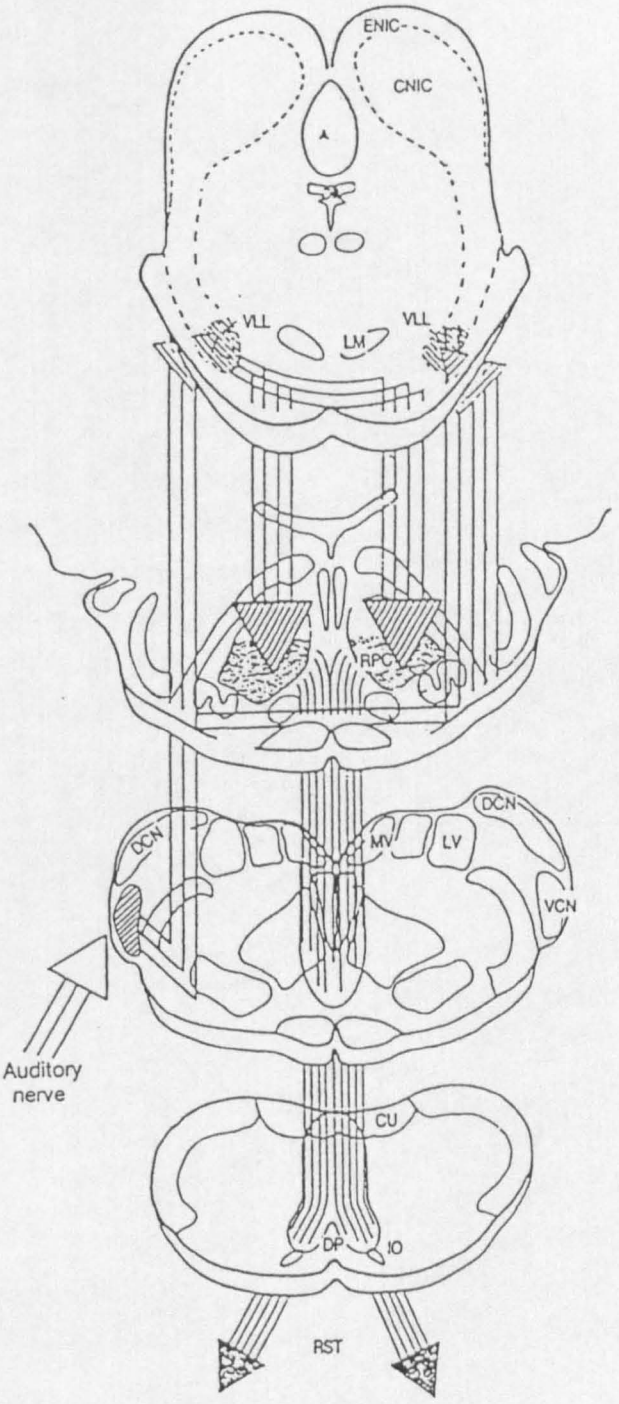
Light-noise trials



Potentiated startle (in light)

**Figure 2**

Schematic diagram of a primary acoustic startle circuit consisting of the ventral cochlear nucleus (VCN); ventral nucleus of the lateral lemniscus (VLL) and nucleus reticularis pontis caudalis (RPC). Other abbreviations used are: A, aqueduct; CNIC, central nucleus of the inferior colliculus; CU, cuneate nucleus; DCN, dorsal cochlear nucleus; DP, decussation of pyramids; DR, dorsal raphe nucleus; ENIC, external nucleus of the inferior colliculus; IO, inferior olive; LL, lateral lemniscus; LM, medial lemniscus; LV, lateral vestibular nucleus; RST, reticulospinal tract. Taken from Davis, 1988.


ENIC
CNIC
VLL
LM
VLL
RPC
DCN
DCN
MV
LV
VCN
Auditory
nerve
CU
DP
IO
RST

points along the startle pathway in order that a startle response could be electrically elicited. Following recovery, all groups were trained to be fearful of a light by pairing it with a foot-shock. The results are shown diagrammatically in Fig. 3. There was potentiation of electrically elicited startle (equivalent to potentiation of acoustic startle) at all locations up and including the VLL. In contrast, startle response elicied from the RPC was not potentiated. These data indicate that the VLL or the RPC is the point in the startle pathway where a visual conditioned stimulus modulates transmission following conditioning, affecting the startle reflex.

**The role of the central nucleus of the amygdala and various amygdala projections in potentiated startle**

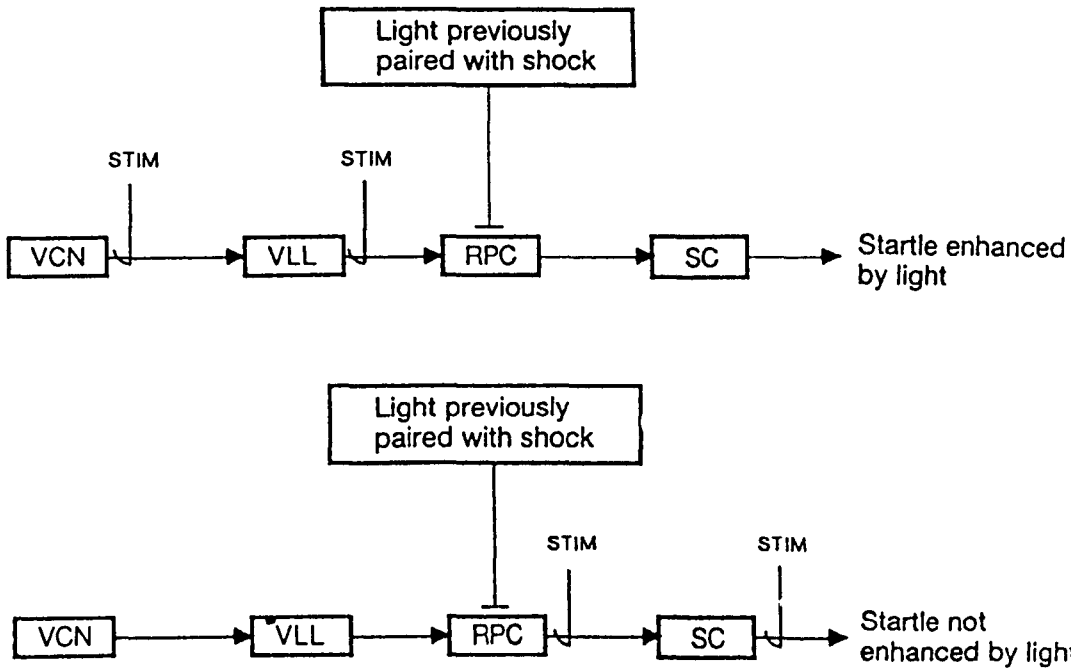
Fear-potentiated startle was completely blocked by lesions of the central nucleus of the amygdala but not by transection of the cerebellar peduncles or lesions of the red nucleus (Hitchcock and Davis, 1986a). Further work showed blockade of potentiated startle in animals with amygdala lesions was not attributable to visual impairment (Hitchcock and Davis, 1986b), suggesting that the amygdala is involved in fear conditioning, given that potentiated startle is a measure of conditioned fear. It is possible that the cerebellum could also modulate potentiated startle because electrical stimulation of the cerebellum has been reported to increase the magnitude of potentiated startle (Albert et al., 1985).

The central nucleus of the amygdala has direct projections to a variety of brainstem areas that might be expected to be involved in fear or anxiety. These include a region of the central grey (involved with freezing behaviour) (Beitz, 1982; Post and Mai, 1980), the dorsal motor nucleus of the vagus (concerned with autonomic measures of fear) (Hopkins and Holstege, 1978; Schwaber et al., 1982; Veening et al., 1984), parabrachial nucleus (concerned with respiratory changes) (Krettek and Price, 1978; Price and Amaral, 1981; Takeuchi et al., 1982), trigeminal and facial motor nuclei (facial expressions of fear), and the RPC (fear-potentiation of the startle reflex) (Berg and Davis, 1985).

Electrical stimulation of the central nucleus of the amygdala produces a cessation of ongoing behaviour (Applegate et al., 1983). Such cessation of behaviour is the critical measure of fear or anxiety in several animal models such as the operant conflict test (Geller and Seifter, 1960), the conditioned

**Figure 3**

Schematic diagram of modulation of electrically elicited startle by presentation of a light previously paired with a shock. The point of electrical elicitation of startle is indicated by the 'stim' symbol. The model predicts that if the light ultimately modulates neural transmission at the RPC, then startle elicited electrically in the VCN or VLL will be enhanced by the light, since the signal produced by electrical stimulation must travel through the area where the light modulates neural transmission. In contrast, startle elicited electrically in the RPC beyond the area of modulation or startle elicited electrically in spinal motoneurons will not be enhanced by light. Refer to Figure 2 for the definition of abbreviations. Taken from Davis, 1988.


Light previously paired with shock
STIM
STIM
VCN
VLL
RPC
SC
Startle enhanced by light
Light previously paired with shock
STIM
STIM
VCN
VLL
RPC
SC
Startle not enhanced by light

emotional response test (Estes and Skinner, 1941) and social interaction (File, 1980). Electrical stimulation of the central nucleus of the amygdala also alters heart rate (Applegate et al., 1983), blood pressure (Morgenson and Calaresu, 1973), and respiration (Applegate et al., 1983). Amygdala stimulation also produces jaw movements (Applegate et al., 1983) and gastric ulceration (Henke 1980a,b; Innes and Tansy, 1980). It has been reported in humans that electrical stimulation of the amygdala elicits feelings of fear or anxiety as well as autonomic reactions indicative of fear (Chapman et al., 1954; Gloor et al., 1981). Low-level electrical stimulation of the amygdala (e.g. 40-400μA, 0.1ms square wave cathodal pulses) increases acoustic startle amplitude in rats (Rosen and Davis, 1986).

As outlined earlier, lesioning of the central nucleus of the amygdala blocks fear-potentiated startle (Hitchcock and Davis, 1986a), and it has also been found to attenuate conditioned changes measured by a cessation of ongoing behaviour such as freezing (Iwata et al., 1986b), reduced bar pressing in the operant conflict test (Shibata et al., 1986) or the conditioned emotional response paradigm (Kellicut and Schwartzbaum, 1963; Spevack et al., 1975). Lesioning the central nucleus of the amygdala also blocks changes in heart rate (Cohen, 1975; Gentile et al., 1986), blood pressure (Iwata et al., 1986a), and ulceration (Henke 1980a and b) in response to startle.

## METHODS

### 1. ELEVATED X-MAZE

#### Animals

Male, Lister hooded rats (200-270g) (Nottingham University Medical School Animal Unit) were housed in groups of 4-6 in a room with a 12hr light : 12 hr dark cycle (0700-1900 lights on), with food and water available *ad libitum*.

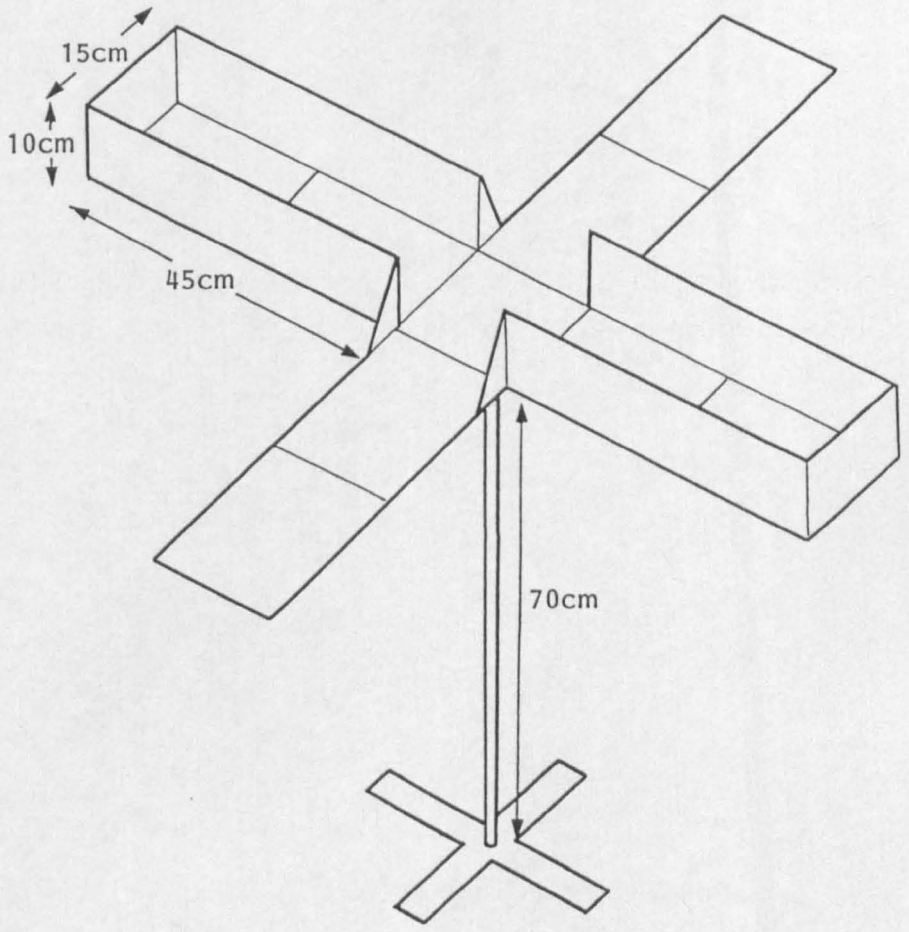
#### Apparatus

The X-maze was elevated to a height of 70 cm and consisted of two open arms, 45 x 15 cm (length x width), and two enclosed arms, 45 x 15 x 10 cm (length x width x height), with an open roof, arranged such that the two pairs of identical arms were opposite each other and is shown diagrammatically in Fig. 4. The maze was constructed out of matt black perspex (6mm thickness), and lines scored on the maze at the entrance and half way along each of the

**Figure 4**

Schematic diagram of the elevated X-maze used in all of the studies throughout this thesis.

ELEVATED PLUS MAZE




MATERIAL : BLACK PERSPEX (6mm thick) MATT FINISH

arms. The animal's behaviour was recorded directly into a microcomputer by an observer sitting 2 metres away in the same room.

The X-maze was carefully wiped with a wet towel after each animal. All experiments were carried out between 10.30 and 15.30. hrs

**Procedure**

Each rat received an injection of either vehicle control (1% methyl cellulose in 0.9% saline), diazepam (1, 2.5 or 5mg/kg), idazoxan (0.25 or 0.5 mg/kg) or FG 7142 (1 or 5 mg/kg) intraperitoneally (i.p.) and was then returned to its home cage; 6 or 8 animals per treatment. After 25 min the animal was isolated in a cage for 5 min and then placed in the centre of the X-maze facing one of the enclosed arms. An entry into an arm or the end of an arm was defined as the animal placing all four paws over the line marking that area. The cumulative time spent in, and the number of entries made into, the open, end of open, closed, or end of closed arms, and the number of rears in the open, closed or neutral area in the centre of the maze (a rear being defined as the elevation of both forepaws off the ground) were recorded during a 5 min test period. The open arm data are expressed as percentage of the total time spent in, and the total number of entries made into, both the open and enclosed arms and the rearing data as the rears per minute in the open arms, closed arms, and central square.

The percentage open:total time and percentage open:total entries, and the actual time in the end of the open arms were used as indices of anxiety, while the total number of arm and end of arm entries was used as an index of locomotor activity.

**Statistics**

All results are expressed as the mean ± standard error of the mean (S.E.M.). The ratio of open:total arm entries and open to total time was calculated for each rat individually and the mean ± S.E.M. for each treatment group presented in order to show group variation.
All data were analysed using 2-tailed Mann-Whitney U-test.

## 2. FEAR-POTENTIATED STARTLE PARADIGM

### Animals

Experimentally naive male Lister hooded rats (Harlan Olac Ltd.) weighing 340-400g were housed in groups of 6 for two weeks prior to experimentation. The rats were maintained on a 12hr light-dark cycle (lights on 0700-1900) with food and water were available *ad libitum*. All experiments were performed in the light phase.

### Apparatus

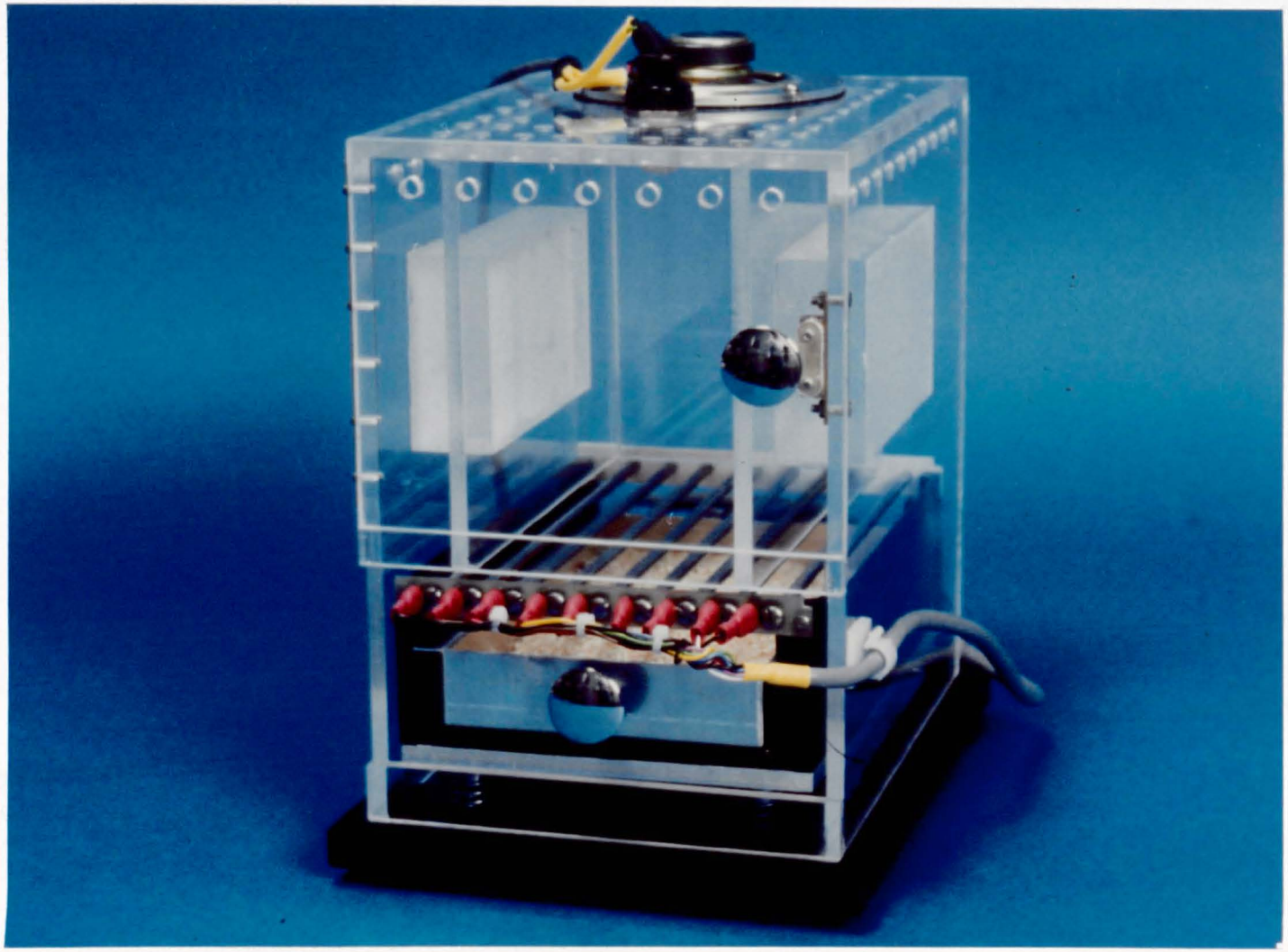
The acoustic startle box (designed by Instrument Engineering Services, SmithKline Beecham, Harlow) consisted of a clear perspex chamber of internal dimensions 18 x 10 x 15 cm (length x width x height) and is photographed in Fig. 5. The floor of the chamber was made of stainless steel bars (4.5 mm diameter) spaced 10 mm apart. The grid was fixed to a metal frame which rested on the base of the startle box by four compression springs. An aluminium tray containing a fixed amount of sawdust was hung from the metal frame directly under the grid floor; the sawdust was changed after each rat was tested.

An accelerometer (Super-G, G-1A, Hakuto International U.K. Ltd.) of sensitivity 200mV/g and frequency response 1-300Hz was fixed underneath the metal platform in the centre of the test chamber. The accelerometer had an internal amplifier and a variable gain setting which was adjusted manually. Movement of the chamber produced a displacement of the accelerometer and the resultant voltage was amplified with an adjustable gain amplifier using a digitally controlled potentiometer. The signal was low pass filtered to eliminate high frequency interference and then passed to an analog to digital converter. The digitised signal was then fed to a BBC 'Master' computer using a 1MHz Bus. The signal was sampled continuously at 0.5 msec intervals for 300 msec after the start of the tone or shock presentation. The samples were stored in the memory of the BBC by a machine code algorithm, and the maximum peak height and associated latency determined using a programme written 'in house' by P. Lloyd.

The noise stimulus (tone) was generated by a sine wave generator integrated circuit and the white noise (present throughout the experiment) was generated by a digital noise source controlled by an analog switch connected to the computer system. The two signals were added together, amplified, and

**Figure 5**

Photograph of the fear-potentiated acoustic startle chamber in which the animals are placed for all studies in this thesis.



delivered via a 2W speaker mounted in the ceiling of the test chamber. The duration, frequency, and intensity of the noise stimulus was controlled via the BBC microcomputer. The intensity of the stimulus tone and background white noise was measured within the chamber using a precision sound level meter (CS 192B, Castle Associates, Scarborough), and the average of five readings (one taken from each wall and one from the ceiling) used.

The conditioned stimulus (CS) consisted of a round MES 4.8W light bulb (International Lamps Ltd., Hertford) mounted in a protective casing in the centre of the startle chamber ceiling. Shocks (unconditioned stimuli, US) were delivered through the grid floor from a shock generator designed 'in house' and located outside the chamber. Shock delivery was controlled by the BBC microcomputer. The startle box was enclosed in a series of sound attenuating boxes to minimise exposure of the rats outside of the test apparatus and experimenter to the startle noise stimulus.

**Calibration**

This was carried out by dropping bags of sand of known masses (2.5 - 180g) from a fixed height on to the metal grid with a 300g bag of sand permanently on the metal grid to allow for the initial mass of the rat. This was repeated 10 times for each weight and the average output of the accelerometer recorded.

**Matching Procedure**

All rats underwent 1 matching session (3 days prior to the first training session) in order to establish their baseline startle responses. Groups of 30-36 rats were weighed the day prior to matching, and any having values outside the range - mean ± 2 standard deviations excluded. Each rat was placed individually in the startle chamber and five minutes later presented with 20 tones (95dB, 4000Hz, 300msec duration) with a mean inter-stimulus interval of 20sec (range 8-32sec). Background white noise (55dB) was present throughout the session. The mean startle amplitude was calculated for each rat, excluding responses occurring less than 25msec or greater than 200msec after the start of the tone presentation. Rats were assigned into 3 groups ensuring that the groups were similar with respect to weight, baseline startle, and contained a representative number of rats from each of the original home cages. Vehicle and drug treatments were randomly assigned to the three groups.

**Training Procedure**

All rats underwent 2 training sessions on consecutive days starting on the third day after matching. Rats were run in the same order, and at approximately the same time of day on both training and testing days. Each rat was placed individually in the startle chamber and after 5 minutes was exposed to 20 light-shock pairings (trials) in which a 0.5mA shock (US) was delivered through the grid floor during the last 0.5sec of the 3sec light (conditioning) stimulus. The trials were presented at an average inter-trial interval of 20sec (range 8-32sec).

**Testing Procedure**

24 hours after the second training session, rats were injected intraperitoneally with either vehicle (1%w/v methyl cellulose in 0.9% saline), diazepam (0.625, 1.25, 2.5 or 5mg/kg), idazoxan (0.125, 0.25 or 0.5 mg/kg) or FG 7142 (2.5 or 5 mg/kg). The pH of all solutions injected was checked and adjusted to approximate neutrality (pH 6-8). Injections were made 25 minutes prior to placing a rat in the startle chamber which resulted in a 30 minute pretreatment time including the usual 5 minute latency period. 20 tones (see matching procedure) were delivered at a mean inter-trial interval of 20sec (range 8-32sec). 10 of the tones were presented alone as in the matching procedure, and 10 were preceded for 2.5sec by the conditioning light stimulus (duration 3sec). The presentation of the 'light on' and 'light off' trials was controlled by the startle programme so that they occurred in a random manner across the 20 trials; no more than 2 consecutive trials of the same type was allowed. No shock is used when the light is presented during the test procedure.

The 'light on' (potentiated startle) responses are used as an index of anxiety and the 'light off' (basal startle) responses used as an indicator of general activity.

**Data Analysis**

The total startle amplitude scores for the 10 'light off' (startle) and 10 'light on' (potentiated startle) responses were calculated for each rat. The startle score used was the maximum response occurring within 200msec of the onset of tone; responses occurring less than 25msec were not included. The mean of the total startle amplitudes for each type of trial were calculated for each treatment group and are presented graphically. The difference between 'light on' and 'light off' responses were analysed using the Wilcoxon matched pairs

signed ranks test for each treatment group to ascertain the significance of the potentiated startle effect. Comparison across groups for each trial type was performed using the Kruskal Wallis one way analysis of variance by ranks test with post-hoc 2 tailed Mann-Whitney U-test.

**Drugs**

For both the X-maze and potentiated startle studies drugs were suspended in 1% methyl cellulose (in 0.9% saline), by sonication. All drugs were prepared immediately prior to use and injected i.p. in a volume of 1 ml/kg.

Compounds were obtained from the following sources: Methyl cellulose (BDH Chemicals); diazepam (Courtin and Warner Ltd.); idazoxan (Reckitt and Coleman); FG 7142 (ß-carboline-3-carboxylate methylamide) (synthesized at SmithKline Beecham).

## RESULTS

### 1. ELEVATED X-MAZE

The vehicle control animals spent less time on the open arms compared to the closed arms indicating the aversive nature of the open arms (these animals had less than 30% open:total entries and 20% open:total time). Diazepam dose-dependently increased the percentage open:total entries and time, and time on the end of the open arms, reaching significance at all doses (1, 2.5 and 5 mg/kg) with the exception of 1 mg/kg on the time on the end of the open arms (Fig. 6A and B). None of these doses had any effect on the rears/min in each area (Fig. 6C) or the total number of arm and end of open arm entries (Table 1). Idazoxan at both doses (0.25 and 0.5 mg/kg) significantly decreased the percentage open:total entries and time, and the time spent on the end of the open arms (Fig. 7A and B). Neither dose had any significant effect on the rears/min in each area (Fig. 7C) or the total number of arm and end of open arm entries, although this index of locomotor activity was reduced with the 0.5 mg/kg dose (Table 1). FG 7142 at both doses (1 and 5 mg/kg) significantly reduced the percentage open:total entries and time (Fig. 8A) with the higher dose causing the greater reduction. Also both doses significantly reduced the time spent on the end of the open arms (Fig. 8B). Neither dose had any significant effect on the rears/min in each area

**Figure 6**

Effects of acute administration of diazepam (1, 2.5 or 5 mg/kg i.p.) on (A) the percentage open:total entries and time, (B) the time spent on the end of the open arms, and (C) the rears/min in the closed arms, open arms and central square. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$, **$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.

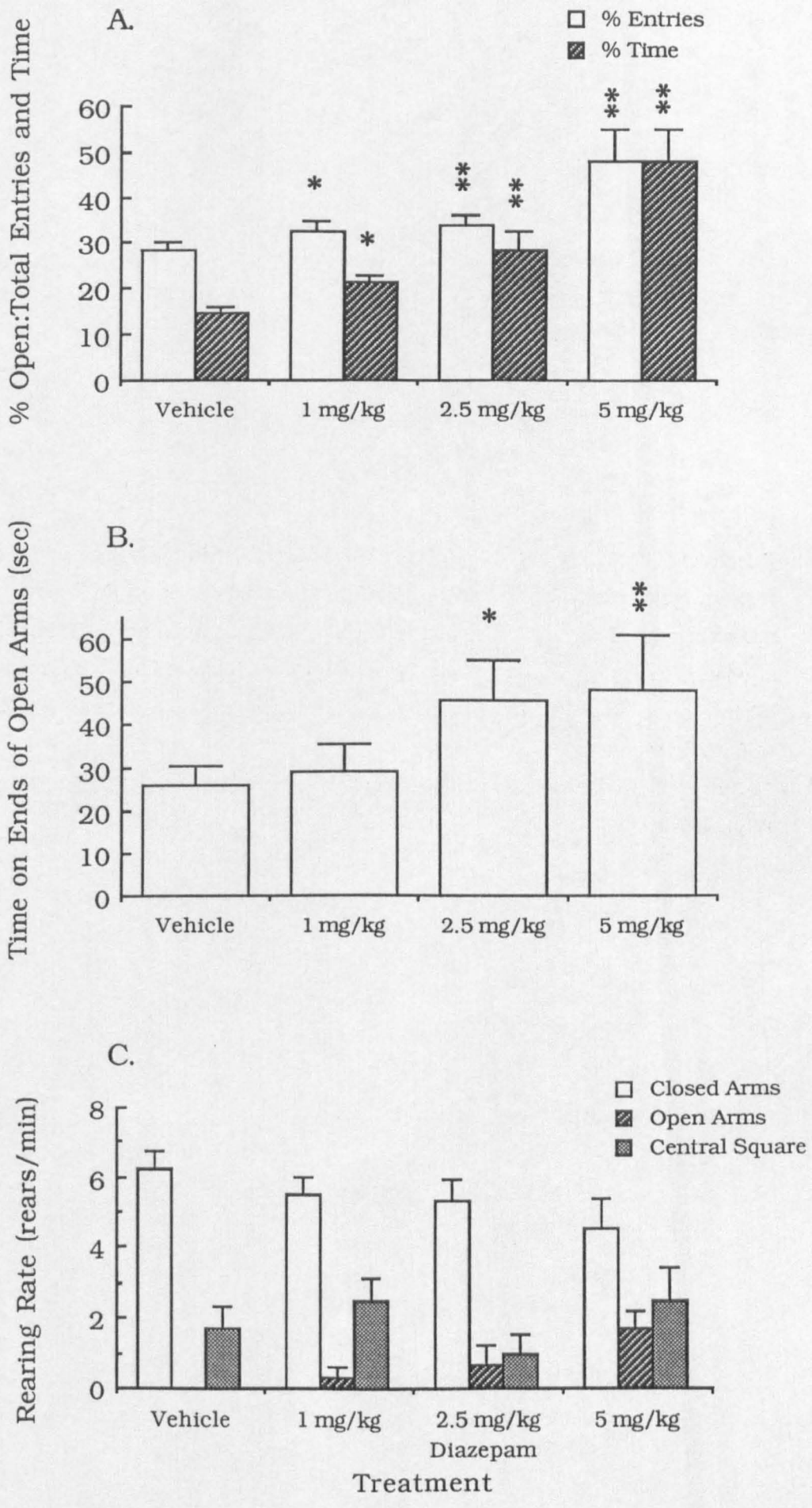

A.
% Entries
% Time
% Open:Total Entries and Time
60
50
40
30
20
10
0
Vehicle
1 mg/kg
2.5 mg/kg
5 mg/kg
B.
Time on Ends of Open Arms (sec)
60
50
40
30
20
10
0
Vehicle
1 mg/kg
2.5 mg/kg
5 mg/kg
C.
Closed Arms
Open Arms
Central Square
Rearing Rate (rears/min)
8
6
4
2
0
Vehicle
1 mg/kg
2.5 mg/kg
Diazepam
5 mg/kg
Treatment

**Figure 7**

Effects of acute administration of idazoxan (0.25 or 0.5 mg/kg i.p.) on (A) the percentage open:total entries and time, (B) the time spent on the end of the open arms, and (C) the rears/min in the closed arms, open arms and central square. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.

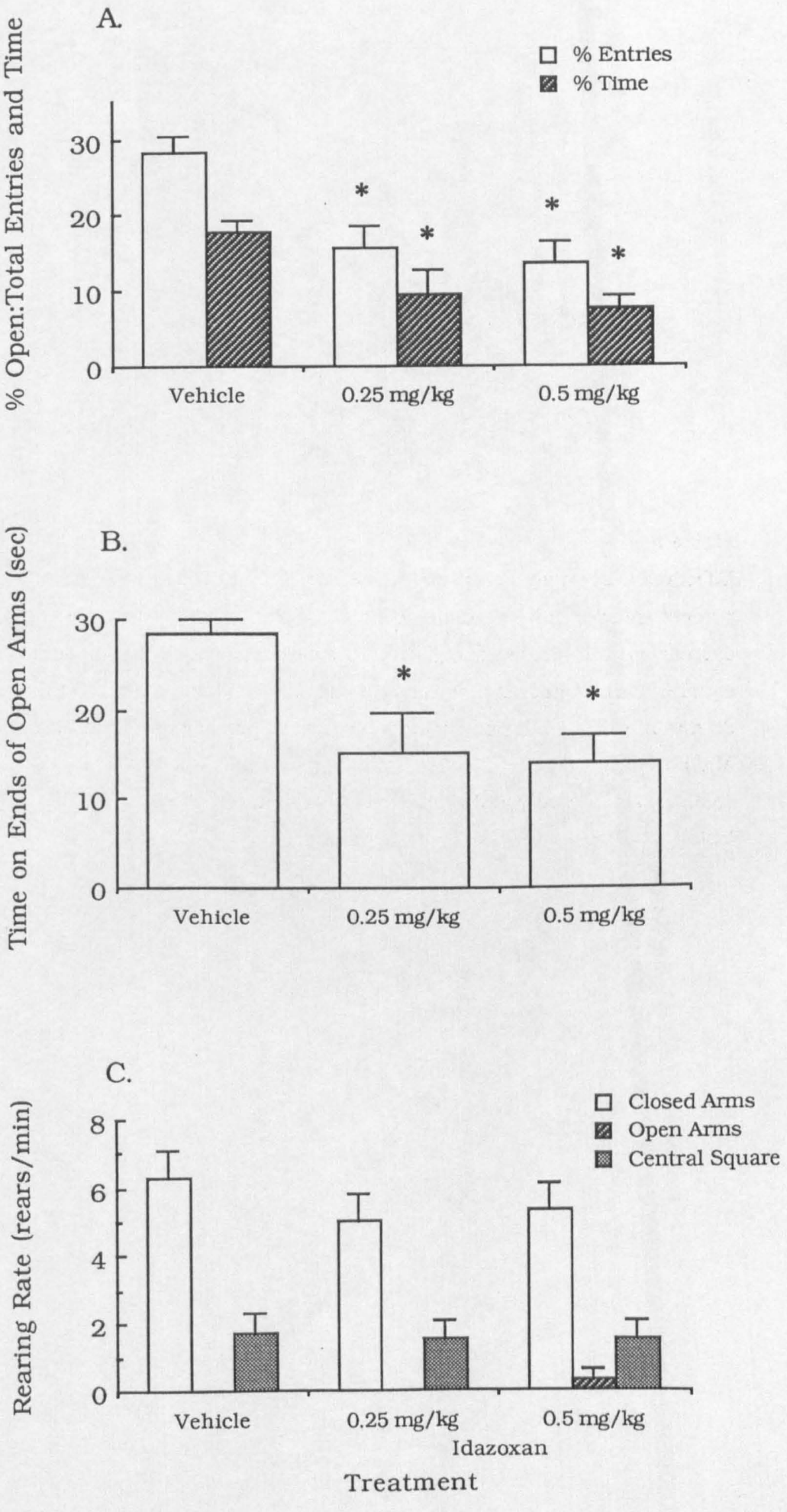

A.
% Entries
% Time
% Open:Total Entries and Time
30
20
10
0
*
*
*
*
Vehicle
0.25 mg/kg
0.5 mg/kg
B.
Time on Ends of Open Arms (sec)
30
20
10
0
*
*
Vehicle
0.25 mg/kg
0.5 mg/kg
C.
Closed Arms
Open Arms
Central Square
Rearing Rate (rears/min)
8
6
4
2
0
Vehicle
0.25 mg/kg
0.5 mg/kg
Idazoxan
Treatment

**Figure 8**

Effects of acute administration of FG 7142 (1 or 5 mg/kg i.p.) on (A) the percentage open:total entries and time, (B) the time spent on the end of the open arms, and (C) the rears/min in the closed arms, open arms and central square. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$, **$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.

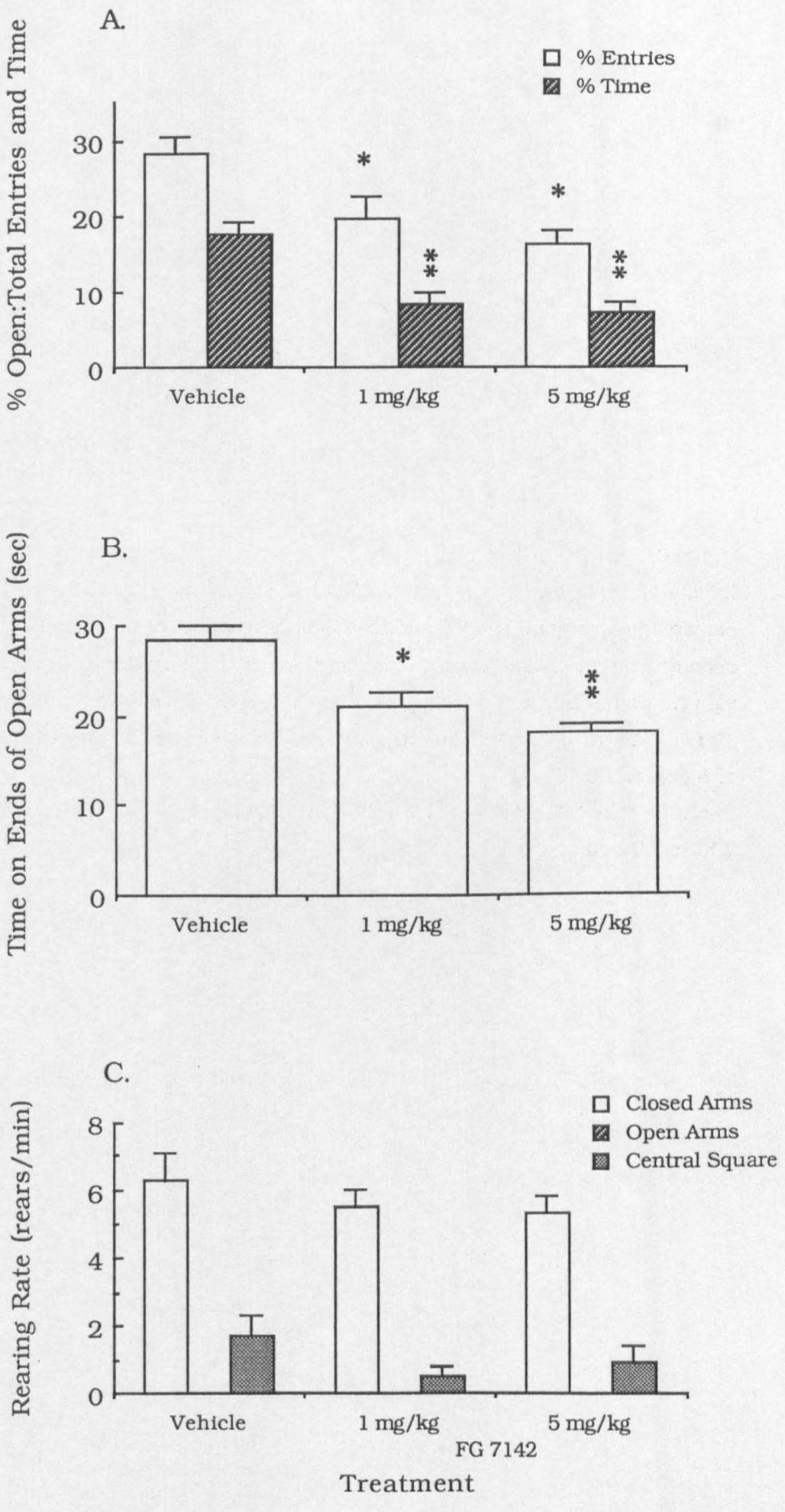

A.
% Entries
% Time
% Open:Total Entries and Time
30
20
10
0
*
**
*
**
Vehicle
1 mg/kg
5 mg/kg
B.
Time on Ends of Open Arms (sec)
30
20
10
0
*
**
Vehicle
1 mg/kg
5 mg/kg
C.
Closed Arms
Open Arms
Central Square
Rearing Rate (rears/min)
8
6
4
2
0
Vehicle
1 mg/kg
5 mg/kg
FG 7142
Treatment

**Table 1**

Number of arm and end of arm entries made by each animal during a 5 minute exposure to the X-maze 30 minutes after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations for vehicle and drug treated groups.

No significant difference from vehicle controls. 2-tailed Mann-Whitney U-test.

| Treatment | No. of Arm and End of Arm Entries |
|---|---|
| Vehicle | 32.5 ± 1.1 |
| Diazepam (1mg/kg) | 31.2 ± 3.6 |
| Diazepam (2.5mg/kg) | 34.2 ± 2.9 |
| Diazepam (5mg/kg) | 35.7 ± 3.0 |
| Idazoxan (0.25mg/kg) | 29.3 ± 3.5 |
| Idazoxan (0.5mg/kg) | 27.2 ± 2.1 |
| FG 7142 (1mg/kg) | 29.8 ± 2.0 |
| FG 7142 (5mg/kg) | 25.2 ± 2.7 |

(Fig. 8C) or the total number of arm and end of arm entries, although again, the higher dose reduced this parameter (Table 1).

## 2. FEAR-POTENTIATED STARTLE PARADIGM

### Calibration

Figure 9 shows that the output of the accelerometer was proportional to the vertical force on the grid and that the relationship was curvilinear over the range of startle responses measured.

### Pharmacological Validation

Figure 10 shows a computer printout of a typical startle response in a typical control rat during a light and tone trial. The y axis represents the amplitude of the deflection (arbitary units) produced when the platform on which the animal is standing moves as a result of a startle response, with the x axis being the time from the onset of the startle tone. The dotted lines indicate the peak amplitude of 76 units occurring 82 msec after the onset of the tone.

Figure 11A shows the mean of the total startle amplitude responses on the tone alone and the light and tone trials during testing after injection of the vehicle or the various doses of diazepam. Analysis of the data for the vehicle treated group using the Wilcoxon matched pairs signed ranks test showed that displacement during the light and tone trials were significantly greater than the tone alone trials ($p<0.01$), indicating a robust potentiation of the startle response. Animals pretreated with diazepam at all doses (0.625, 1.25, 2.5 and 5 mg/kg) still showed a significant potentiated startle response ($p<0.05$) (Fig. 11A). The 0.625 and 1.25 mg/kg doses were not significantly different from the vehicle control group in the mean of the total startle amplitudes for both the light and tone and the tone alone trials (Fig. 11A); however, the light and tone response amplitudes tended to be reduced resulting in a reduction in the difference between the light and tone and the tone alone trials at the 1.25 mg/kg dose (the 'potentiated startle') (Fig. 11B). Diazepam at 2.5 and 5 mg/kg significantly reduced the mean of the total startle amplitudes for the light and tone trials ($p<0.01$) (Fig. 11A) and the 2.5 mg/kg dose significantly reduced the mean of the total startle amplitudes in the tone alone trials (Fig. 11A). These doses of diazepam reduced the difference between the two types of trial (Fig. 11B).

**Figure 9**

Calibration of the fear-potentiated startle platform. Each different weight of sandbag was dropped from a set height and the amplitude of the deflection (arbitary units) was recorded with a 300g bag of sand permanently on the metal grid floor to allow for the weight of a rat under experimental conditions. Data points are the mean ± S.E.M. of 10 drops.

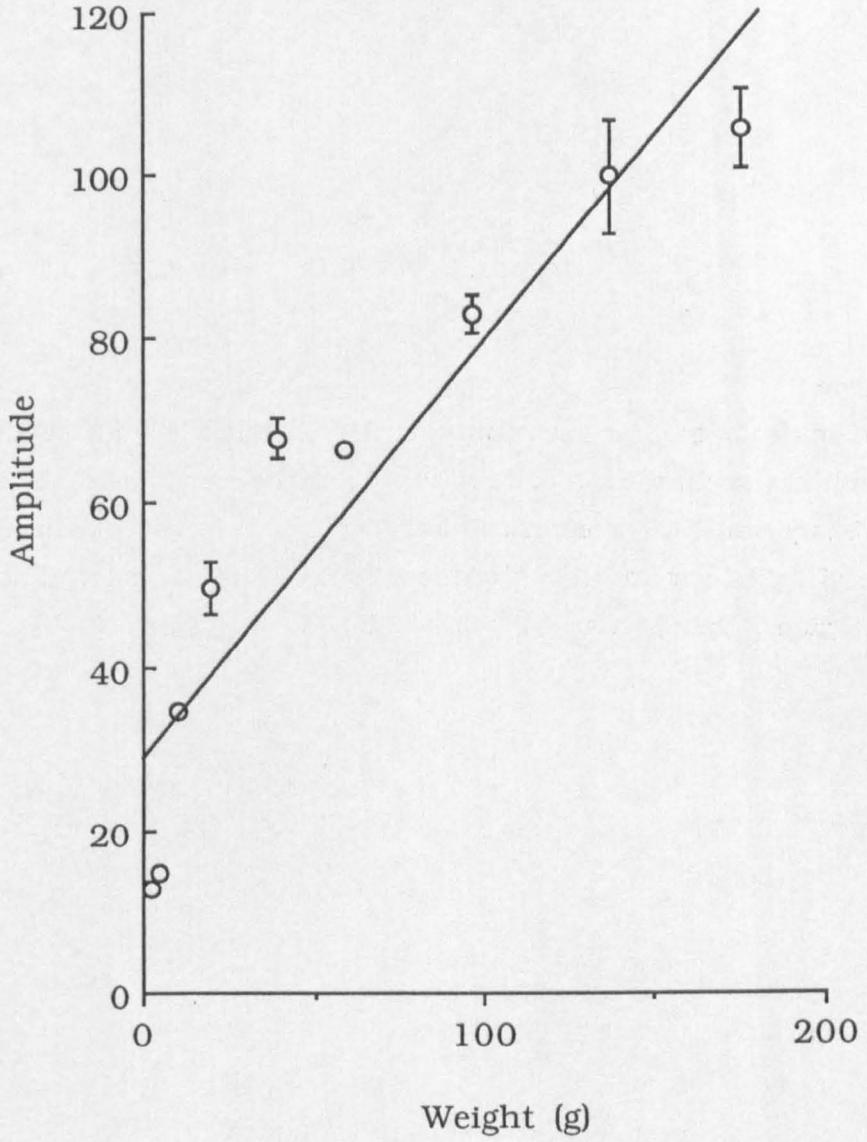

120
100
80
60
40
20
0
Amplitude
0
100
200
Weight (g)

**Figure 10**

A computer printout of a typical acoustic startle response for a rat in the acoustic startle chamber. The y axis represents the amplitude of the deflection (arbitary units) produced when the platform on which the animal is standing moves as a result of a startle response, with the x axis being the time from the onset of the startle tone. The dotted lines indicate the peak amplitude of 76 units occurring 82 msec after the onset of the tone in this trial.

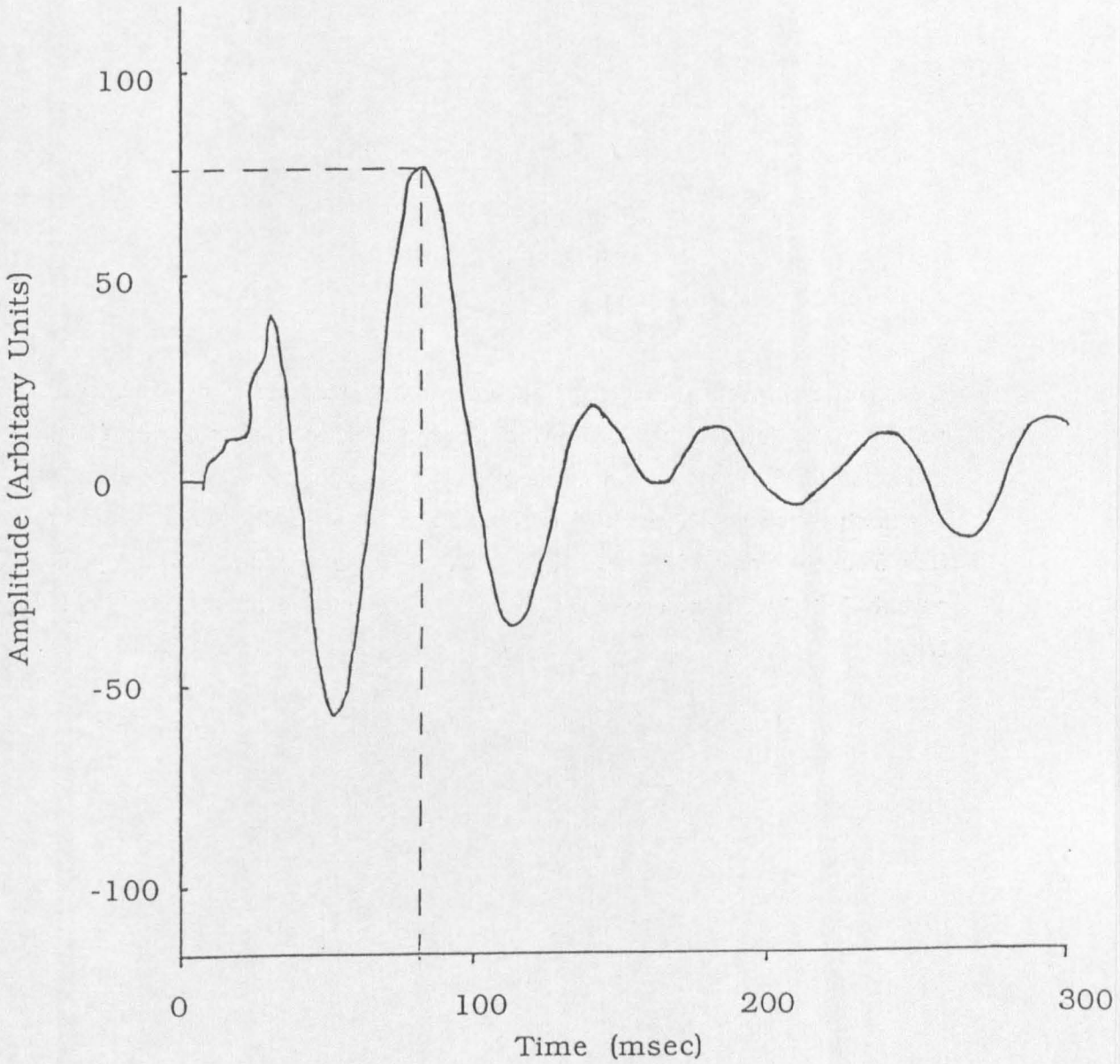
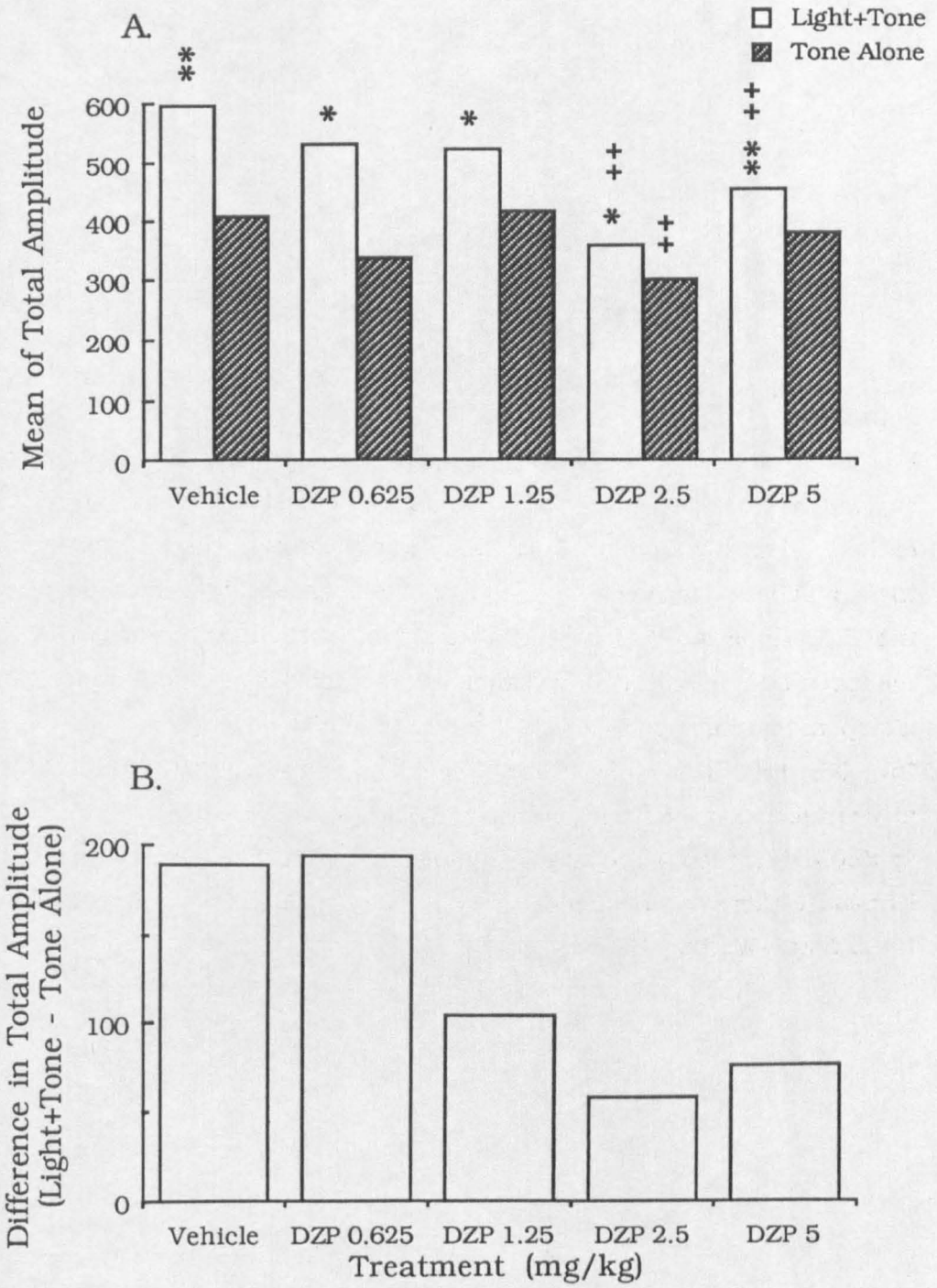

100
50
0
-50
-100
Amplitude (Arbitary Units)
0
100
200
300
Time (msec)

**Figure 11**

Effect of vehicle (1% methyl cellulose i.p.) or diazepam (DZP 0.625, 1.25, 2.5 or 5 mg/kg i.p.) administered 30 minutes prior to testing to rats on (A) mean of the total startle amplitudes of 10 light and tone trials and 10 tone alone trials, and (B) the mean of the difference in total amplitude of the two types of trial (light+tone - tone alone) (i.e. 'potentiated' startle). Each bar represents the mean total amplitude of 20 animals for vehicle control and 10 for each diazepam treatment group.

*$p<0.05$, **$p<0.01$ significantly different between light and tone and tone alone trials using the Wilcoxon matched pairs signed ranks test.

$^{+}p<0.05$, $^{++}p<0.01$ significantly different from vehicle control using the Kruskal Wallis one way analysis of variance by ranks test with post-hoc 2 tailed Mann-Whitney U-test.


A.
Light+Tone
Tone Alone
Mean of Total Amplitude
600
500
400
300
200
100
0
Vehicle
DZP 0.625
DZP 1.25
DZP 2.5
DZP 5
B.
Difference in Total Amplitude
(Light+Tone - Tone Alone)
200
100
0
Vehicle
DZP 0.625
DZP 1.25
DZP 2.5
DZP 5
Treatment (mg/kg)

Animals pretreated with idazoxan at all doses (0.125, 0.25 and 0.5 mg/kg) produced a significant potentiated startle response ($p<0.01$) (Fig. 12A) and showed an increase, but not to significance, in the mean of the total startle amplitudes during the light and tone trials ($p<0.1$ for 0.25 mg/kg). None of the doses of idazoxan had any effect on the mean of the total startle amplitudes during the tone alone trials (Fig. 12A). Fig. 12B shows the dose-dependent increase in the difference in mean of the total startle amplitudes between the two types of trial with idazoxan pretreatment.

Animals pretreated with FG 7142 (2.5 and 5 mg/kg) had a significant potentiated startle reponse ($p<0.05$) (Fig. 13A). The mean of the total startle amplitudes for both the light and tone and tone alone trials in vehicle controls was lower in this experiment compared to the two previous studies. FG 7142 at both doses significantly increased both the mean of the total startle amplitudes in the light and tone and tone alone trials compared to vehicle control ($p<0.05$) (Fig. 13A), and the net result was a decrease in the difference between the light and tone and tone alone trials (Fig. 13B).

**DISCUSSION**

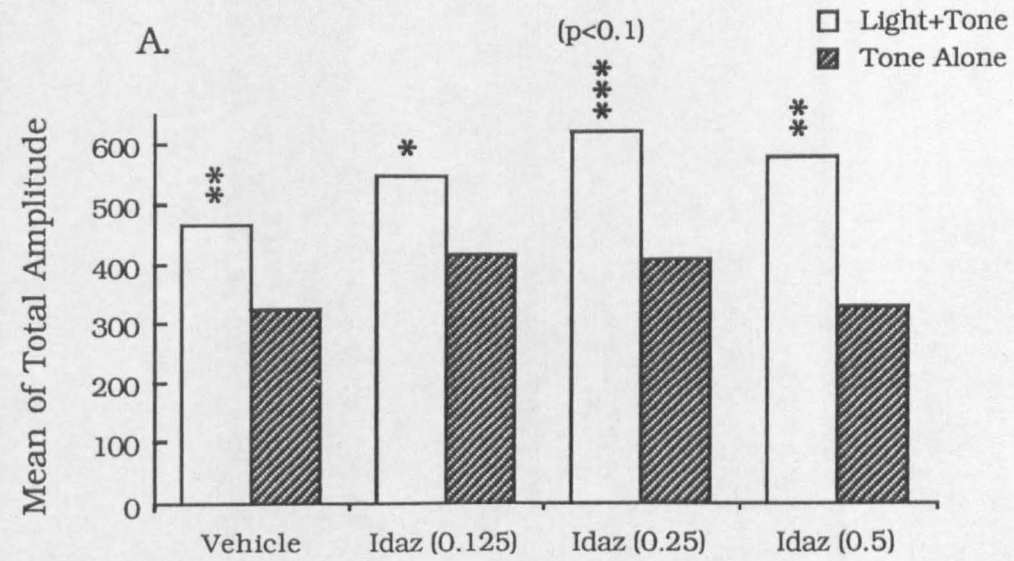
The present study has confirmed that both the elevated X-maze and the potentiated startle paradigms can detect the anxiolytic activity of benzodiazepine-like compounds (Pellow et al., 1985; Davis, 1979a, b; 1984). First, considering the elevated X-maze, acute diazepam treatment significantly increased the percentage open:total entries and time and time on the ends of the open arms in a dose-dependent manner without changing the total number of arm and end of arm entries. Several authors (e.g. Pellow et al., 1985; Critchley and Handley, 1987) have previously reported an increase in the total arm entries with diazepam and this is thought to be a result of enhanced locomotor activity occurring from an increase in exploration. However, the marking of a line half-way along each of the arms allows activity within a particular arm to be monitored and helps rule out the possibility of false positives occurring as a result of increased locomotor activity. The control animals did not show a significant reduction in locomotor activity compared to the diazepam treated animals, suggesting that they explore within an enclosed arm to a greater extent than diazepam treated animals and the increase in percentage open:total entries observed

**Figure 12**

Effect of vehicle (1% methyl cellulose i.p.) or idazoxan (Idaz 0.125, 0.25 or 0.5 mg/kg i.p.) administered 30 minutes prior to testing to rats on (A) mean of the total startle amplitudes of 10 light and tone trials and 10 tone alone trials, and (B) the mean of the difference in total amplitude of the two types of trial (light+tone - tone alone) (i.e. 'potentiated' startle). Each bar represents the mean total amplitude of 20 animals for vehicle control and 10 for each idazoxan treatment group.

*$p<0.05$, **$p<0.01$, ***$p<0.005$ significantly different between light and tone and tone alone trials using the Wilcoxon matched pairs signed ranks test.

($p<0.1$) significantly different from vehicle control using the Kruskal Wallis one way analysis of variance by ranks test with post-hoc 2 tailed Mann-Whitney U-test.


A.
(p<0.1)
Light+Tone
Tone Alone
Mean of Total Amplitude
600
500
400
300
200
100
0
Vehicle
Idaz (0.125)
Idaz (0.25)
Idaz (0.5)

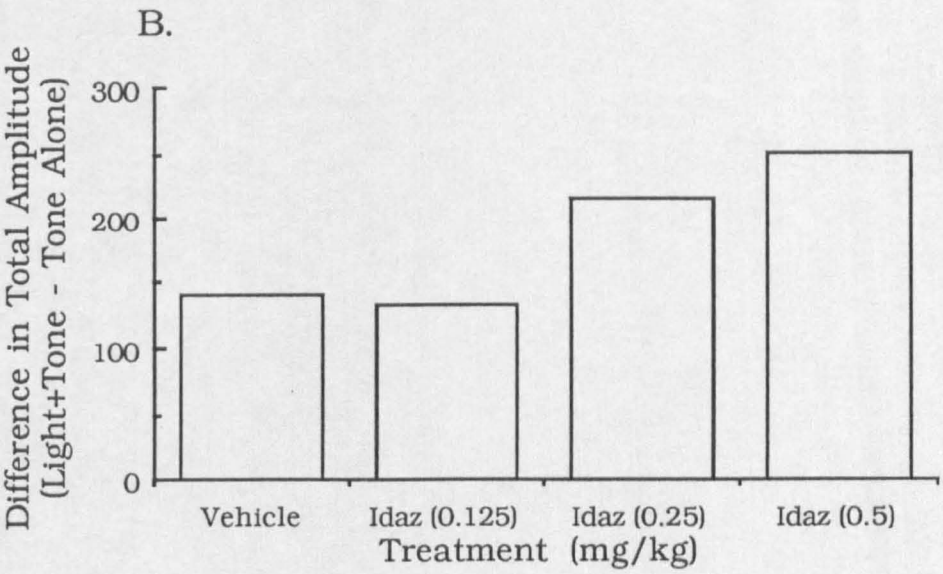
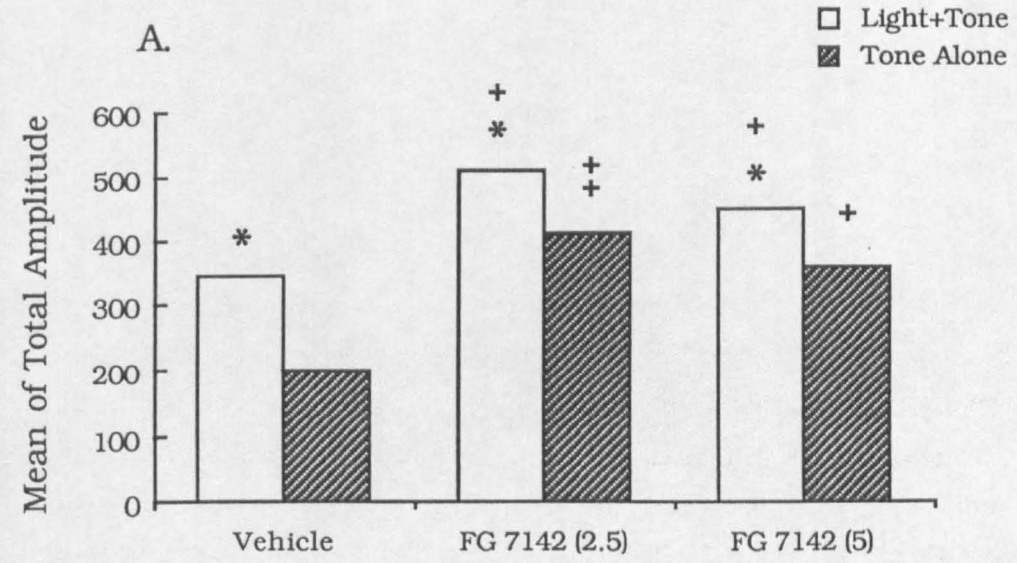

B.
Difference in Total Amplitude
(Light+Tone - Tone Alone)
300
200
100
0
Vehicle
Idaz (0.125)
Idaz (0.25)
Idaz (0.5)
Treatment (mg/kg)

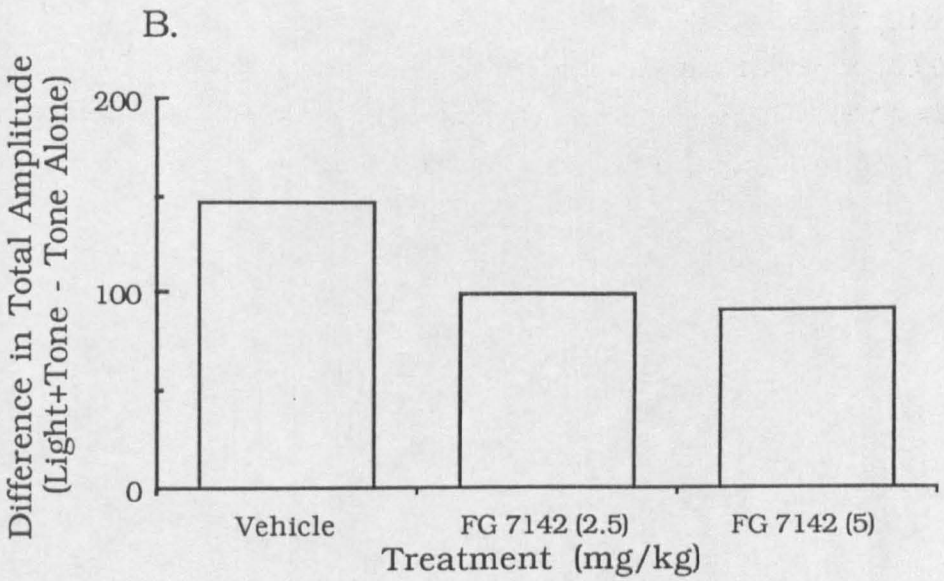
**Figure 13**

Effect of vehicle (1% methyl cellulose i.p.) or FG 7142 (2.5 or 5 mg/kg i.p.) administered 30 minutes prior to testing to rats on (A) mean of the total startle amplitudes of 10 light and tone trials and 10 tone alone trials, and (B) the mean of the difference in total amplitude of the two types of trial (light+tone - tone alone) (i.e. 'potentiated' startle). Each bar represents the mean total amplitude of 20 animals for vehicle control and 10 for each FG 7142 treatment group.

*$p<0.05$ significantly different between light and tone and tone alone trials using the Wilcoxon matched pairs signed ranks test.

$^{+}p<0.05$, $^{++}p<0.01$ significantly different from vehicle control using the Kruskal Wallis one way analysis of variance by ranks test with post-hoc 2 tailed Mann-Whitney U-test.


A.
Light+Tone
Tone Alone
Mean of Total Amplitude
600
500
400
300
200
100
0
Vehicle
FG 7142 (2.5)
FG 7142 (5)


B.
Difference in Total Amplitude
(Light+Tone - Tone Alone)
200
100
0
Vehicle
FG 7142 (2.5)
FG 7142 (5)
Treatment (mg/kg)

with the benzodiazepine is most likely a result of increased exploration of the open arms (occurring possibly as a result of reduced fear) and does not merely reflect an increase in general locomotor activity. Acute diazepam at all doses had no effects on the rears/min in the open or enclosed arms of the maze suggesting that measurement of rearing alone cannot be used as an index of anxiety in the rat. In the potentiated startle, the effects of the benzodiazepines are well established with low doses of diazepam (up to 2.5 mg/kg i.p.) reducing only the potentiated startle and higher doses (e.g. 5-10 mg/kg i.p.) reducing both potentiated and baseline startle responses (Davis, 1979a, b). However, in this study diazepam a caused significant reduction in potentiated startle at doses which also reduced baseline startle, suggesting that the system used in the present study was not as sensitive to the benzodiazepines as those described in the literature.

As previously reported (e.g. Handley and Mithani, 1984) the $\alpha_2$-adrenoceptor antagonist idazoxan reduced the percentage open:total entries and time with no significant reduction in the number of arm and end of arm entries, indicating that the reduced exploration of the open arms (indicating increased fear) was not due to decreased locomotor activity. In the potentiated startle paradigm, idazoxan did not produce a significant increase in the potentiated startle nor did it effect the baseline startle. Similar studies using other $\alpha_2$-adrenoceptor antagonists such as piperoxane and yohimbine have shown enhancement of the potentiated startle alone (Davis et al., 1979), and conversely, the $\alpha_2$-adrenoceptor agonist clonidine has been found to selectively reduce the potentiated startle (Davis et al., 1977; Handley and Thomas, 1979). Thus, as seen with diazepam, there is a trend to the standard response reported in the literature but due to variations between individual animals these changes failed to reach significance. In clinical studies yohimbine has been reported to produce anxiety and induce panic attacks (Charney et al., 1984), and clonidine to reduce anxiety (Redmond, 1982). It has been suggested (Marsden, 1989) that these drugs may have their effects by indirect actions on 5-HT systems, rather than a direct action on noradrenergic systems. $\alpha_2$-Adrenoceptors situated on 5-HT nerve cells or terminals have been shown to regulate 5-HT release in rats (Gothert *et al.* 1980) and in man (Raiteri et al., 1990). Application of idazoxan directly into the dorsal raphe nucleus by microinjection has been shown to increase 5-HT dorsal raphe neuronal firing, suggesting that idazoxan increases 5-HT activity by either blocking

with the benzodiazepine is most likely a result of increased exploration of the open arms (occurring possibly as a result of reduced fear) and does not merely reflect an increase in general locomotor activity. Acute diazepam at all doses had no effects on the rears/min in the open or enclosed arms of the maze suggesting that measurement of rearing alone cannot be used as an index of anxiety in the rat. In the potentiated startle, the effects of the benzodiazepines are well established with low doses of diazepam (up to 2.5 mg/kg i.p.) reducing only the potentiated startle and higher doses (e.g. 5-10 mg/kg i.p.) reducing both potentiated and baseline startle responses (Davis, 1979a, b). However, in this study diazepam a caused significant reduction in potentiated startle at doses which also reduced baseline startle, suggesting that the system used in the present study was not as sensitive to the benzodiazepines as those described in the literature.

As previously reported (e.g. Handley and Mithani, 1984) the $\alpha_2$-adrenoceptor antagonist idazoxan reduced the percentage open:total entries and time with no significant reduction in the number of arm and end of arm entries, indicating that the reduced exploration of the open arms (indicating increased fear) was not due to decreased locomotor activity. In the potentiated startle paradigm, idazoxan did not produce a significant increase in the potentiated startle nor did it effect the baseline startle. Similar studies using other $\alpha_2$-adrenoceptor antagonists such as piperoxane and yohimbine have shown enhancement of the potentiated startle alone (Davis et al., 1979), and conversely, the $\alpha_2$-adrenoceptor agonist clonidine has been found to selectively reduce the potentiated startle (Davis et al., 1977; Handley and Thomas, 1979). Thus, as seen with diazepam, there is a trend to the standard response reported in the literature but due to variations between individual animals these changes failed to reach significance. In clinical studies yohimbine has been reported to produce anxiety and induce panic attacks (Charney et al., 1984), and clonidine to reduce anxiety (Redmond, 1982). It has been suggested (Marsden, 1989) that these drugs may have their effects by indirect actions on 5-HT systems, rather than a direct action on noradrenergic systems. $\alpha_2$-Adrenoceptors situated on 5-HT nerve cells or terminals have been shown to regulate 5-HT release in rats (Gothert *et al.* 1980) and in man (Raiteri et al., 1990). Application of idazoxan directly into the dorsal raphe nucleus by microinjection has been shown to increase 5-HT dorsal raphe neuronal firing, suggesting that idazoxan increases 5-HT activity by either blocking

$\alpha_2$-adrenoceptors on 5-HT terminals in the dorsal raphe, or by blocking $\alpha_2$-adrenoceptors on noradrenergic terminals in the dorsal raphe resulting in enhanced noradrenaline release,and consequently increased 5-HT neuronal activity (Garratt et al., 1991). If 5-HT levels increase in anxiety (Chopin and Briley, 1987), $\alpha_2$-adrenoceptor antagonists, by increasing 5-HT release would be predicted to have an anxiogenic profile, and $\alpha_2$-adrenoceptor agonists by decreasing 5-HT release, an anxiolytic profile.

FG 7142 is a well established partial inverse agonist at the GABA-benzodiazepine receptor complex. The present study has confirmed that the elevated X-maze, as well as detecting the anxiolytic activity of benzodiazepine agonists can also detect the anxiogenic activity of partial inverse agonists at the GABA-benzodiazepine receptor complex (Pellow et al., 1987). In the potentiated startle paradigm, FG7142 significantly increases both the potentiated startle and the basal startle. As a result of the increased basal startle it cannot be determined whether the raised potentiated startle is a result of an anxiogenic effect of FG 7142 or a result of increased arousal caused by the drug. Lower doses of FG 7142 may be useful in determining whether this compound has an anxiogenic effect. The ß-carboline has also been found to induce anxiety in man (Dorow et al., 1983). It has been suggested that one of the mechanisms of the anxiolytic effects of the benzodiazepines is via a decrease in central serotonergic transmission (Stein et al., 1977) and evidence exists which shows that benzodiazepines inhibit 5-HT synthesis (Saner and Pletscher, 1979) and release (Pei et al., 1989b). Thus, one of the mechanisms by which the partial inverse agonist FG 7142 may be expected to produce anxiety is by increasing 5-HT function.

Both models have their own advantages, and for the elevated X-maze these are: firstly, it is a non-shock procedure, relying not on electrical stimulation but on the new (novel) environment producing a conflict between fear and exploration. Second, the rats are not deprived of food or water unlike the Geller-Seifter and Vogel tests respectively, thus the effects of drugs on appetite and thirst need not be considered. Third, the animal's behaviour is natural, it is not something it has to learn. Fourth, no training of the rats is necessary and hence testing the animals is relatively easy.

For the potentiated startle paradigm, potentiated startle is defined as a within-subjects difference in startle amplitude in the presence (light-noise

trials) versus the absence (noise-alone trials) of the visual conditioned stimulus. Thus, each animal acts as its own control and hence the problem of between-subjects variability in startle is reduced, making the test more sensitive. Second, it allows evaluation of specific effects (reduction of startle on light-noise trials) versus non-specific effects (reduction of startle on noise-alone trials), so that qualitative as well as quantitative drug profiles can be compared. Third, no shocks are given during testing, thus any drug effects observed cannot be explained by changes in sensitivity to shock. Fourth, the separation between training and testing sessions allows one to determine if a drug alters original learning or performance, and testing for state-dependent learning can be evaluated (Davis, 1979a). Fifth, potentiated startle does not involve any obvious operant. Thus drug effects that might be expected to alter operant performance (e.g. rate-dependent, motivational, disinhibitory motor effects) are avoided.

Additionally, most animal tests of fear of anxiety involve a suppression of ongoing behaviour in the presence of a fear stimulus (e.g. suppression of bar-pressing in the Geller-Seifter operant conflict test; suppression of licking in the licking-suppression test; suppression of normal activity in the social interaction test; suppression of exploration of open arms in X-maze). Hence, certain treatments (e.g. those causing decreases in 5-HT transmission) may appear anxiolytic in these tests if they interact with neural systems common to each of these tests (e.g. response inhibition), even if this is not so in the clinic. Because fear in the potentiated startle paradigm is reflected by enhanced response output, it may provide an important alternative test to analyse potential anxiolytic compounds. A further advantage of the potentiated startle paradigm is that fear is being measured by a change in a simple reflex. Hence, with potentiated startle, fear is expressed through some neural circuit that is activiated by the conditioned stimulus and ultimately impinges on the startle circuit.

Both tests showed that they could detect both anxiolytic and anxiogenic compounds. However, the elevated X-maze produced more reproducible and dose-dependent results in comparison to the potentiated startle, in which there was a great amount of variation in baseline startle response. For this reason, and the simplicity of procedure in the elevated X-maze it was chosen as the behavioural model of anxiety for use in the studies in this thesis. Furthermore, with the X-maze being open-roofed it is possible to combine

this test with microdialysis, thus allowing simultaneous neurochemical and behavioural changes to be measured in the same animal.

# CHAPTER 3

## COMPARISON OF ACUTE AND CHRONIC TREATMENT OF VARIOUS AGENTS THAT ACT DIRECTLY ON 5-HT RECEPTORS WITH THOSE OF DIAZEPAM AND IDAZOXAN IN THE ELEVATED X-MAZE

## INTRODUCTION

In the General Introduction evidence is presented for drugs affecting central serotonergic neurones as putative anxiolytic compounds. The most widely used anxiolytics, the benzodiazepines, are known to influence 5-HT neurotransmission but there is still controversy over whether this action is related to their anxiolytic effect (see Soubrie, 1986; Thiebot, 1986; Kahn et al., 1988). Studies of anxiety disorders in humans (Kahn and Westenberg, 1985; Charney et al., 1987) point towards the involvement of 5-HT in anxiety.

The 5-$HT_{1A}$ receptor partial agonist buspirone has been found to be effective clinically in the treatment of anxiety in benzodiazepine-naive patients (Goldberg and Finnerty, 1979), and the more recently developed 5-$HT_{1A}$ receptor partial agonists gepirone and ipsapirone appear to have clinical activity regardless of the patient's previous drug treatment (Traber and Glaser, 1987). Other clinical studies have reported that buspirone and the 5-$HT_2$ receptor antagonist ritanserin to be effective in the treatment of generalized anxiety disorder (Ceulemans et al., 1985; Taylor et al., 1985). The 5-$HT_3$ antagonists seem to be effective in some behavioural models of anxiety (Tyers, 1989) and clinical trials studying the anxiolytic potential of ondansetron (originally GR38032F) are at present being carried out. However, these classes of 5-HT compounds have produced inconsistent results in various animal models (see Chopin and Briley, 1987).

Using the elevated X-maze model of anxiety described in the General Introduction and Chapter 2, the 5-$HT_{1A}$ receptor partial agonist ipsapirone given acutely has been reported to produce either an anxiolytic (Soderpalm et al., 1989) or an anxiogenic (Moser, 1989a) behavioural profile, or to have no effect (File et al., 1987). Similarly, the 5-$HT_2$ antagonist, ritanserin, has shown either an anxiolytic (Critchley and Handley, 1986) or an anxiogenic (File et al., 1987) profile in the elevated X-maze. Ondansetron has been demonstrated to have an anxiolytic profile on the elevated X-maze (Costall et al., 1989a) and also displays an anxiolytic profile in other behavioural models of anxiety based on exploration such as the black and white box (Costall et al., 1989b) and the social interaction models (Jones et al., 1988). Other 5-$HT_3$ antagonists, such as BRL 46470A, have also displayed an anxiolytic profile in the X-maze (Upton and Blackburn, 1991).

In the clinic, ipsapirone and ritanserin show greater activity after chronic administration (Traber and Glaser, 1987; Ceulemans et al., 1985) and hence the aim of this study was to compare acute and chronic treatments of these two compounds and also ondansetron with those of established anxiolytic (diazepam) and anxiogenic (idazoxan) compounds using the elevated X-maze. In the ondansetron study a group of animals received chronic vehicle followed by acute ondansetron to determine the effects of the chronic dosing schedule on the acute effects of the compound. One of the problems associated with the benzodiazepines are the withdrawal effects sometimes observed on cessation of chronic treatment. In the present study animals were tested 24 hours after the last dose of the chronic study to determine if diazepam withdrawal effects can be observed using the X-maze and whether similar effects are produced by ipsapirone, ritanserin and ondansetron.

## METHODS

### Animals

Male, Lister hooded rats (200-270g) (Nottingham University Medical School Animal Unit) were housed in groups of 4-6 in a room with a 12hr light : 12 hr dark cycle (0700-1900 lights on), with food and water available *ad libitum*.

### Apparatus

The X-maze used was the same one as described in Chapter 2.

### Procedure

Each rat received an injection of either vehicle control (1% methyl cellulose in 0.9% saline), ipsapirone (0.01, 0.1 and 1 mg/kg), ritanserin (0.05 and 0.25 mg/kg), ondansetron (0.01, 0.1 and 1 mg/kg), diazepam (5mg/kg) or idazoxan (0.25 mg/kg) intraperitoneally (i.p.) and was then returned to its home cage; 6 or 8 animals per treatment group. After 25 minutes the animal was isolated in a cage for 5 minutes and then placed in the centre of the X-maze facing one of the enclosed arms and monitored on the X-maze as described in chapter 2.

### Chronic Treatment

Groups of 6 or 8 rats were treated twice daily for 14 days with either vehicle, ipsapirone (0.01, 0.1 and 1 mg/kg), ritanserin (0.25 mg/kg), ondansetron (0.01 mg/kg), or diazepam (5mg/kg) i.p. between 0730-0900 and between 1700-1830 hrs. As a result of the rapid metabolism of idazoxan, two groups

of rats had Alzet osmotic mini-pumps placed (under halothane anaesthesia) below the skin in the middle of the back containing either idazoxan (0.8 mg/kg/hr at a flow rate of 5.5µl/hr) or saline (5.5µl/hr). The osmotic minipumps were still in place during the behavioural testing of these animals. On the morning of the 15th day the rats received their final injection of either drug or vehicle and were tested 30 min later in the X-maze as described above. In the ondansetron study, two groups of animals received either vehicle or ondansetron throughout whilst a third group received chronic vehicle treatment and were then injected with ondansetron (0.01 mg/kg) on the test day.

In the withdrawal study animals were retested on the X-maze 1 (24 hours) and 7 days after the last treatment as described above. No withdrawal study was carried out with the idazoxan treated animals.

Body weight was monitored throughout the treatment period. Housing conditions during the period of chronic treatment were identical to those before chronic dosing (described above).

**Drugs**

Drugs were suspended in 1% methyl cellulose (in 0.9% saline), by sonication. All drugs were prepared immediately prior to use and injected i.p. in a volume of 1 ml/kg.

Compounds were obtained from the following sources: Methyl cellulose (BDH Chemicals); ipsapirone (Troponwerke Gmbh and Co.); ritanserin (Janssen Pharmaceutica); ondansetron (Glaxo); diazepam (Courtin and Warner Ltd.); idazoxan (Reckitt and Coleman).

**Statistics**

All results are expressed as the mean ± S.E.M.. The ratio of open:total arm entries and open to total time was calculated for each rat individually and the mean ± S.E.M. for each treatment group presented.

All data were analysed using 2-tailed Mann-Whitney U-test.

**RESULTS**

**(a) Acute Studies**

Diazepam (5 mg/kg) significantly increased the percentage open:total entries and time, and the time in the end of the open arms (Fig. 1A and 1B), but had no effect on the total number of arm and end of arm entries (Table 1). Idazoxan (0.25 mg/kg) significantly decreased the percentage open:total entries and time (Fig. 1A), and reduced, though not significantly, the time in the ends of the open arms (Fig. 1B), but had no effect on the total number of arm and end of arm entries (Table 1). Ipsapirone at doses of 0.01 and 1mg/kg had no significant effect on the percentage open:total entries and time (Fig. 2A), or on the time spent in the ends of the open arms (Fig. 2B), or on the total number of arm and end of arm entries (Table 1). However, ipsapirone (0.1 mg/kg) significantly decreased the percentage open:total entries and time, and the time spent in the ends of the open arms (Fig. 2). This dose of ipsapirone had no effect on the number of arm and end of arm entries (Table 1). Acute ritanserin (0.05 mg/kg) produced no change in the percentage open:total entries and time, time in the ends of the open arms (Fig. 3A and 3B), and total number of arm and end of arm entries (Table 1). However, ritanserin (0.25 mg/kg) tended to reduce the percentage open:total entries and time, and time in the end of the open arms (Fig. 3A and 3B) with the reduction in open:total time approaching significance ($p<0.06$). This dose of ritanserin had no effect on the total number of arm and end of arm entries (Table 1). Acute ondansetron (0.01, 0.1 and 1 mg/kg) had no significant effect on any of the parameters scored (Fig. 4).

**(b) Chronic Studies**

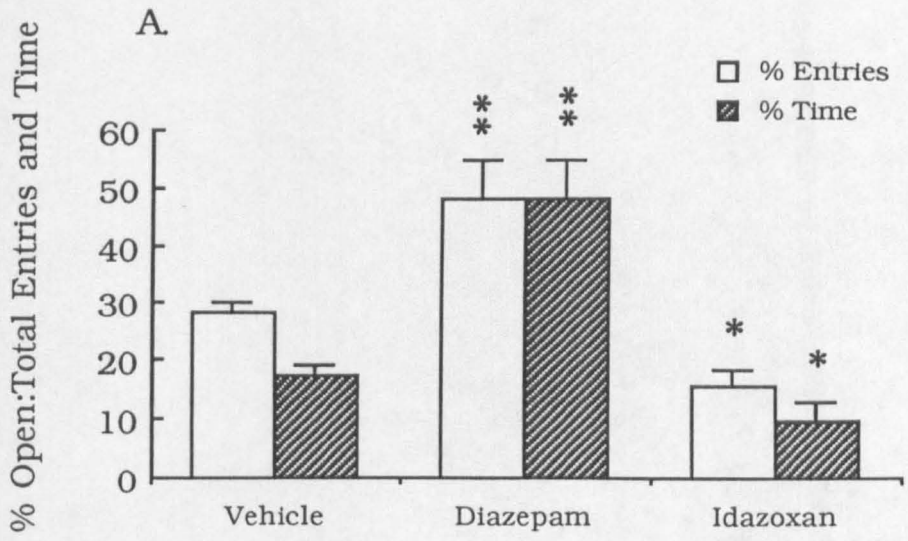
Chronic treatment, twice daily for 14 days, with diazepam, ipsapirone, ritanserin and ondansetron had no effect on the body weights of animals compared with those receiving vehicle control (data not shown). Chronic administration of idazoxan or saline via the mini-pump was also observed to have no effect on body weight (data not shown). The number of arm and end of arm entries decreases with increase in duration of the experimental protocol regardless of the treatment.

Chronic diazepam treatment increased the percentage open:total entries and time, and time spent in the ends of the open arms compared to the control group (Fig. 5A and 5B). There was no difference in the total number of arm and end of arm entries between these two groups (Table 1). Chronic idazoxan significantly decreased the percentage open:total entries and time and the time spent in the ends of the open arms (Fig. 6A and 6B) but had no effect on

**Figure 1**

Effects of acute administration of diazepam (5 mg/kg i.p.) and idazoxan (0.25 mg/kg i.p.) on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$, **$p<0.01$ significantly different from vehicle controls. 2-tailed Mann-Whitney U-test.


A.
% Open:Total Entries and Time
% Entries
% Time
60
50
40
30
20
10
0
Vehicle
Diazepam
Idazoxan

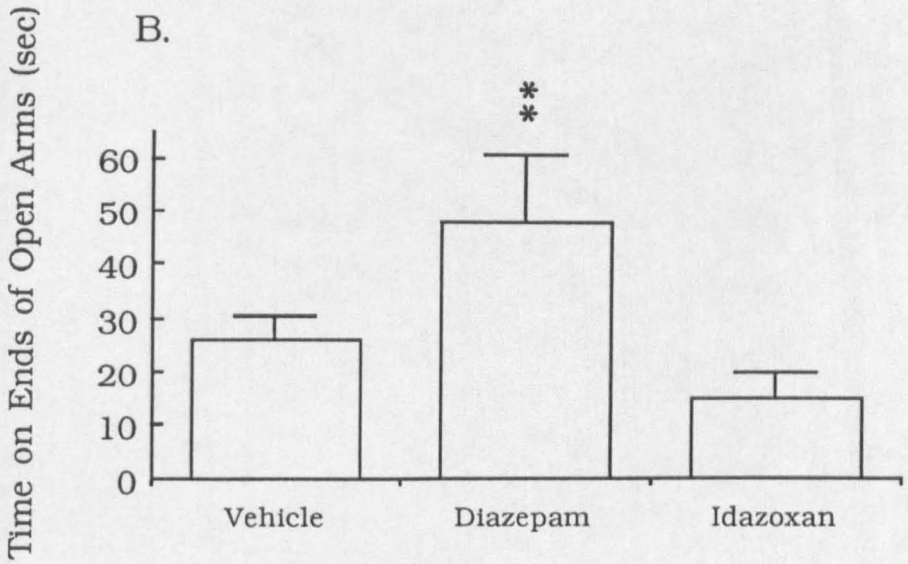

B.
Time on Ends of Open Arms (sec)
60
50
40
30
20
10
0
Vehicle
Diazepam
Idazoxan

**Figure 2**

Effects of acute administration of ipsapirone (0.01, 0.1 and 1 mg/kg i.p.) on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$ significantly different from vehicle controls. 2-tailed Mann-Whitney U-test.

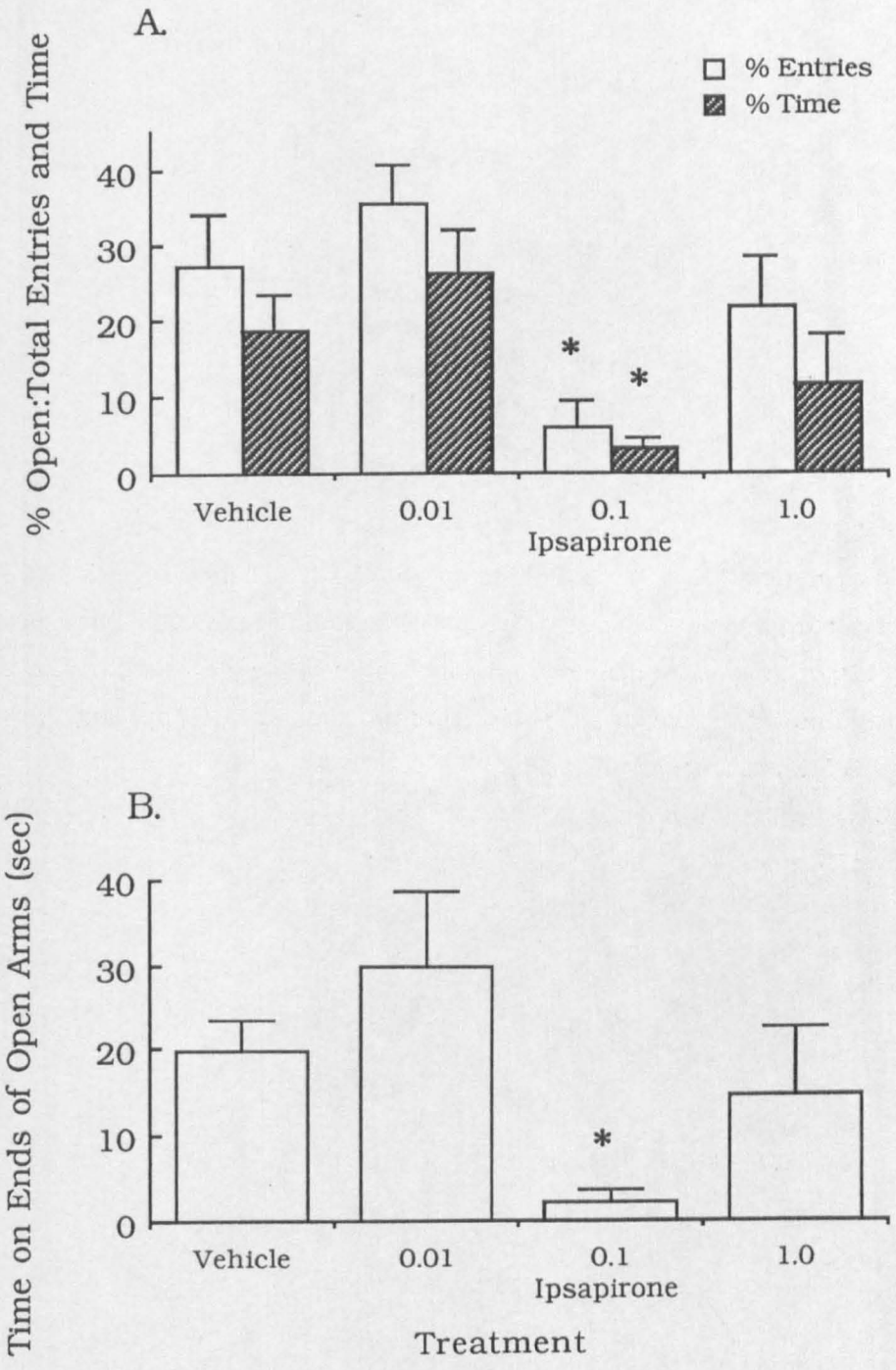

A.
% Entries
% Time
% Open:Total Entries and Time
40
30
20
10
0
*
*
Vehicle
0.01
0.1
1.0
Ipsapirone
B.
Time on Ends of Open Arms (sec)
40
30
20
10
0
*
Vehicle
0.01
0.1
1.0
Ipsapirone
Treatment

**Figure 3**

Effects of acute administration of ritanserin (0.05 and 0.25 mg/kg i.p.) on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 6 observations.

There was no significant difference at the $p<0.05$ level between either of the drug treated groups and the vehicle control. 2-tailed Mann-Whitney U-test.

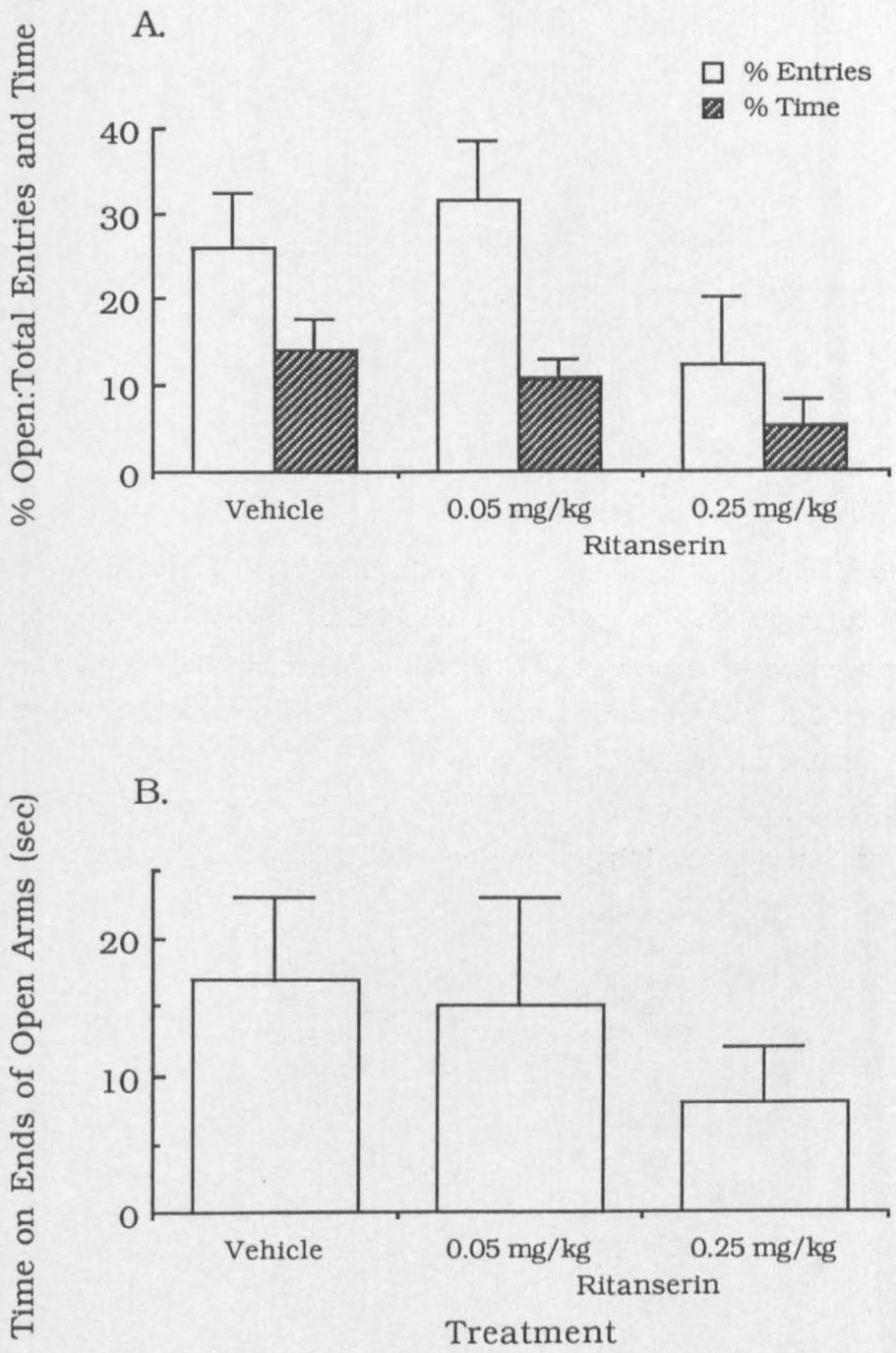

A.
% Open:Total Entries and Time
% Entries
% Time
40
30
20
10
0
Vehicle
0.05 mg/kg
0.25 mg/kg
Ritanserin
B.
Time on Ends of Open Arms (sec)
20
10
0
Vehicle
0.05 mg/kg
0.25 mg/kg
Ritanserin
Treatment

**Figure 4**

Effects of acute administration of ondansetron (0.01, 0.1 and 1 mg/kg i.p.) on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period 30 min after drug administration. Data are expressed as the mean ± S.E.M. of 8 observations.

There was no significant difference at the $p<0.05$ level between any of the drug treated groups and the vehicle control. 2-tailed Mann-Whitney U-test.

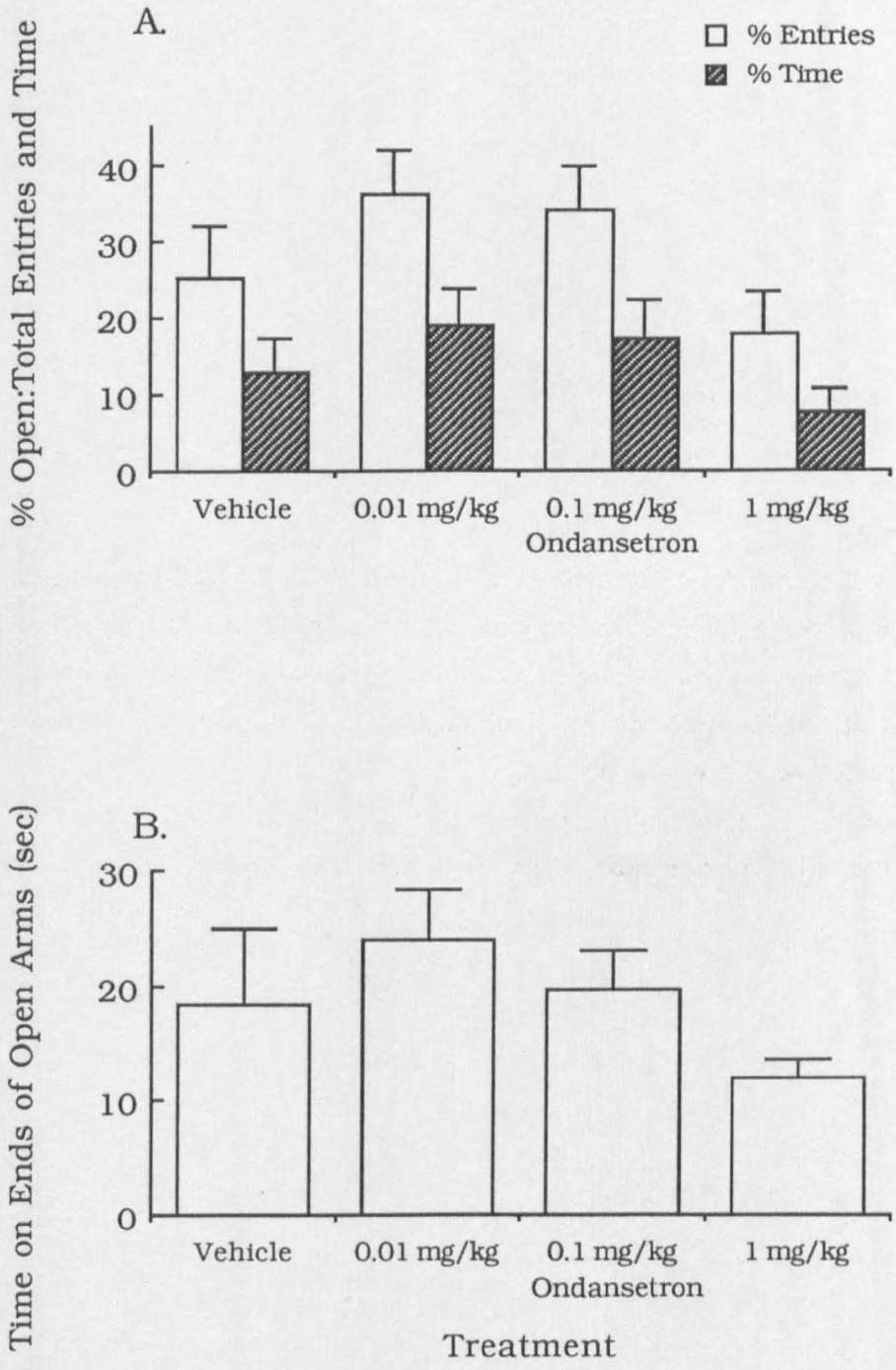
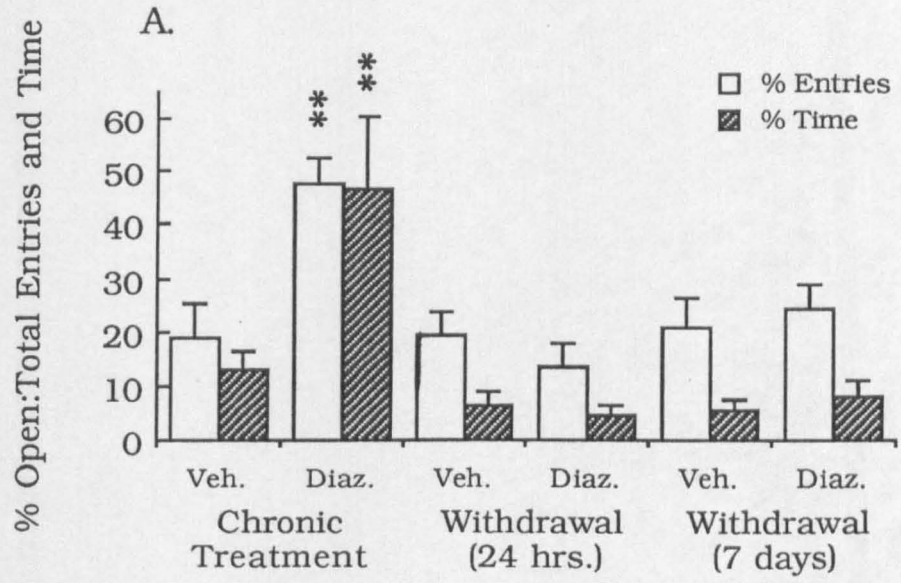

A.
% Entries
% Time
% Open:Total Entries and Time
40
30
20
10
0
Vehicle
0.01 mg/kg
0.1 mg/kg
1 mg/kg
Ondansetron
B.
Time on Ends of Open Arms (sec)
30
20
10
0
Vehicle
0.01 mg/kg
0.1 mg/kg
1 mg/kg
Ondansetron
Treatment

**Figure 5**

Effects of chronic treatment of diazepam (Diaz. 5 mg/kg i.p.) twice daily for 14 days, with behaviour monitored 30 min after last injection on the 15th day, and effects after 24 hours withdrawal and 7 days withdrawal of chronic treatment on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period. Data are expressed as the mean ± S.E.M. of 6 observations.

**$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
% Open:Total Entries and Time
% Entries
% Time
60
50
40
30
20
10
0
**
**
Veh.
Diaz.
Veh.
Diaz.
Veh.
Diaz.
Chronic Treatment
Withdrawal (24 hrs.)
Withdrawal (7 days)

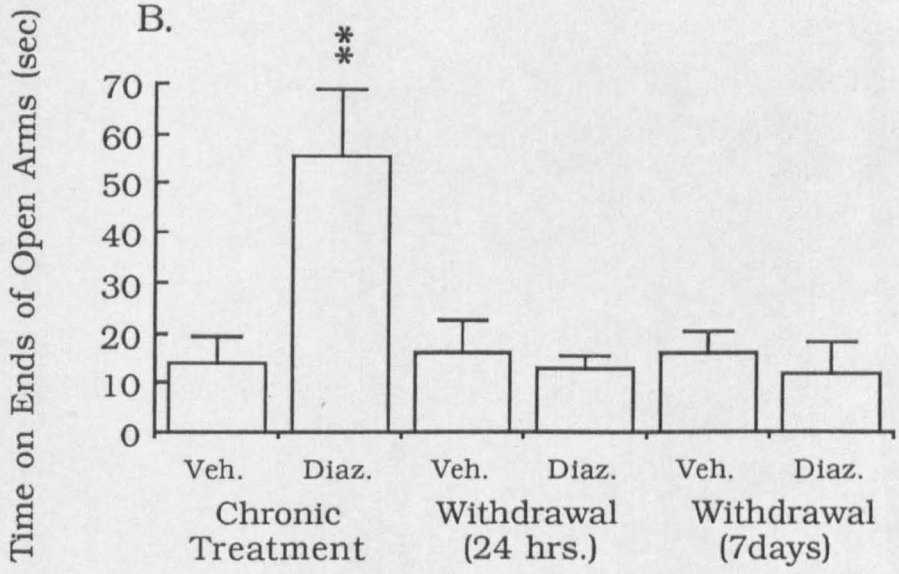
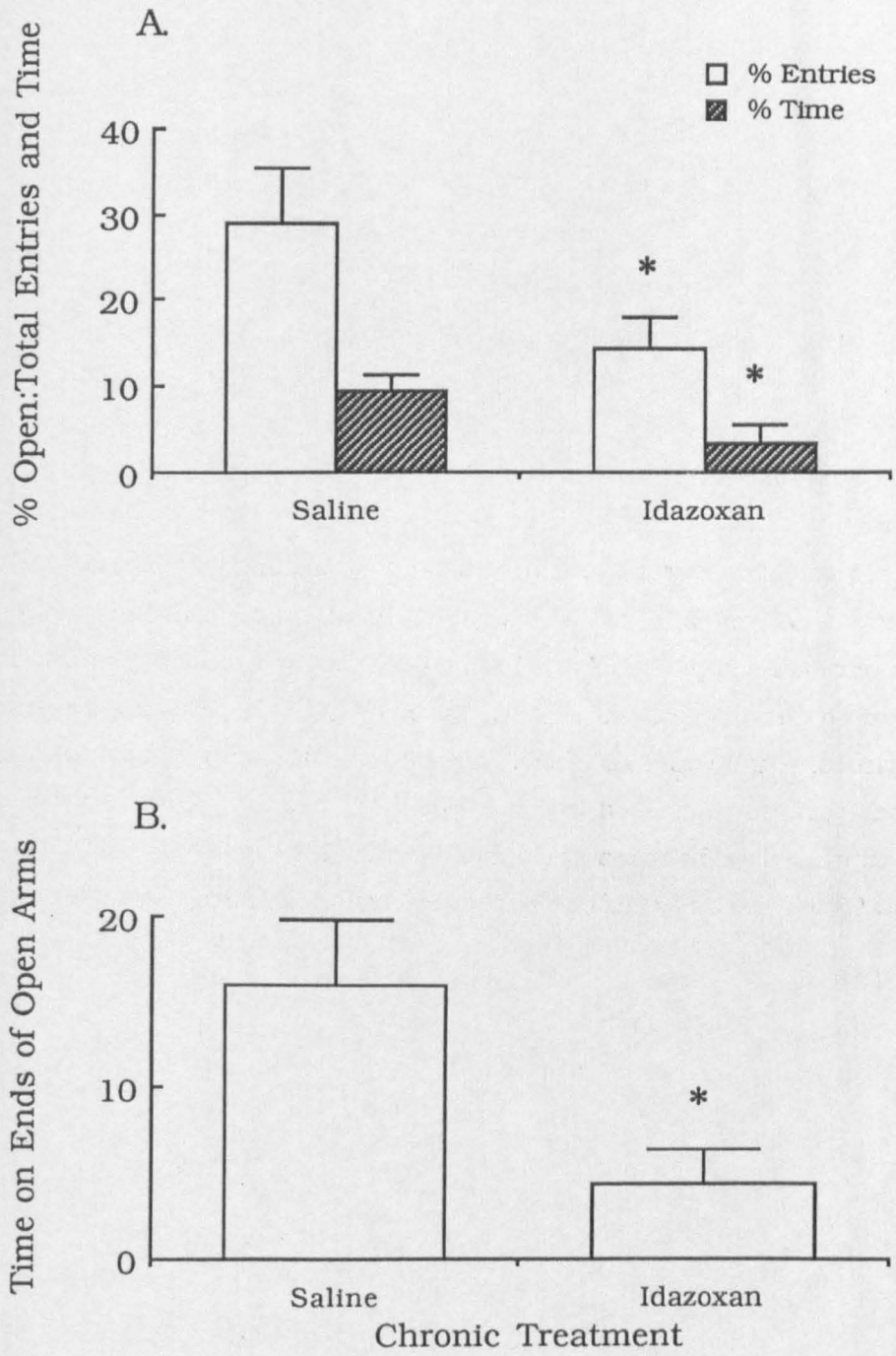

B.
Time on Ends of Open Arms (sec)
70
60
50
40
30
20
10
0
**
Veh.
Diaz.
Veh.
Diaz.
Veh.
Diaz.
Chronic Treatment
Withdrawal (24 hrs.)
Withdrawal (7days)

**Figure 6**

Effects of chronic treatment of vehicle control (0.9% saline, 5.5µl/hr) or idazoxan (0.8 mg/kg/hr at a flow rate of 5.5µl/hr) administered using Alzet minipumps for a period of 14 days, with behaviour monitored on the 15th day with the minipumps still in place, on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
% Entries
% Time
% Open:Total Entries and Time
40
30
20
10
0
*
*
Saline
Idazoxan
B.
Time on Ends of Open Arms
20
10
0
*
Saline
Idazoxan
Chronic Treatment

the total number of arm and end of arm entries (Table 1). Chronic treatment with ipsapirone at all of the doses studied had no effect on any of the behavioural parameters measured (Fig. 7). Chronic ritanserin (0.25 mg/kg) significantly increased the percentage open:total entries and time, and time on the ends of the open arms (Fig. 8A and 8B) but had no effect on the total number of arm and end of arm entries compared to control animals (Table 1). Chronic ondansetron significantly increased the percentage open:total entries and time (Fig. 9A), and also increased the time spent in the ends of the open arms (Fig. 9B) but not to significance while having no effect on the number of arm and end of arm entries (Table 1). On the whole the increases in the indices used as measures of anxiety seen with chronic ritanserin and ondansetron treatments were not as great as those observed with chronic diazepam treatment.

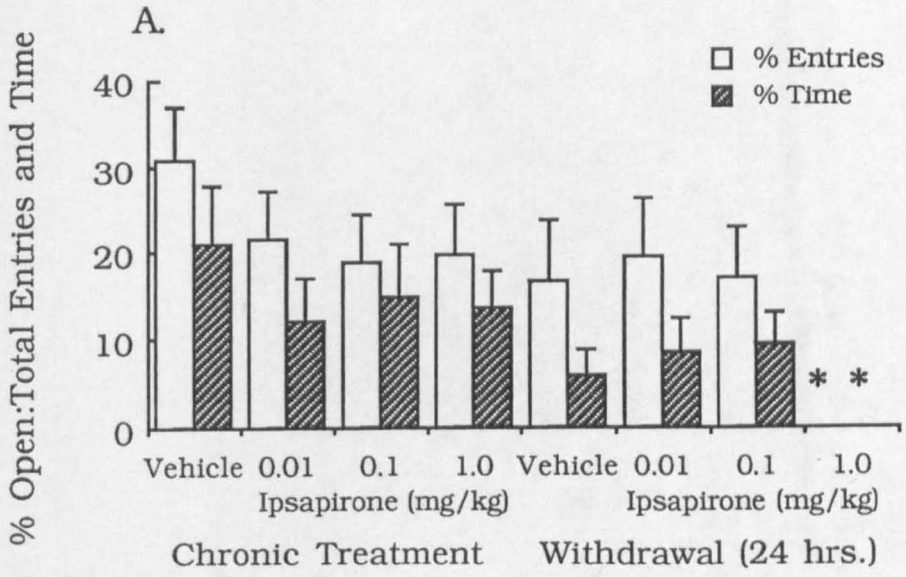
**(c) Withdrawal Studies**

24 hours or 7 days after completion of chronic diazepam treatment there were no significant differences in the behaviour of these rats compared to vehicle controls (Fig. 5). 24 hours after the last dose of ipsapirone, the rats that had received 0.01 and 0.1 mg/kg showed no behavioural difference from their controls (Fig. 7) but ipsapirone (1 mg/kg) reduced scores for all of the parameters measured except for the total number of arm and end of arm entries (Table 1). This anxiogenic profile observed with ipsapirone (1 mg/kg) was lost 7 days after cessation of chronic treatment when these animals displayed behaviour no different from the controls (data not shown). 24 hours after completion of chronic ritanserin treatment these animals still had significantly greater percentage open:total entries and time compared to the control group (Fig. 8A), but there was no difference in the total number of arm and end of arm entries between these two groups (Table 1). 7 days after cessation of chronic ritanserin treatment these rats displayed behaviour that was not significantly different from the control animals, although the percentage open:total entries was still somewhat greater than the control values (Fig 8A and 8B). 24 hours after cessation of chronic ondansetron treatment there was no difference in the behaviour of these animals compared to those of vehicle control (data not shown).

**Figure 7**

Effects of chronic treatment of ipsapirone (0.01, 0.1 and 1 mg/kg i.p.) twice daily for 14 days, with behaviour monitored 30 min after last injection on the 15th day, and effects after 24 hours withdrawal of chronic treatment on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
% Open:Total Entries and Time
% Entries
% Time
40
30
20
10
0
* *
Vehicle 0.01 0.1 1.0 Vehicle 0.01 0.1 1.0
Ipsapirone (mg/kg)
Ipsapirone (mg/kg)
Chronic Treatment
Withdrawal (24 hrs.)

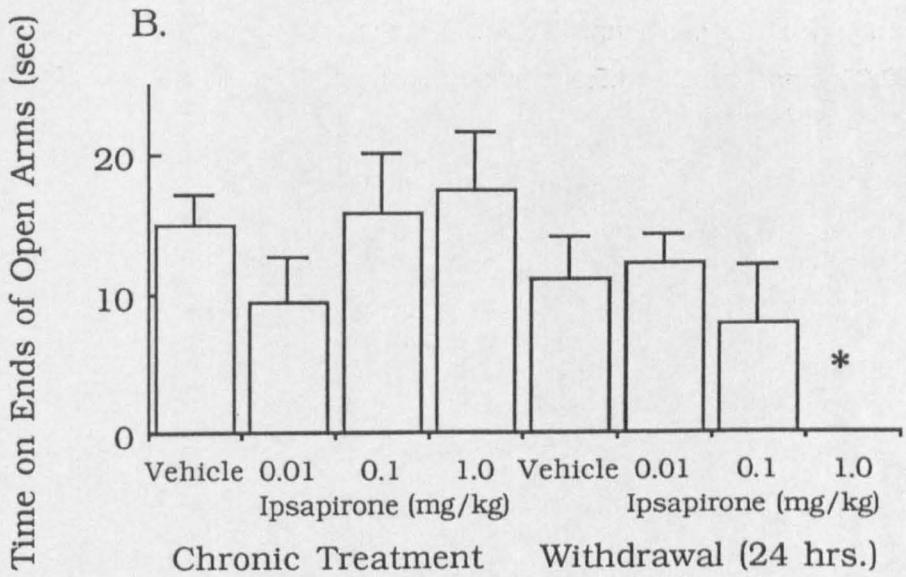
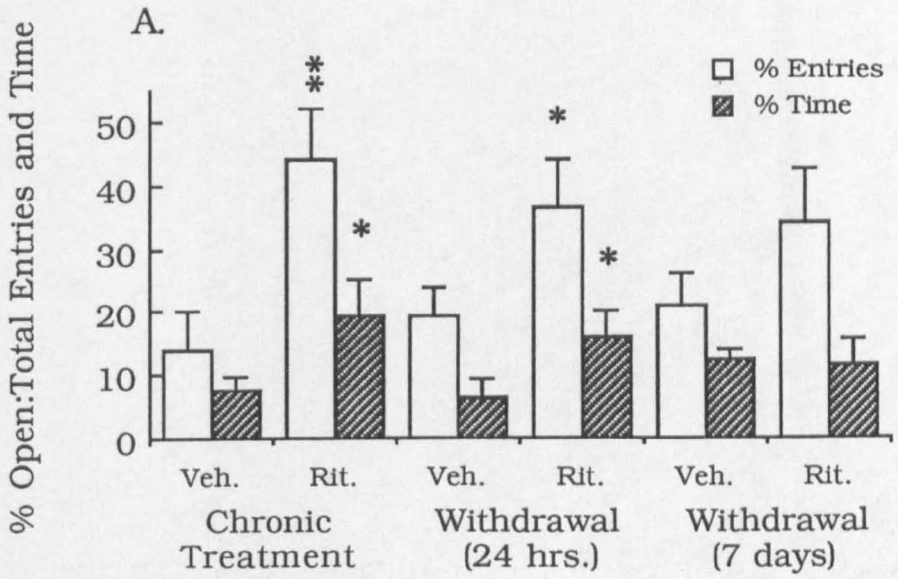

B.
Time on Ends of Open Arms (sec)
20
10
0
*
Vehicle 0.01 0.1 1.0 Vehicle 0.01 0.1 1.0
Ipsapirone (mg/kg)
Ipsapirone (mg/kg)
Chronic Treatment
Withdrawal (24 hrs.)

**Figure 8**

Effects of chronic treatment of ritanserin (Rit. 0.25 mg/kg i.p.) twice daily for 14 days, with behaviour monitored 30 min after last injection on the 15th day, and effects after 24 hours withdrawal and 7 days withdrawal of chronic treatment on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period. Data are expressed as the mean ± S.E.M. of 6 observations.

*$p<0.05$, **$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
% Open:Total Entries and Time
% Entries
% Time
50
40
30
20
10
0
Veh.
Rit.
Veh.
Rit.
Veh.
Rit.
Chronic Treatment
Withdrawal (24 hrs.)
Withdrawal (7 days)

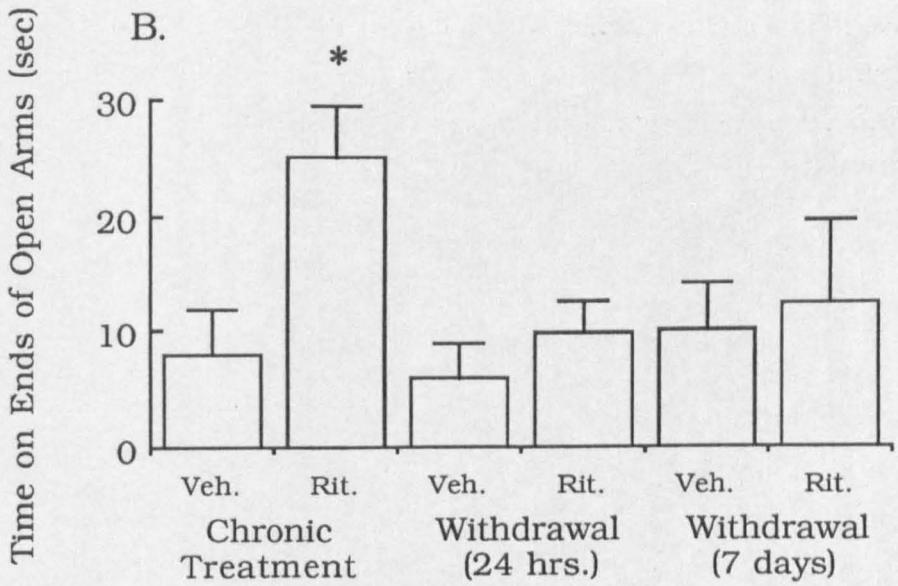

B.
Time on Ends of Open Arms (sec)
30
20
10
0
Veh.
Rit.
Veh.
Rit.
Veh.
Rit.
Chronic Treatment
Withdrawal (24 hrs.)
Withdrawal (7 days)

**Figure 9**

Effects of chronic treatment of vehicle control (1% methyl cellulose), twice daily for 14 days followed by a single injection of either vehicle (Vehicle) or ondansetron (0.01 mg/kg i.p.) (Acute Ondan.) on the test day (day 15), and ondansetron (0.01 mg/kg i.p.) twice daily for 14 days (Chronic Ondan.) with behaviour monitored 30 min after last injection on the 15th day on (A) the percentage open:total entries and time and (B) the time spent on the end of the open arms. Behaviour was monitored in the elevated X-maze for a 5 min period. Data are expressed as the mean ± S.E.M. of 8 observations.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.

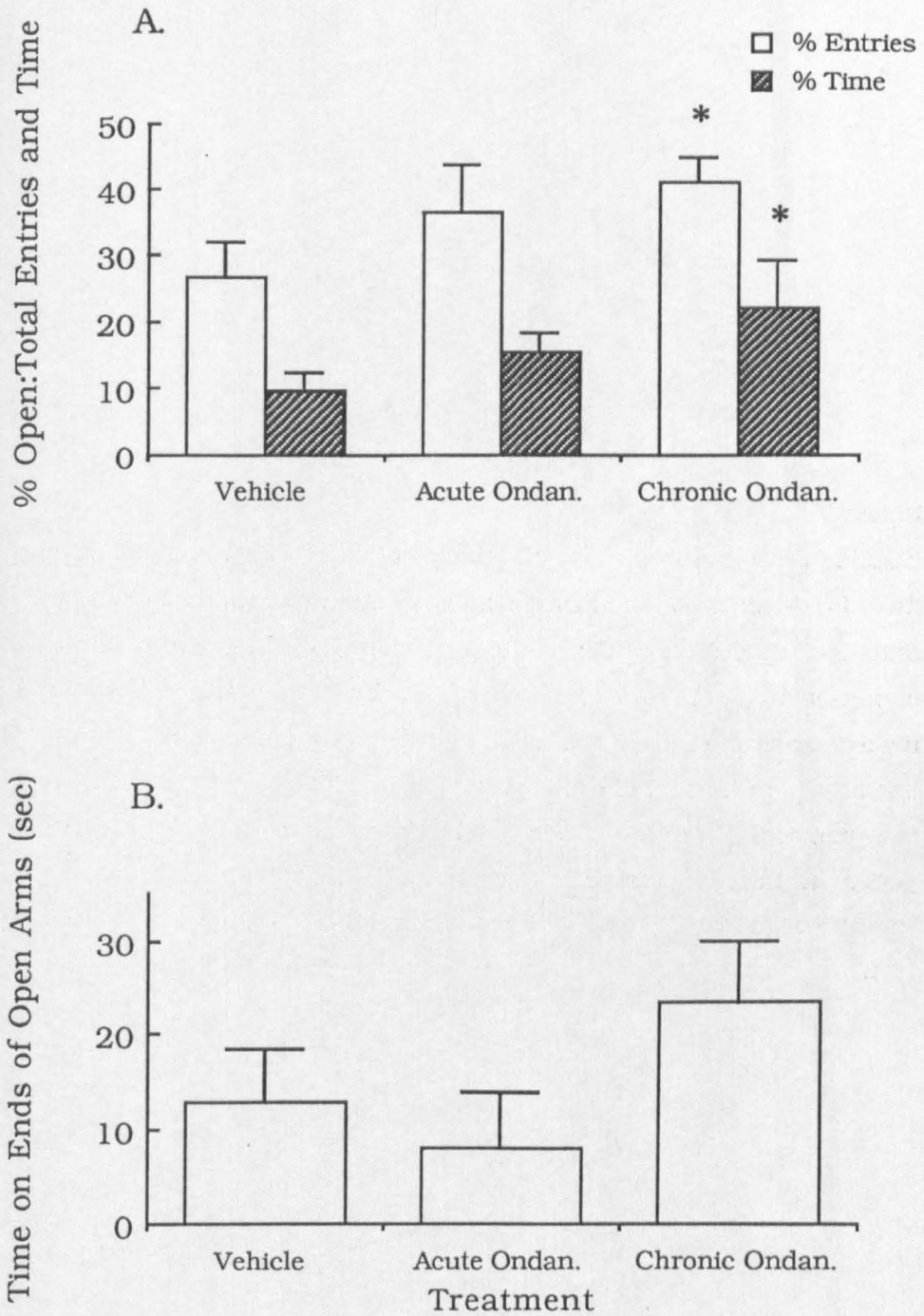

A.
% Entries
% Time
% Open:Total Entries and Time
50
40
30
20
10
0
*
*
Vehicle
Acute Ondan.
Chronic Ondan.
B.
Time on Ends of Open Arms (sec)
30
20
10
0
Vehicle
Acute Ondan.
Chronic Ondan.
Treatment

**Table 1**

Effect of diazepam (DZP, 5mg/kg), idazoxan (Idaz, 0.25mg/kg), ipsapirone (Ipsap, 0.01,0.1 and 1mg/kg), ritanserin (Rit 0.05 and 0.25mg/kg) and ondansetron (Ondan 0.01, 0.1 and 1mg/kg) after acute or chronic (twice daily for 14 days) treatment, or 24 hours or 7 days after cessation of chronic treatment on the total number of arm and end of arm entries during a 5 minute exposure to the X-maze. Results are expressed as the mean ± S.E.M. for 6-8 observations when animals were exposed on the X-maze for a 5 minute period 30 minutes after injection i.p.. The number of arm and end of arm entries decreases with increase in duration of the experimental protocol regardless of the treatment.

Key: n.t. = not tested.

There were no significant differences at the $p<0.05$ level. 2-tailed Mann-Whitney U-test, each treatment group was compared to its vehicle control.

| Treatment | No. of Arm and End of Arm Entries | | | |
|---|---|---|---|---|
| | Acute | Chronic | 24 hrs. after chronic | 7 days after chronic |
| Vehicle | 32.5 ± 2.1 | 24.5 ± 3.6 | 20.3 ± 3.1 | 18.7 ± 1.9 |
| DZP (5mg/kg) | 35.7 ± 3.0 | 28.8 ± 5.3 | 18.7 ± 1.6 | 20.9 ± 3.4 |
| Idaz (0.25mg/kg) | 29.3 ± 3.5 | n.t. | n.t. | n.t. |
| | | | | |
| Vehicle (0.9% saline) | n.t. | 18.0 ± 2.5 | n.t. | n.t. |
| Idaz (0.8mg/kg/hr) | n.t. | 16.0 ± 2.2 | n.t. | n.t. |
| | | | | |
| Vehicle | 30.6 ± 2.9 | 24.1 ± 2.7 | 17.4 ± 2.7 | 15.0 ± 1.8 |
| Ipsap (0.01mg/kg) | 27.3 ± 2.6 | 19.7 ± 4.9 | 16.0 ± 1.9 | n.t. |
| Ipsap (0.1mg/kg) | 25.2 ± 5.9 | 22.0 ± 3.2 | 17.7 ± 1.1 | n.t. |
| Ipsap (1mg/kg) | 28.0 ± 2.6 | 22.2 ± 2.4 | 14.2 ± 2.5 | 15.6 ±2.0 |
| | | | | |
| Vehicle | 32.5 ± 2.1 | 24.5 ± 3.6 | 20.3 ± 3.1 | 18.7 ± 1.9 |
| Rit (0.05mg/kg) | 26.0 ± 5.7 | n.t. | n.t. | n.t. |
| Rit (0.25mg/kg) | 26.2 ± 4.9 | 24.2 ± 4.7 | 19.8 ± 1.6 | 17.3 ± 3.7 |
| | | | | |
| Vehicle | 28.6 ± 1.8 | 23.7 ± 4.0 | 18.7 ± 1.7 | n.t. |
| Ondan (0.01mg/kg) | 29.8 ± 2.0 | 28.7 ± 3.3 | n.t. | n.t. |
| Ondan (0.1mg/kg) | 29.4 ± 3.8 | n.t. | n.t. | n.t. |
| Ondan (1mg/kg) | 27.5 ± 2.8 | n.t. | n.t. | n.t. |
| Chronic Vehicle + Acute Ondan (0.01mg/kg) | | 26.3 ± 5.3 | 19.0 ± 3.1 | n.t. |

## DISCUSSION

### (a) Acute Studies

It has been demonstrated previously by others (Pellow et al., 1985) and in this thesis (Chapter 2) that the elevated X-maze can detect the anxiolytic activity of benzodiazepine-like compounds. This was confirmed in the experiments described in this chapter showing that acute diazepam treatment significantly increased the percentage open:total entries and time and time on the ends of the open arms without changing the total number of arm and end of arm entries. Again, as described in Chapter 2 and by other authors (e.g. Handley and Mithani, 1984), the $\alpha_2$-adrenoceptor antagonist idazoxan reduced the percentage open:total entries and time with no significant reduction in the number of arm and end of arm entries, indicating that the reduced exploration of the open arms (indicating increased fear) was not a result of decreased locomotor activity. The proposed mechanisms of action of these two compounds has been discussed in Chapter 2.

While the lowest and highest doses of ipsapirone (0.01 and 1 mg/kg) had no effect on behaviour in the X-maze the middle dose (0.1 mg/kg) significantly decreased both the percentage open:total entries and time. Using the X-maze Soderpalm et al. (1989) have shown an anxiolytic profile for ipsapirone at a dose of 0.01 mg/kg s.c. and also for the other 5-$HT_{1A}$ partial agonists over a narrow dose range with similar concentrations to ipsapirone. In contrast, Pellow et al. (1987) showed that ipsapirone had an anxiogenic profile at doses of 2.5 - 10 mg/kg i.p., producing similar results to those found in this study. Looking at other animal models of anxiety, ipsapirone has been shown to antagonise passive avoidance behaviour with equipotent activity compared to diazepam (Traber et al., 1984), and in the potentiated startle paradigm (Davis, 1979a and b) ipsapirone has been found to be anxiolytic (Davis et al., 1988) but at doses which are at least 10 fold greater than those found to be anxiolytic by Soderpalm and co-workers (1989). The anxiolytic effects of ipsapirone and the other pyrimidinylpiperazine derivatives (e.g. buspirone and gepirone) are thought to be related to a supression of 5-HT neuronal activity in the dorsal raphe nucleus (Aghajanian et al., 1987; Glaser and Traber, 1987). However in the CNS, 5-$HT_{1A}$ receptors are found not only on the cell bodies/dendrites of 5-HT neurones in the raphe nuclei but also at postsynaptic sites such as the limbic terminal regions (Hoyer et al. 1986a). 5-$HT_{1A}$ partial agonists have been shown to reduce 5-HT

synthesis, turnover and release in limbic regions, presumably via a preferential stimulation of 5-$HT_{1A}$ autoreceptors (Dourish et al., 1986; Traber and Glaser, 1987; Broadhurst and Briley, 1988; Sharp et al., 1989c). However, stimulation of postsynaptic 5-$HT_{1A}$ receptors in the limbic regions would tend to increase 5-HT neuronal activity and may produce an anxiogenic-like profile under certain behavioural conditions. It is possible that the balance of receptor stimulation of these two sites could vary depending on the dose of ipsapirone, with low doses acting predominantly at pre-synaptic sites (probably the somatodenritic autoreceptor) and higher doses acting at a mixture of pre- and postsynaptic sites. From the results of this study two possible explanations for the effects of ipsapirone are that either ipsapirone at 0.1 mg/kg i.p. is a high enough dose to cause postsynaptic receptor stimulation and produce an anxiogenic profile on the X-maze (with the reason for no activity with the 1 mg/kg dose being unknown), or stimulation of the presynaptic receptors at the 0.1 mg/kg dose produces an anxiogenic profile which is blocked by increasing the dose to 1 mg/kg and stimulating both pre- and postsynaptic 5-$HT_{1A}$ sites. Overall, the effects seen with ipsapirone do not fit well with those in the literature. A further complication is that 1-pyrimidinylpiperazine (1-PP), the main metabolite of ipsapirone, is a potent $\alpha_2$-adrenoceptor antagonist (Bianchi et al., 1988) which, from the results of the idazoxan experiment, suggesting that this would produce anxiogenic profile. However, it has been reported that 1-PP does not produce an anxiogenic profile on the X-maze (Moser, 1989b).

Acute treatment with ritanserin (0.05 mg/kg) had no effect (either anxiolytic or anxiogenic) on the animals performance on the X-maze. One possible explanation for the difference between these results and the anxiolytic profile with acute ritanserin observed by Critchley and Handley (1987) at doses similar to the one used in this study is that they monitored the animals on the maze for a 10 min period. The higher dose of ritanserin (0.25 mg/kg) produced a non-significant anxiogenic profile, supporting the observations by File et al. (1987). This observation is somewhat unexpected as administration of a 5-$HT_2$ selective agonist such as 1-(2, 5-dimethoxy-4-iodophenyl) -2-aminopropane (DOI) is also seen to produce an anxiogenic profile in the X-maze (unpublished results) and social interaction models (Kennett et al., 1989). Clinically, the 5-$HT_{1C/2}$ agonist mCPP has been shown to induce panic attacks in both healthy subjects and in patients with panic disorder (Charney et al., 1987). It suggests that ritanserin after acute

administration may be acting at a different 5-$HT_{1C/2}$ site compared with that seen with the selective agonists.

Ondansetron given acutely (0.01, 0.1 or 1 mg/kg) did not produce behaviour different from vehicle controls in the X-maze. The results reported in the literature with this 5-$HT_3$ antagonist vary greatly with the particular animal model of anxiety. Jones et al. (1988) found that ondansetron produced an anxiolytic-like profile in a social interaction test in rats and monkeys but not in the rat water-lick conflict test. However, a more recent study (File and Johnston, 1989) found that acute administration of ondansetron at 0.1 and 1 mg/kg p.o. and two other 5-$HT_3$ antagonists (BRL 43694 (0.1 and 1 mg/kg p.o.) and zacopride (0.01, 0.1 and 1 mg/kg p.o.)) had no significant effects on social interaction. Ondansetron and BRL 46470A, another 5-$HT_3$ antagonist, have been shown to have an anxiolytic profile in the X-maze when the animals are monitored for a 10 minute period (Costall et al., 1989a; Upton and Blackburn, 1991). Thus, increasing the time the animal is on the X-maze may produce an anxiolytic profile.

**(b) Chronic and Withdrawal Studies**

Chronic treatment with the vehicle control (1% methyl cellulose) resulted in a slight fall in exploratory behaviour, observed as reduced percentage open:total entries and time. It cannot be determined from this experiment whether this was a result of the methyl cellulose or the effects of handling and injecting the animals twice daily. It is interesting to note that infusion of 0.9% w/v saline via an osmotic mini-pump also appeared to produce a reduction in the number of arm and end of arm entries even though these animals were not handled on a daily basis. Chronic diazepam treatment (5 mg/kg twice daily for 14 days) produced an anxiolytic profile, the percentage open:total entries and time being similar to those observed in the acute study, indicating no tolerance to the anxiolytic effects of diazepam. This may be due to the selected dose of diazepam not being large enough to induce tolerance using the X-maze, or that the dose of diazepam produced a supramaximal effect on the X-maze and hence even if some tolerance developed it was still possible to observe a maximal effect on the X-maze. 24 hours after the last dose of diazepam no anxiolytic activity was observed and behaviour did not differ significantly from controls. However, using a different design of elevated X-maze File and Baldwin (1989) have been able to detect anxiogenic withdrawal effects using chlordiazepoxide at doses of 5 and

20 mg/kg i.p.. It may be necessary to use higher (i.e. 10 or 20 mg/kg) doses of diazepam to cause significant withdrawal behaviour as indicated by previous studies (Costall et al., 1990a). From the present results it would appear possible in an animal test to produce clear anxiolytic effects with diazepam without obvious concomittant withdrawal effects, however, increasing the baseline values of the vehicle controls may produce a greater difference between control and diazepam treated animals allowing withdrawal to be observed.

Chronic administration of idazoxan by osmotic mini-pumps still resulted in an anxiogenic profile, with the reduction in percentage open:total entries and time being more marked than with acute treatment. Electrophysiological studies have shown that chronic administration of idazoxan elevated the basal 5-HT neuronal firing rate in the dorsal raphe nucleus and it remained above the control basal level 24 hours after cessation of treatment (Garratt et al., 1991). The elevated basal firing rate could be further increased with an acute dose of idazoxan; the percentage increase obtained being equivalent to that observed in animals given the same dose acutely, indicating that tolerance to idazoxan does not develop after 14 days (Garratt et al., 1991). The lack difference between acute and chronic idazoxan may be a result of no tolerance or because of the different doses used in the two studies.

Chronic ipsapirone at all doses showed no significant effect in the X-maze. Why these results are different from the acute study in which 0.1 mg/kg ipsapirone produced anxiogenic effects is not clear; it is possible that chronic administration of ipsapirone alters somatodendritic and/or postsynaptic 5-$HT_{1A}$ receptor sensitivity or number. Chronic treatment with ipsapirone has been shown to have no effect on the 5-$HT_{1A}$ stimulated inhibition of adenylyl cyclase activity in the hippocampus (Varrault et al., 1991), suggesting that there is no down-regulation of this receptor in this region. In an autoradiographic study using another 5-$HT_{1A}$ partial agonist, buspirone, it has been found that after chronic treatment there was a down regulation of 5-$HT_{1A}$ receptors in the dorsal raphe with no changes observed in the hippocampal postsynaptic 5-$HT_{1A}$ receptors (Mennini and Gobbi, 1990; Gobbi et al., 1991). Furthermore, chronic administration of buspirone (1 mg/kg i.p. twice daily for 14 days), produced no significant effects on behaviour in the X-maze (Moser, 1989b) although the drug has activity in the clinic (Goldberg and Finnerty, 1979; Goa and Ward, 1984). The present

behavioural study supports previous work suggesting that the X-maze cannot reliably detect anxiolytic activity with 5-$HT_{1A}$ partial agonists after acute or chronic treatment. The effects of 24 hours withdrawal of ipsapirone were notable as animals withdrawn from 0.01 and 0.1 mg/kg ipsapirone were not significantly different from controls but those withdrawn from 1 mg/kg displayed an absence of activity on the open arms (which reached significance). Re-testing after 7 days showed these animals to be the same as controls. This result could be due to possible changes in 5-$HT_{1A}$ receptor function after chronic treatment or to some unexplained withdrawal effect of that specific dose.

The anxiolytic behavioural profile displayed by the animals receiving chronic ritanserin indicate that the elevated X-maze can detect putative anxiolytics from chemical classes other than the benzodiazepines. The increase in the indices used as a measure of anxiety were not as great for chronic ritanserin as those seen with either acute or chronic diazepam, although it is possible that different doses and regime with ritanserin may improve the profile. 24 hours after ritanserin administration the anxiolytic profile was still observed. It cannot be determined from the present experiment whether this observation is a result of 5-$HT_2$ receptor down-regulation (Leysen et al., 1986) or relates to the pharmacokinetics of ritanserin which has a long half-life and receptor occupancy time (Leysen et al., 1985), so the drug may be present in the body 24 hours after completion of chronic treatment. All detectable anxiolytic activity was lost 7 days after completion of chronic ritanserin treatment, a time when the drug would have cleared from the body. Unfortunately, due to the very nature of the elevated X-maze measurement of anxiety, retesting animals everyday is impossible as the maze is no longer a novel enviroment; this results in reduced exploration and sensitivity to benzodiazepines (File, 1990). Hence, in this study, it was not possible to determine when, between days 1 and 7 post-chronic dosing, the anxiolytic activity was lost.

Chronic ondansetron treatment produced an anxiolytic profile with significant increases in percentage open:total entries and time and a non-significant increase in the time spent on the open arms. This effect is not a result of the handling or the chronic treatment procedure as chronic vehicle followed by acute ondansetron (0.01 mg/kg) on the day of testing did not produce a significant anxiolytic profile (the profile produced was very

similar to that of acute ondansetron at this dose). The reason for the anxiolytic profile being observed with either ritanserin or ondansetron only after chronic treatment is not certain, especially as some (Critchley and Handley, 1987; Jones et al. 1988) but not all (File et al., 1987) studies show both compounds producing anxiolytic profiles on acute treatment. Though there is still some controversy over the fact that 5-$HT_3$ antagonists are capable of producing anxiolytic profiles on the elevated X-maze (File and Johnston, 1989) there is increasing evidence suggesting that stimulation of central 5-$HT_3$ receptors modifies the functioning of specific neurotransmitter systems (Barnes et al., 1989b; Hagan et al., 1987). The anxiolytic profile of ondansetron, though less pronounced than that of diazepam, is lost after 24 hours withdrawal, indicating that ondansetron may not cause withdrawal effects (Costall et al., 1990a). There is evidence to suggest that ondansetron reduces the anxiogenic withdrawal effects observed with chronic benzodiazepine treatment (Costall et al., 1990a), However, it has very little or no effect on other symptoms of withdrawal such as weight loss and reduced food intake (Leathley et al., 1990).

The results of these experiments demonstrate that the elevated X-maze can be used to detect anxiolytic activity in non-benzodiazepine drugs. The 5-$HT_2$ and 5-$HT_3$ antagonists, ritanserin and ondansetron respectively, showed anxiolytic profiles but only after chronic treatment. Thus, for ritanserin, effects in the X-maze correlate with effects observed in the clinic (Ceulemans et al., 1985). At present preliminary clinical trial data has been published but the necessary statistical analysis on this data has not been carried out to establish how the current findings with ondansetron in the X-maze relate to those obtained in humans (Abuzzahab, 1991). Further studies are required to determine whether there is any causal relationship between ritanserin induced down-regulation of 5-$HT_2$ receptors (Leysen et al., 1986) and the anxiolytic profile observed in the present study. In contrast, the X-maze failed to detect anxiolytic activity with the 5-$HT_{1A}$ partial agonist ipsapirone after either acute or chronic treatment, and greater range of doses may be necessary to observe some activity. Ipsapirone displays a different clinical profile compared to ritanserin and ondansetron and it is possible that the X-maze is unable to easily detect compounds with 5-$HT_{1A}$ receptor partial agonist activity.

# CHAPTER 4

# CHARACTERISATION OF 5-HT RELEASE MEASURED *IN VIVO* BY INTRACEREBRAL MICRODIALYSIS

## INTRODUCTION

The technique of intracerebral microdialysis was originally used to study the release of dopamine (Ungerstedt et al., 1982; Hernandez et al., 1983; Imperato and Di Chiara, 1984; Ungerstedt, 1984). 5-HT has only been measured more recently as the levels are lower than those of dopamine and therefore more difficult to detect (Sharp et al., 1984; Marsden, 1985; Marsden et al., 1986).

There is some debate in the literature about the length of time after implantation of a dialysis probe at which neuronal 5-HT release can be measured. Kalen and co-workers (1988) suggested that neuronal release cannot be measured until a period of at least 24 hours has elapsed after implantation. Also Westerink et al. (1987a) implied that any 5-HT measured by dialysis may originate from non-neuronal sources, i.e. platelets, glia or mast cells (Sneddon, 1973; Austen, 1978; Kimelberg, 1986). However, much evidence has accumulated which suggests that 5-HT measured after 24 hours in freely moving animals or that measured 2 hours after implantation in anaesthetised animals is of neuronal origin. Both types of protocol are used in this thesis but in this chapter only experiments in the freely-moving animal are studied. Inclusion of tetrodotoxin (TTX) in the perfusate to block sodium channels and thus inhibit action potential conduction (Hille, 1968), reduced hippocampal 5-HT release in anaesthetised (Daszuta et al., 1989; Sharp et al., 1989c) and cortical and hypothalamic release in conscious animals (Carboni and Di Chiara, 1989; Auerbach et al., 1989) measured using microdialysis. Perfusion with $Ca^{2+}$-free CSF helps demonstrate the origin of the transmitter as neuronal release is $Ca^{2+}$-dependent (Augustine et al., 1987) whereas platelet release is not (Pannocchia et al., 1987). The $Ca^{2+}$-dependency of 5-HT release has been shown in conscious rats in various brain regions such as the cortex (Carboni and Di Chiara, 1989), hippocampus (Pei et al., 1989), caudate-putamen (Kalen et al., 1988) and hypothalamus (Auerbach et al., 1989). In anaesthetised rats electrical stimulation of the dorsal raphe nucleus has been shown to increase hippocampal 5-HT release (Sharp et al., 1989c) indicating that the 5-HT release is of neuronal origin. Drugs such as fenfluramine and p-chloramphetamine, which release 5-HT, increase extracellular 5-HT in the cortex of anaesthetised (Sharp et al., 1986; Crespi et al., 1990) and conscious rats (Schwartz et al., 1989), and the effects of fenfluramine were attenuated in animals lesioned with the selective

serotonergic neurotoxin 5,7-DHT (Crespi et al., 1990). However, these drugs also release 5-HT from platelets (Sneddon, 1973; Buckzo et al., 1975). Using compounds such as RU24969, which is thought to act at the 5-$HT_{1B}$ terminal autoreceptor (Middlemiss, 1985), inhibition of 5-HT release has been observed in anaesthetised rats (Brazell et al., 1985; Sharp et al., 1989a). The selective 5-$HT_{1A}$ agonist 8-OH-DPAT (Middlemiss and Fozard, 1983) also inhibited 5-HT release in anaesthetised animals after either systemic administration (Routledge and Marsden, 1985; Sharp et al., 1989a, b) or local infusion into the dorsal raphe nucleus (Hutson et al., 1989; Sharp et al., 1989c). In conscious rats systemic 8-OH-DPAT has been found to inhibit hypothalamic (Auerbach et al., 1989) and cortical 5-HT release (Carboni and Di Chiara, 1989).

This study was designed to show that the 5-HT release measured in animals implanted with a dialysis probe in the ventral hippocampus, and allowed 24 hours to recover, is of neuronal origin. This was examined using perfusion with artificial CSF containing TTX or perfusion with $Ca^{2+}$-free CSF. Also, basal dialysis samples had exogenous 5-HT and 5-HIAA of a known concentration added to them and were then assayed via high-performance liquid chromatography with electrochemical detection (HPLC-ECD) to determine whether the endogenous and exogenous 5-HT and 5-HIAA elute off the column at the same time and oxidise at the same potential.

## METHODS

### Preparation of Dialysis Probes

Dialysis tubing (Hospal, U.K. Ltd. 300μm o.d., 220μm i.d.), made of acrylonitrile-sodium methallyl sulphonate, with a molecular cut-off point of 20,000 Daltons, was obtained from a renal dialysis pack. In the series of experiments later in this chapter which look at recovery, methylcellulose tubing (Gambro, 250μm o.d.), molecular weight cut off of 6,000 Daltons was obtained from a similar renal dialysis pack. The dialysis probes were manufactured in a similar manner regardless of the type of dialysis membrane used. A piece of tungsten wire was threaded into the lumen of a length of dialysis tubing (~120mm) to act as support during the initial preparation of the probe. The dialysis tubing was then inserted (~5mm) into a steel cannula (23 gauge) and secured with epoxy resin. The tungsten wire was then removed, the dialysis tubing cut (~8mm in length), and the epoxy

resin allowed to dry for several hours. A 20mm length of portex-tubing (1.02mm o.d., 0.58mm i.d.) was pushed over the other end (i.e. the end without the dialysis tubing) of the steel cannula and this was secured in place with cyanoacrylic adhesive. A small hole was then made in the portex-tubing using a 25G needle. Fine fused silica-glass capillary tubing (Scientific Glass Engineering) was then inserted inside the dialysis membrane and pushed through the steel cannula and out through the hole (made with the 25G needle) in the portex-tubing. The end of the dialysis tubing was sealed with epoxy-resin and left to dry for 2 hrs. Finally, the hole in the portex tubing, through which the silica-glass tubing emerges was sealed with epoxy resin. The dialysis probes were then stored until used. The dialysis probe is shown diagrammatically in Fig. 1.

**Measurement of Relative Recovery *in vitro***

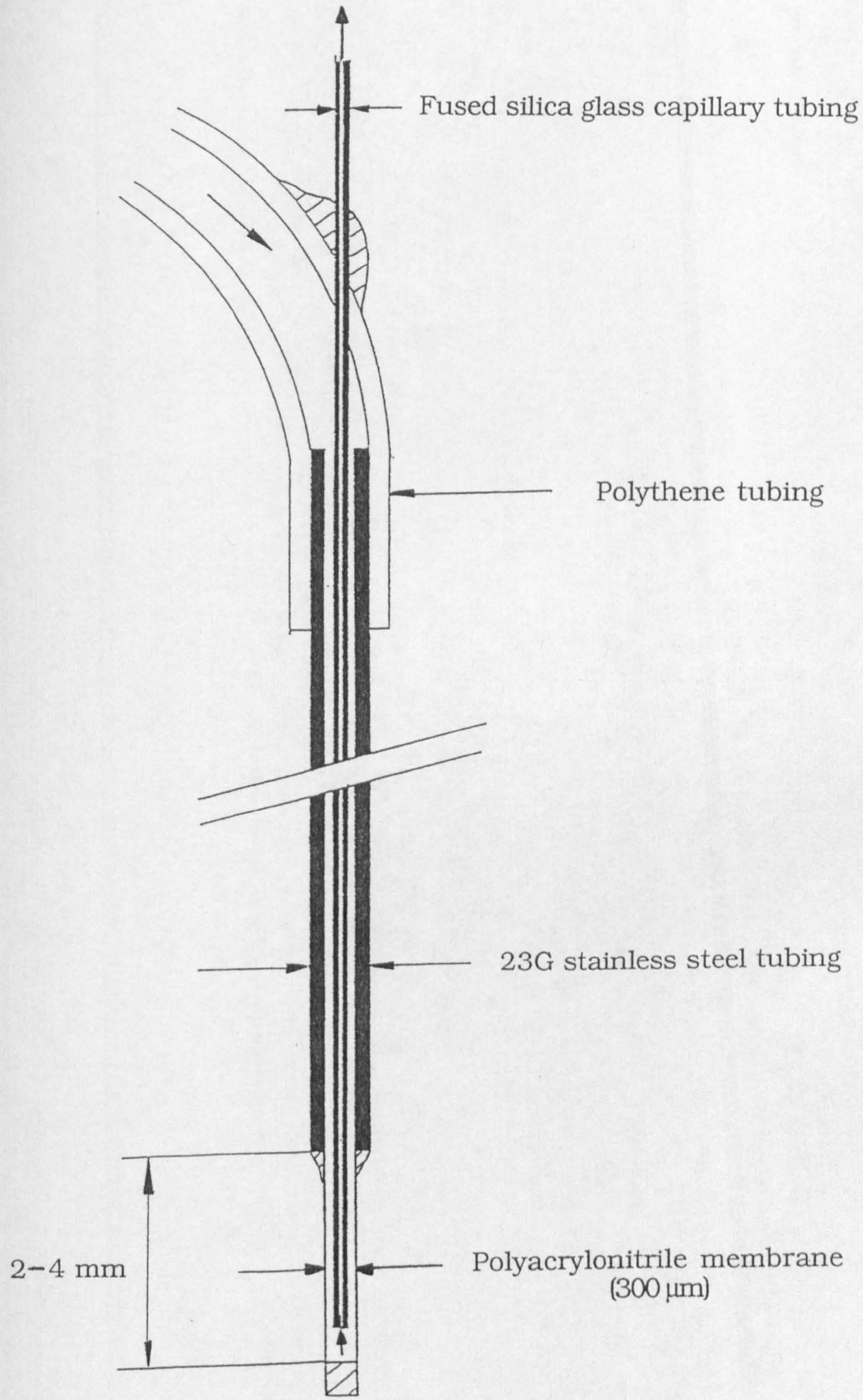
Dialysis probes were perfused at a rate of 1µl/min (Scientific and Research Instruments Ltd., slow infusion pump Ref. 5200) and suspended in a solution containing 2.5 x $10^{-7}$M 5-HT and 5-HIAA in artificial CSF (Composition: 5mM glucose, 125mM NaCl, 2.5mM KCl, 0.5mM $NaH_2PO_4.2H_2O$, 1.2mM $Na_2HPO_4$, 27mM $NaHCO_3$, 0.5mM $Na_2SO_4$ (anhydrous), 1mM $MgCl_2.6H_2O$ and 1mM $CaCl_2.2H_2O$, pH adjusted to 7.4 with 1M o-phosphoric acid). The probes were left to equilibrate for 30 minutes and then three 20 minute samples were collected. The samples were assayed directly using HPLC-ECD to measure 5-HT and 5-HIAA. Comparison with the 5-HT and 5-HIAA standards enabled calculation of the concentration of 5-HT and 5-HIAA in the perfusate and by dividing this with the concentration in the bulk solution enabled the relative recovery to be determined. The relative recoveries of six probes were calculated for each type of probe.

**Analysis of Dialysis Perfusates**

HPLC-ECD was used to analyse the levels of 5-HT and 5-HIAA in all experiments. Separation of the two indoles was by ion-pair, reverse phase chromatography on a 10cm column (Hichrom, internal diameter 2mm) packed with 3µm Shandon Hypersil (Technicol). The mobile phase was made up in double-distilled deionised water further purified in an ELGA purifier and contained 0.15M $NaH_2PO_4.2H_2O$, 0.1mM diaminoethanetetra-acetic acid (EDTA), 0.5mM sodium octyl suphate and 15% methanol with the pH adjusted to 3.8 with o-phosphoric acid. Before use it was filtered through a 0.45µm filter (Millipore) and degassed by sonication for 30 minutes. It was

**Figure 1**

Diagrammatic representation of the structure of the intracerebral microdialysis probe used in this thesis.


Fused silica glass capillary tubing
Polythene tubing
23G stainless steel tubing
2–4 mm
Polyacrylonitrile membrane
(300 µm)

pumped through the column at a flow rate of 0.3ml/min using an HPLC Technology Pump (RR 1066L). A Rheodyne injection unit (7125) fitted with a 20µl injection loop was used to inject samples onto the column. The glassy carbon dual electrode (MF 1000, Bioanalytical Systems) was held at a potential of +0.65V versus an Ag/AgCl reference electrode and the currents produced by the oxidation of 5-HT and 5-HIAA were detected using a Bioanalytical Systems LC-4 electrochemical detector set at 2nA/V and recorded on a SP4290 integrator (Spectra-Physics). Standard curves were constructed for 5-HT and 5-HIAA in a combined solution over the range of 0.025 - 1.0 pmoles/20µl and the peak heights were measured. In all experiments the dialysis samples were injected using the technique of partial loop filling to avoid injecting air into the system. This was done by filling the loop with double-distilled deionised water just prior to injection of the dialysis sample. The resulting peak heights were compared to injections of standards containing 0.5pmoles/20µl of 5-HT and 5-HIAA. For the injection of dialysis samples, the sensitivity of the system had to be increased four-fold to detect 5-HT after the 5-HIAA peak had been eluted as there is a lower level of extracellular 5-HT compared with 5-HIAA.

**Implantation of Dialysis Probes**

Naive male Lister hooded rats (240-300g) (Nottingham University Medical School Animal Unit) were housed in groups of four on a 12 hour light/dark cycle (lights on 0700-1900), with food and water available *ad libitum*. Rats were anaesthetised with 1.5% halothane in a 50:50 $O_2/N_2O$ gas mixture using a Boyle's apparatus, placed in a stereotaxic frame with the mouth piece set at -3.3mm. The body temperature of the animals was maintained using a lamp and cotton wool. Each animal's head was shaved and an incision made using a scalpel blade from behind the eyes to between the ears. The skin was held back with artery clips and the surface of the skull scraped to remove the connective tissue. Three holes were drilled using a hand-held drill with a twist drill bit, two for skull screws to help secure the cement and one for the probe which was placed above the ventral hippocampus using co-ordinates derived from Paxinos and Watson (1986) (from bregma: anterior/posterior -5.6mm, lateral ±4.6mm, ventral -7.5mm from the surface of the brain). The dura was broken using a needle tip. This was necessary as the dialysis probe, which is pliable at the tip, would bend upon contact with the tough dura. The screws were inserted, followed by stereotaxic implantation of the probe into the ventral hippocampus which was then secured in place with dental

cement. Care was taken to ensure that the drill hole for the loop was not bleeding before implantation as otherwise the samples could be contaminated with 5-HT from the blood. Once the dental cement had hardened, the artery clips were removed and the incision was sutured. The incision was then sprayed with an anti-bacterial (Polybactrin) and a plastic wound dressing (Nobecutane). The animal was then placed in a cage on its own with food and water available *ad libitum* and allowed to recover.

24 hours after implantation fine Portex tubing (approximately 25cm in length) (o.d. 0.75mm, i.d. 0.25mm) was placed over the capillary outlet and secured in place with cyanoacrylic adhesive. The inlet was connected via a small steel cannula to a long piece of Portex tubing connected to a 5ml syringe containing artificial CSF mounted in a slow infusion pump (set at 1µl/min). The inlet and outlet were held together by means of small pieces of masking tape. After the probe had been flowing for 30 minutes, samples of the perfusate were collected every 20 minutes in the cut-off tip of an Eppendorf tube containing 5µl of 0.1M perchloric acid to prevent oxidation of the 5-HT and 5-HIAA and analysed by HPLC-ECD. The cut-off tip of the Eppendorf was held in place at the end of the outlet tubing with a small piece of masking tape. At the end of the experiment the rats were killed and the position of the probe in the ventral hippocampus verified by eye in the sectioned brain.

**Local Administration of TTX and Zero $Ca^{2+}$ in the Perfusate**

After 30 minutes of flow and 80 minutes of basal release collection, the perfusion medium was changed from normal CSF to CSF containing either TTX (1µM) or zero $Ca^{2+}$ (plus 0.1mM ethylene glycol-bis (ß-aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA) and increasing the $Na^{+}$ by 1mM in the $Ca^{2+}$-free solution) using a liquid switch (Carnegie Medicine, CMA/110). After 60 minutes the perfusion medium was changed back to normal CSF and samples collected for a further 2 hours. In these experiments the inlet tubing was identical to the fine outlet tubing so that the dead-space between the liquid switch and the probe was as small as possible.

**Addition of Exogenous 5-HT and 5-HIAA to Dialysis Samples**

After 80 minutes of basal release collection in a separate group of animals 0.1pmoles/20µl of both 5-HT and 5-HIAA were added to the samples and they were then analysed by HPLC-ECD.

**Drugs**

5-Hydroxytryptamine creatinine sulphate (5-HT, Sigma) and 5-hydroxyindole-3-acetic acid (5-HIAA, Sigma) were dissolved in double-distilled deionised water and diluted in artificial CSF. Tetrodotoxin (TTX, Sigma) was dissolved in glacial acetic acid and diluted in artificial CSF. All necessary precautions were taken when using TTX (laboratory coat, gloves and mask) to avoid inhalation or skin exposure as the compound is fatal in low doses. Formaldehyde (Vickers Laboratories Ltd.) was diluted in 0.9% saline.

**Data Analysis**

All the results for the experiments were expressed as the mean ± S.E.M.. After administration of vehicle or drug the values were expressed as the percentage change from the preinjection values, calculated separately for each animal. The groups given zero $Ca^{2+}$ or TTX were analysed separately using a one-way ANOVA with repeated measures followed by a Dunnett's t-test to identify any significant changes from the control group.

**RESULTS**

**Calibration of the HPLC**

Fig. 2 shows the standard curves obtained after injection of increasing concentrations of 5-HT and 5-HIAA in a combined solution onto the HPLC. A linear relationship can be seen between the concentration of the standard and the peak height. The minimum detectable amount of 5-HT and 5-HIAA in a standard, taken as 3 times the basal noise was found to be 20fmoles/20µl. From the standard curve a concentration of 0.5pmoles/20µl was chosen to be used as the standard concentration in all future experiments.

**Measurement of Relative Recovery *in vitro***

In Table 1 the relative recoveries for 5-HT and 5-HIAA are shown for dialysis probes made of either Hospal tubing or methylcellulose. It can be seen that the recovery for both indoles is higher in probes made from the Hospal membrane; 5-HT:- 15.1% compared to 4.9% and 5-HIAA :- 12.9% compared to 3.7%.

**Figure 2**

Graph showing the linear relationship between peak heights for 5-HT (r = 0.983) and 5-HIAA (r = 0.996) and their concentrations measured by HPLC-ECD. Each point is the mean of three injections, and the line of best fit (plotted) calculated using linear regression. A standard concentration of 0.5pmoles/20µl was chosen from these curves for use in all future experiments.

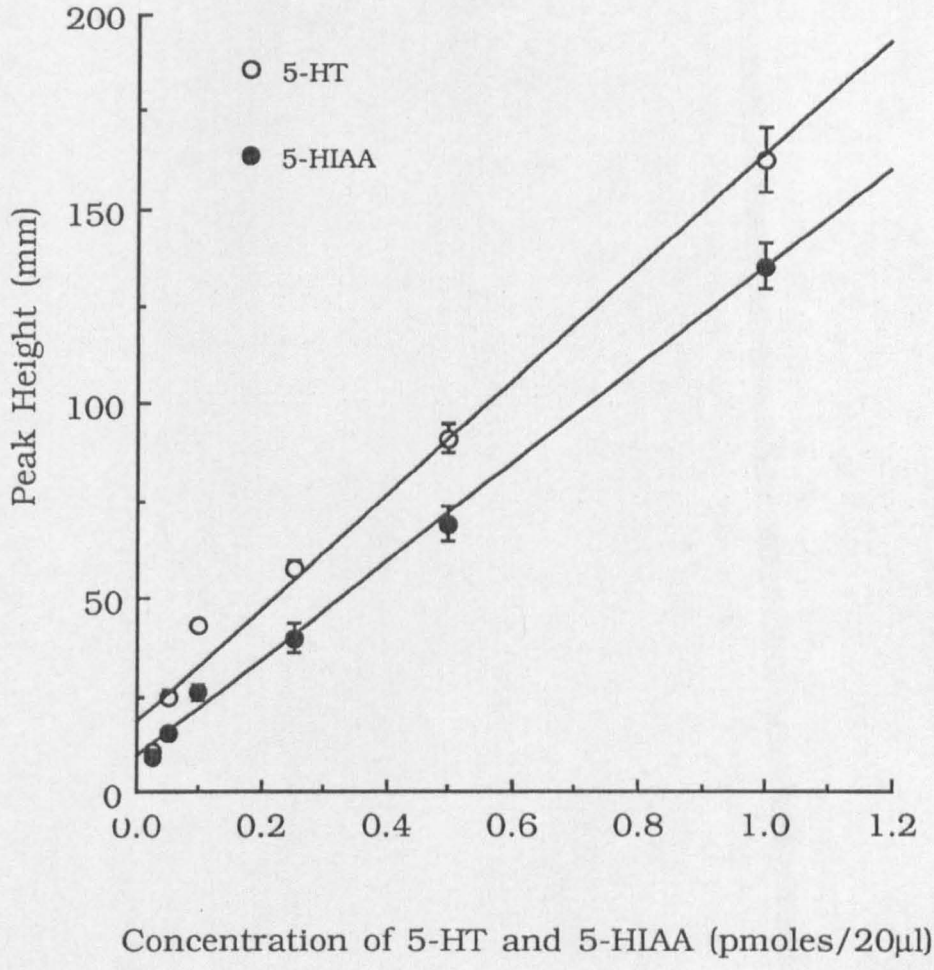

200
150
100
50
0
Peak Height (mm)
5-HT
5-HIAA
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Concentration of 5-HT and 5-HIAA (pmoles/20µl)

**Table 1**

Comparison of percentage relative recoveries for 5-HT and 5-HIAA measured *in vitro* using dialysis probes made of Hospal or methylcellulose tubing. The recoveries were determined by immersion of the probes in a solution containing $2.5 \times 10^{-7}$M 5-HT and 5-HIAA. After a 30 minute equilibration period three 20 minute samples were collected and assayed via HPLC-ECD. Comparison with the 5-HT and 5-HIAA standards enabled calculation of the concentration of 5-HT and 5-HIAA in the perfusate and by dividing this with the concentration in the bulk solution enabled the relative recovery to be determined. The relative recoveries of six probes were calculated for each type of probe.

| Membrane | Mean % Recovery ± S.E.M. | |
|---|---|---|
| | 5-HT | 5-HIAA |
| Hospal Tubing | 15.1 ± 1.9 | 12.9 ± 2.5 |
| Methylcellulose Tubing | 4.9 ± 0.7 | 3.7 ± 1.4 |

**Basal Release of 5-HT and 5-HIAA**

A diagram of a dialysis probe in place in the ventral hippocampus of a rat brain is shown in Fig. 3. The HPLC traces in Fig. 4 show typical results following injection of a standard solution containing 0.5pmoles/20µl 5-HT and 5-HIAA and the injection of a sample taken from the basal release of a control rat. The traces show clear separation of 5-HT and 5-HIAA in both cases and the difference between the levels of the two indoles in the rat brain.

**Local Administration of TTX in the Perfusate**

Perfusion with TTX (1µM) produced a dramatic decrease in the levels of 5-HT (43%) in the first sample after the TTX infusion started (Fig. 5). After this initial large drop the maximal fall in the 5-HT level was 83% occurring 80 minutes after commencing TTX perfusion. Once the perfusion medium was changed back to normal CSF the release of 5-HT did not recover during the following 2 hours (Fig. 5). During the TTX perfusion there was a small, significant decrease in the levels of 5-HIAA (15%), returning towards basal levels upon reperfusion with CSF (Fig. 5). There was a slight fall in the level of 5-HT when the liquid switch is turned and normal CSF perfused, this was probably a result of a fall in pressure on turning the switch leading to a smaller sample volume.

**Local Administration of $Ca^{2+}$-free Solution**

Perfusion with zero $Ca^{2+}$ produced a maximal fall in the 5-HT levels of 67% occurring 80 minutes after commencement (Fig. 6). Once the perfusion medium was changed back to normal CSF the release of 5-HT began to recover in the following 2 hours (Fig. 6). During the zero $Ca^{2+}$ perfusion there was a small non-significant decrease in the levels of 5-HIAA of 11% before returning towards basal levels upon reperfusion with CSF (Fig. 6). The inhibition observed with the $Ca^{2+}$-free solution was more delayed and not as great as that seen with TTX. Again, there was a slight fall in the level of 5-HT when the liquid switch was turned and normal CSF perfused.

**Addition of Exogenous 5-HT and 5-HIAA**

Figure 7 shows typical HPLC traces before and after the addition of exogenous 5-HT and 5-HIAA to the dialysis sample. Table 2 shows that addition of exogenous 5-HT and 5-HIAA increased the peak height of the 5-HT and 5-HIAA respectively by the amount equivalent to the concentration of the exogenous solution.

**Figure 3**

A diagram of a coronal section of a rat brain showing a dialysis probe in the ventral hippocampus (co-ordinates from bregma: A/P -5.6mm, L ±4.6mm, V -7.5mm from the skull surface (Paxinos and Watson, 1986). The diagram was adapted from the stereotaxic atlas of the rat brain by Paxinos and Watson (1986). Length of steel cannula of dialysis probe is not to scale.

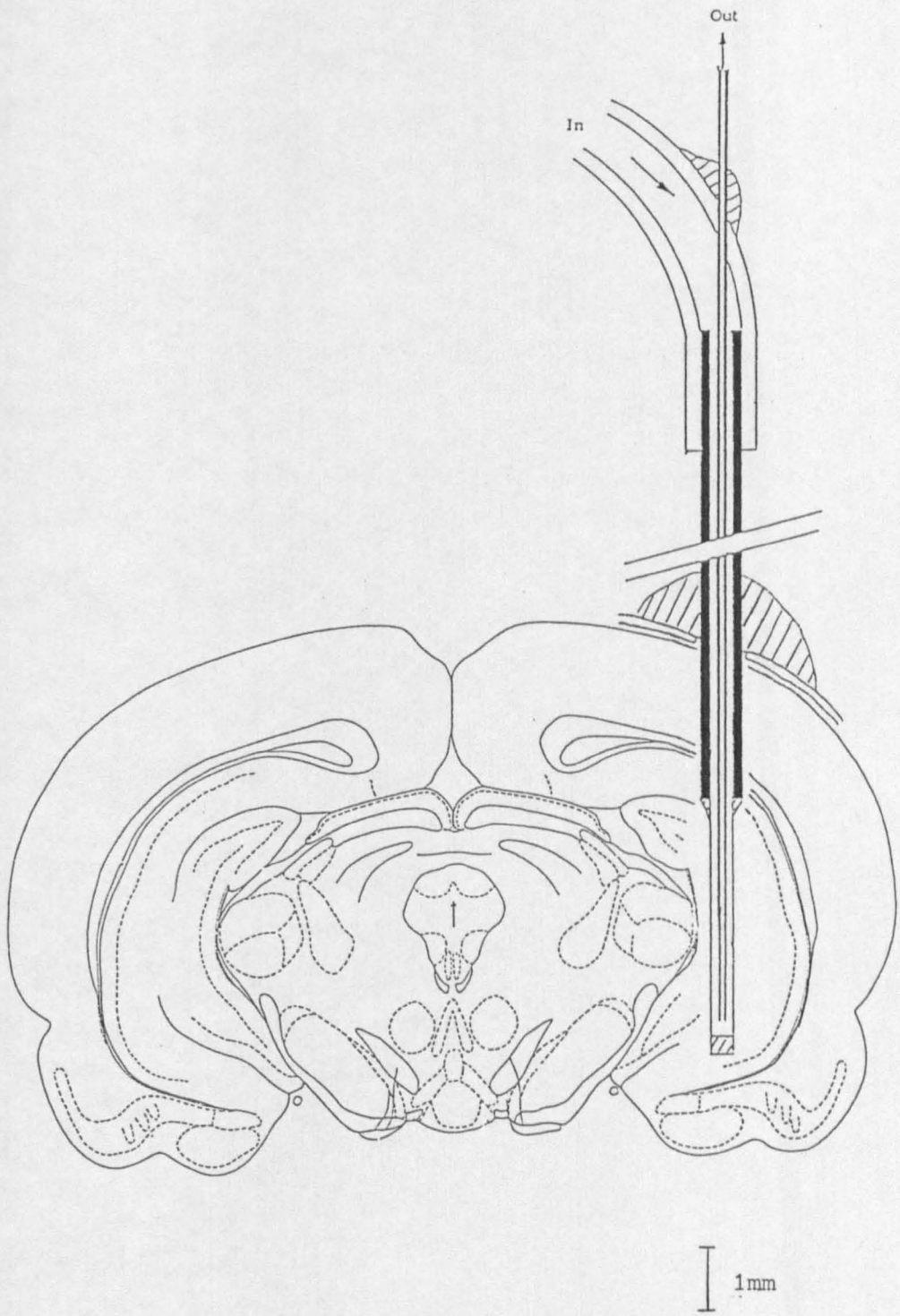

Out
In
1mm

**Figure 4**

Typical HPLC traces obtained after the injection of a standard (5-HT and 5-HIAA 0.5pmoles/20µl) or a 20µl dialysis sample of extracellular levels of 5-HT and 5-HIAA. The arrows on both traces indicate the point of injection. The asterisk on the sample trace after the 5-HIAA peak indicates the time when the sensitivity of the system was increased four-fold for easier detection of 5-HT. Basal levels of 5-HT = 59 ± 8 fmol/20µl and 5-HIAA = 1.26 pmol/20µl.

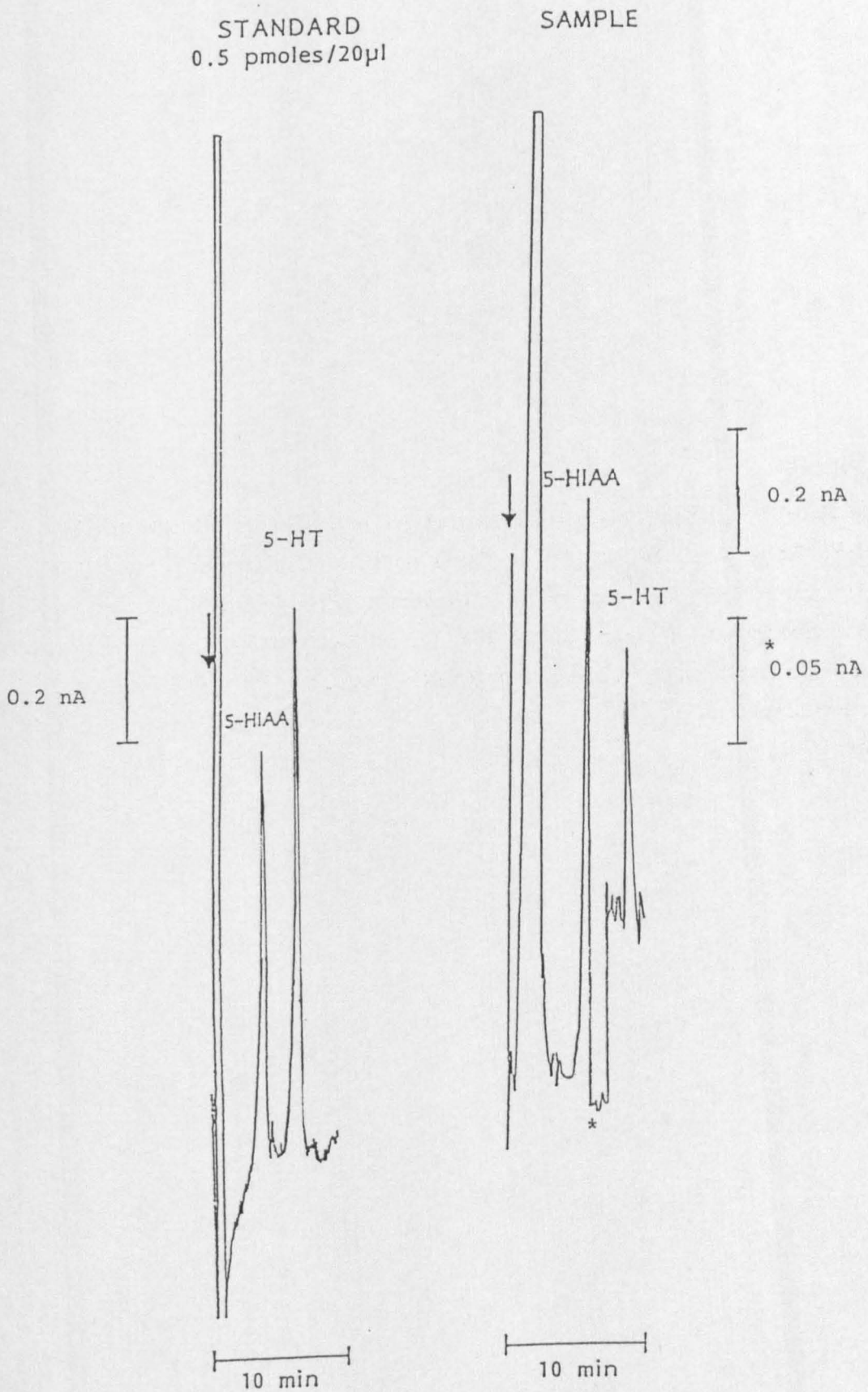
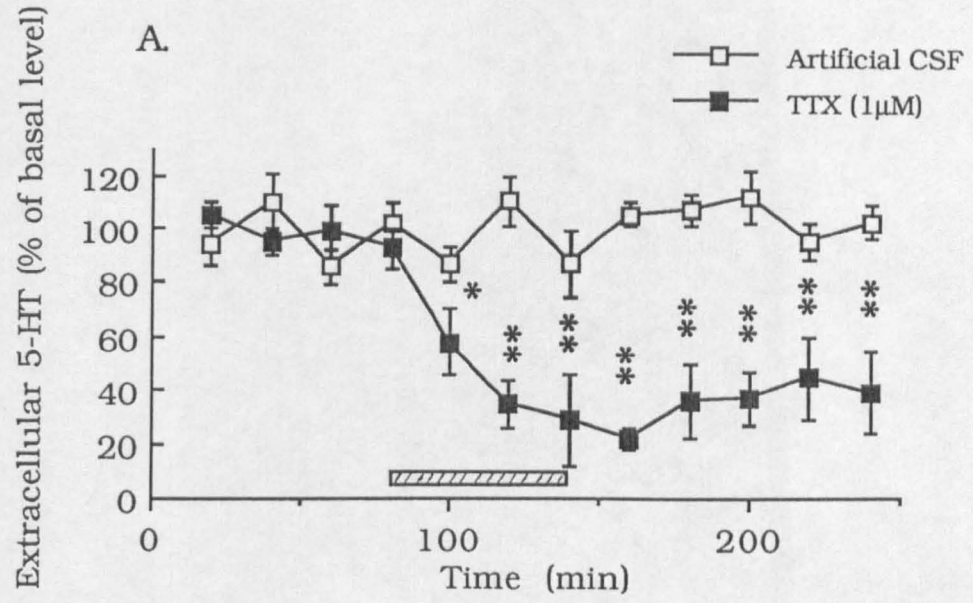

STANDARD
0.5 pmoles/20µl
SAMPLE
5-HT
5-HIAA
0.2 nA
5-HIAA
5-HT
0.2 nA
*
0.05 nA
*
10 min
10 min

**Figure 5**

Changes in the extracellular levels of (A) 5-HT and (B) 5-HIAA in the hippocampus of naive rats before and after local application of tetrodotoxin (TTX, 1μM) in the perfusate. The perfusate was changed by means of a liquid switch from normal artificial CSF to CSF containing TTX for 60 minutes (marked with ▨) and then the probe was reperfused with the TTX-free solution. The results are expressed as the mean extracellular level of 5-HT compared to the basal samples ± S.E.M., n=5. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures (5-HT: $F = 9.91$, $p<0.01$; 5-HIAA: $F = 2.82$, $p<0.13$) followed by a Dunnett's t-test to identify any significant differences between control and TTX groups; *$p<0.05$, **$p<0.01$.


A.
Artificial CSF
TTX (1μM)
Extracellular 5-HT (% of basal level)
120
100
80
60
40
20
0
0
100
200
Time (min)

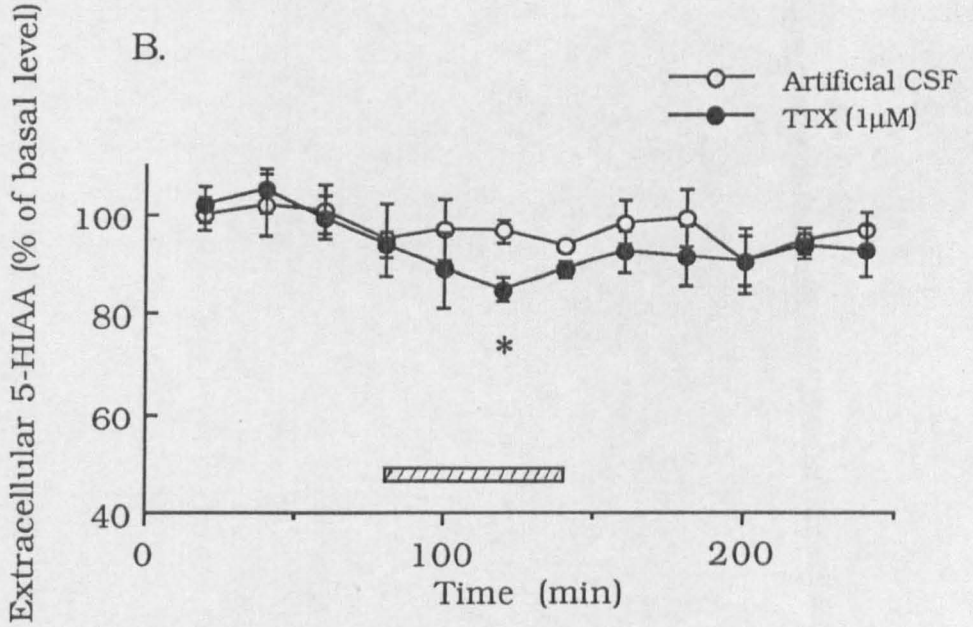
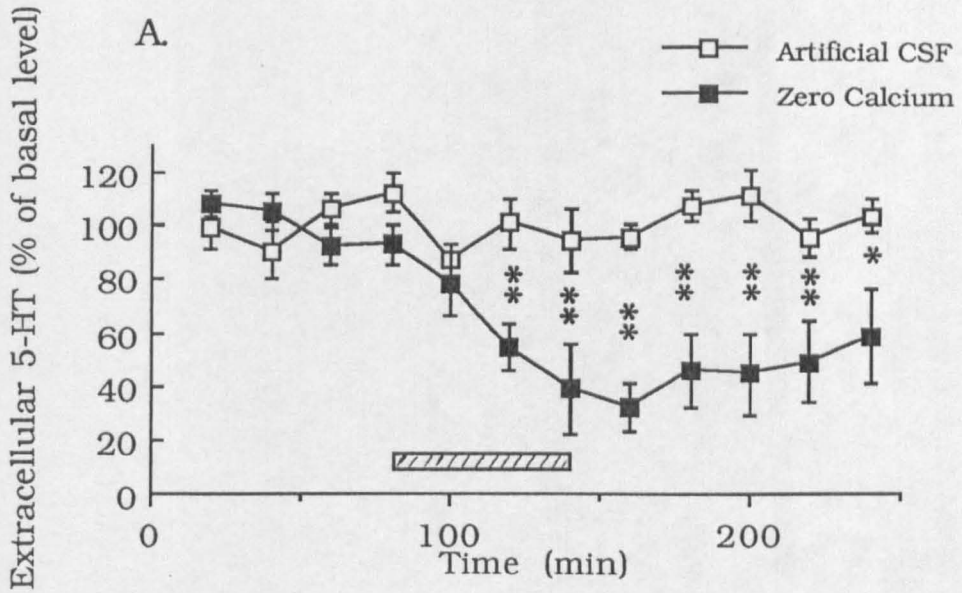

B.
Artificial CSF
TTX (1μM)
Extracellular 5-HIAA (% of basal level)
100
80
60
40
0
100
200
Time (min)

**Figure 6**

Changes in the extracellular levels of (A) 5-HT and (B) 5-HIAA in the hippocampus of naive rats before and after local application of a $Ca^{2+}$-free perfusate. The perfusate was changed by means of a liquid switch from normal artificial CSF to $Ca^{2+}$-free CSF for 60 minutes (marked with ▨) and then the probe was reperfused with CSF containing $Ca^{2+}$. The results are expressed as the mean extracellular level of 5-HT compared to the basal samples ± S.E.M., n=5. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures (5-HT: $F = 7.39$, $p<0.01$; 5-HIAA: $F = 1.84$, $p<0.24$) followed by a Dunnett's t-test to identify any significant differences between control and $Ca^{2+}$-free groups; $^{*}p<0.05$, $^{**}p<0.01$.


A.
Artificial CSF
Zero Calcium
Extracellular 5-HT (% of basal level)
120
100
80
60
40
20
0
0
100
200
Time (min)

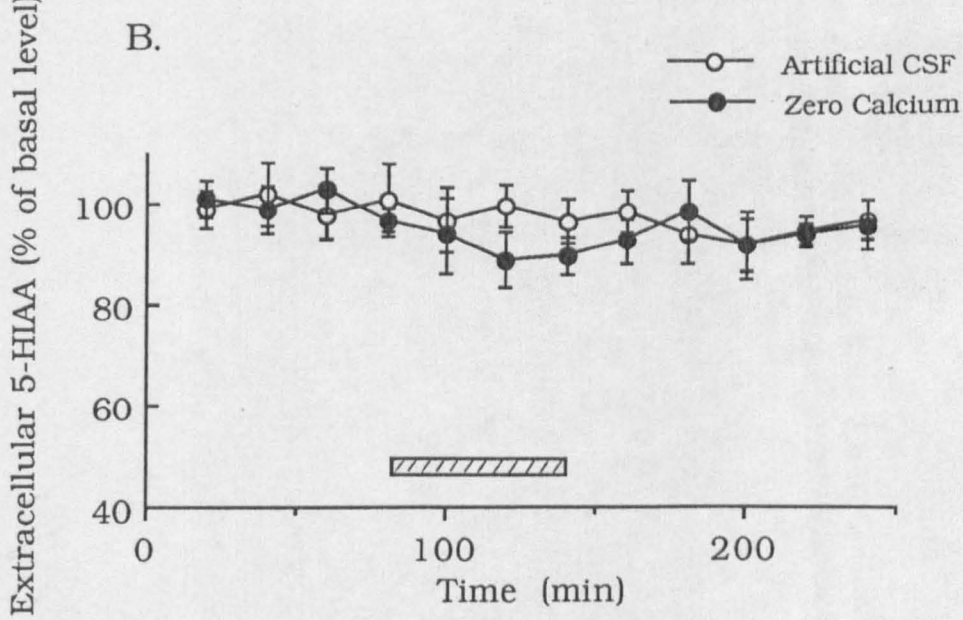

B.
Artificial CSF
Zero Calcium
Extracellular 5-HIAA (% of basal level)
100
80
60
40
0
100
200
Time (min)

**Figure 7**

Typical HPLC traces obtained after the injection of: (A) 20µl of 5-HT and 5-HIAA (0.1pmoles/20µl), (B) a 20µl dialysis sample of extracellular levels of 5-HT and 5-HIAA, and (C) a 20µl dialysis sample with 10µl of 0.2pmoles/20µl of 5-HT and 5-HIAA solution added to it. The arrows on the traces indicate the point of injection. The asterisk on the traces after the 5-HIAA peak indicates the time when the sensitivity of the system was increased four-fold for easier detection of 5-HT.

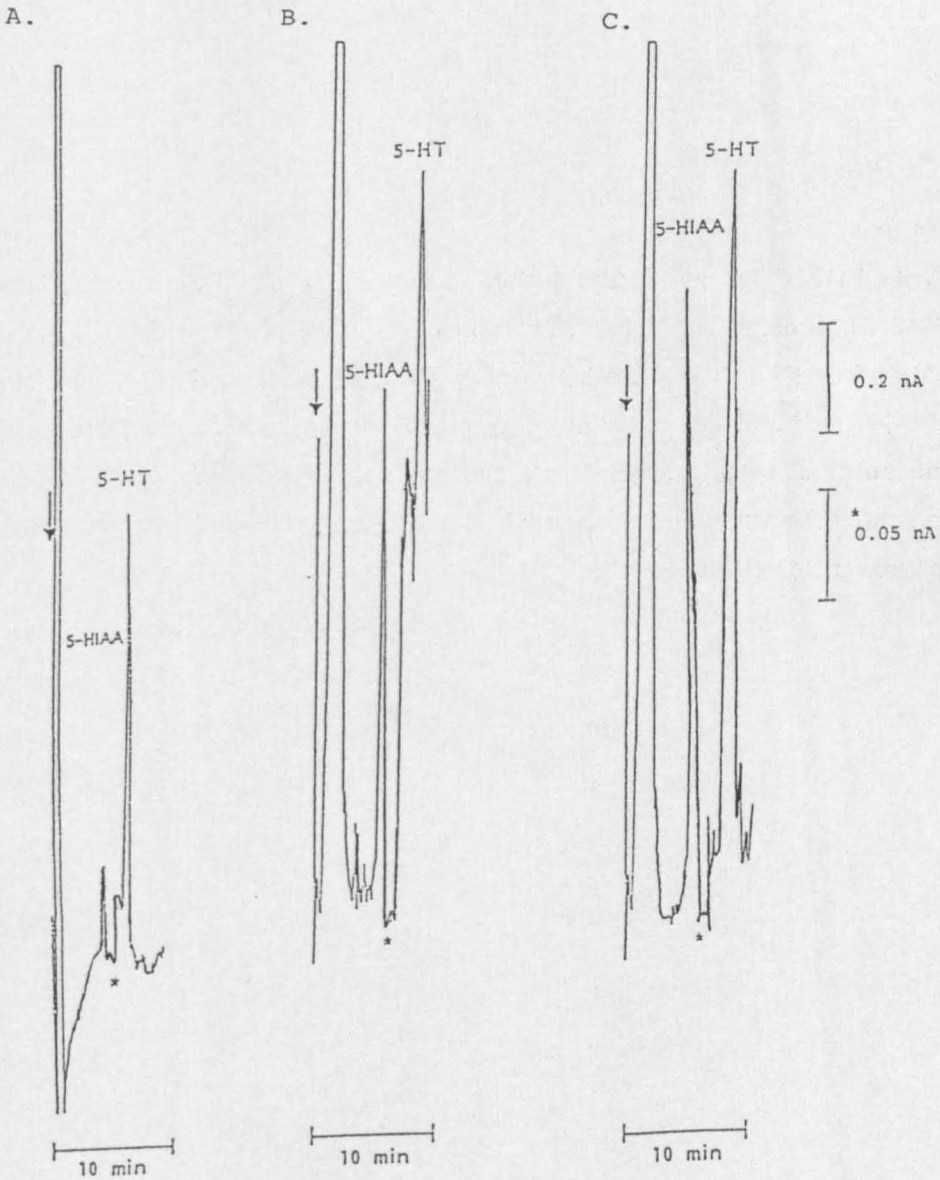

A.
B.
C.
5-HT
5-HIAA
5-HT
5-HIAA
5-HT
5-HIAA
0.2 nA
* 0.05 nA
10 min
10 min
10 min

**Table 2**

Peak heights of basal 5-HT and 5-HIAA dialysis samples (20µl) before and after the addition of 10µl exogenous 5-HT and 5-HIAA (0.1pmoles/20µl), n=6 per group.

| Injection | Peak Height (mm) | |
|---|---|---|
| | 5-HT | 5-HIAA |
| 20µl 0.1pmoles/20µl mixed standard | 67 ± 7 | 14 ± 4 |
| 20µl Basal Sample | 45 ± 7 | 126 ± 13 |
| 20µl Basal Sample + 10µl 0.2pmoles/20µl mixed standard | 102 ± 11 | 138 ± 15 |

## DISCUSSION

### HPLC Measurements and Measurement of *In Vitro* Recovery

The minimum detection level of 20fmoles/20µl for both indoles was lower than the basal levels in the brain thus the assay of HPLC-ECD could be used for these experiments.

The mean relative recovery *in vitro* using dialysis probes made with Hospal membrane was better than that for probes made with the methylcellulose tubing. These results show the importance of determining the relative recovery *in vitro* and of trying to obtain the best possible recovery. The results from the dialysis experiments were not corrected for recovery as *in vitro* recovery is not comparable to *in vivo* recovery (Alexander et al., 1988; Benveniste, 1989; Benveniste et al., 1989; Benveniste and Huttemeier, 1990). Therefore dialysis is not a useful way of accurately determining extracellular concentrations but is excellent for monitoring quantitative neurotransmitter changes.

### Neuronal Release of 5-HT

The evidence in the literature reviewed in the Introduction to this chapter supports the idea that 5-HT measured by dialysis is of neuronal origin, in both anaesthetised and conscious rats. The validation of the neuronal origin of 5-HT in this chapter is only for conscious animals and later work concerning anaesthetised animals is based on the validation by Kidd (1990) who worked in the same laboratory. The experiments described here show that the release of 5-HT in the ventral hippocampus in conscious rats is of neuronal origin. This conclusion is based on the results of (a) the ability of TTX to decrease markedly the levels of 5-HT, (b) the decrease in 5-HT levels seen after removal of $Ca^{2+}$ from the CSF, and (c) the oxidation peak corresponding to the oxidation of endogenous 5-HT coinciding with that of exogenous 5-HT.

The release of 5-HT was inhibited by local perfusion with 1µM TTX while 5-HIAA levels were only slightly affected. As TTX blocks conduction in neurones (Hille, 1968) resulting in reduced neurotransmitter release, this provides good evidence that the 5-HT release is of neuronal origin. There was no sign of a return to basal levels after the perfusion medium had been switched back to normal CSF. This was probably a result of a long-lasting action of TTX binding to the nerves and continuing to prevent action

potential conduction. Other studies have found a return to basal levels after cessation of TTX perfusion (Auerbach et al., 1989; Carboni and Di Chiara, 1989), but Sharp et al. (1989c) only demonstrated a partial return to basal levels two hours after cessation of TTX. TTX did not totally abolish the 5-HT release, indicating that either the 5-HT came from an area beyond that perfused with TTX or that this residual component, approximately 20%, was derived from non-neuronal sources such as glial cells, mast cells or platelets (Sneddon, 1973; Austen, 1978; Kimelberg, 1986). 5-HIAA was hardly affected by TTX and thus very little appears to be released by action potentials and is likely to be the product of intraneuronal metabolism of 5-HT which spills over into the synapse. This agrees with earlier findings that the 5-HIAA measured is not a product of the released 5-HT but is from the intraneuronal metabolism of unreleased 5-HT (Grahame-Smith, 1971, 1974; Kuhn et al., 1986; Kalen et al., 1988; Auerbach et al., 1989; Sharp et al., 1989c; Crespi et al., 1990). Thus changes in 5-HIAA levels are not a good indication of corresponding changes in 5-HT levels. Similar work on the dopaminergic system has found that dopamine release is inhibited by the addition of TTX to the perfusion medium in conscious animals (Westerink et al., 1987b).

Perfusion with $Ca^{2+}$-free CSF (and 0.1mM EGTA to chelate extracellular $Ca^{2+}$) decreased 5-HT levels by 67%. $Ca^{2+}$ is required for exocytotic release of neurotransmitters (Augustine et al., 1987) and thus this result further confirms the neuronal origin of 5-HT in this study. Two hours after returning to normal CSF the basal levels had started to return to normal but had not yet reached the original level. As with TTX, the effect of $Ca^{2+}$-free CSF on the 5-HIAA levels was only small, supporting other evidence suggesting that the 5-HIAA is a result of intraneuronal metabolism of 5-HT spilling over into the synapse. Perfusion with $Ca^{2+}$-free CSF did not reduce the 5-HT peak down to zero, and doubt has been expressed concerning the validity of the use of $Ca^{2+}$-free solutions as an indicator for neuronal release of any neuronal transmitter (Di Chiara, 1990). It is possible that there is sufficient endogenous $Ca^{2+}$ present, even with a $Ca^{2+}$ chelator such as EGTA included in the perfusion medium, to maintain some release of neurotransmitters. $Ca^{2+}$-free CSF has also been used to demonstrate neuronal release of dopamine in both anaesthetised (Imperato and Di Chiara, 1984) and conscious rats (Westerink and De Vries, 1988).

A third method of demonstrating neuronal transmitter release is by local stimulation with a high $K^+$ concentration in the perfusate which depolarises the nerve terminals directly. High $K^+$ has been shown to increase dopamine release in anaesthetised (Imperato and Di Chiara, 1984) and conscious rats (Westerink and Tuinte, 1986). Kalen et al. (1988) showed that a high $K^+$ solution could still release 5-HT even in the absence of $Ca^{2+}$ and the presence of EGTA, thus it is not surprising that the release of 5-HT was only partially affected by perfusion with a $Ca^{2+}$-free solution.

Addition of exogenous 5-HT and 5-HIAA to basal dialysate samples increased the peak heights correlating to 5-HT and 5-HIAA respectively. This indicates that the retention time on the column and oxidation at the working electrode is the same for exogenous 5-HT and the endogenous compound (presumed to be 5-HT) and exogenous 5-HIAA and its respective endogenous peak.

**Conclusions**

These experiments have shown that the 5-HT measured in the ventral hippocampus by intracerebral microdialysis is released from neurones as it is TTX-sensitive and affected by the removal of $Ca^{2+}$. The 5-HIAA measured is predominently as a result of intraneuronal metabolism of 5-HT which subsequently spills over into the synapse and is not a good correlate of 5-HT levels.

# CHAPTER 5

# EFFECT OF DIAZEPAM, IPSAPIRONE AND F2692 ON EXTRACELLULAR 5-HT AND 5-HIAA RELEASE IN THE VENTRAL HIPPOCAMPUS OBSERVED IN RATS ON THE ELEVATED X-MAZE MODEL OF ANXIETY USING *IN VIVO* MICRODIALYSIS

**INTRODUCTION**

In the Introduction to this thesis evidence was presented for the involvement of the central serotonergic systems as one of the mechanisms of anxiolytic action of the benzodiazepines (Kahn et al., 1988; Iversen, 1984; Soubrie, 1986; Stein et al., 1975; Thiebot, 1986). Thus, an important question is whether changes in serotonergic function can be related to the anxiolytic action of the benzodiazepines. Benzodiazepines have been reported to reduce 5-HT turnover after acute and chronic treatment (Chase et al., 1970; Lidbrink et al., 1973; Wise et al., 1972), suggesting that the decrease in 5-HT turnover reflects reduced activity of 5-HT-containing neurones and thus decreased release of 5-HT. A more recent study using *in vivo* microdialysis showed that both systemic and local administration of benzodiazepine agonists (diazepam and flurazepam) inhibited the release of 5-HT from the ventral hippocampus, and this effect could be reversed by administration of flumazenil (Ro 15-1788) (Pei et al., 1989b). *In vitro* studies have shown that benzodiazepines inhibit potassium-evoked [$^3$H]-5-HT release from hippocampal synaptosomes (Balfour, 1980; Colline and Pycock, 1982), suggesting that 5-HT release can be inhibited via an action at the nerve terminals. Electrophysiological findings support an interaction between benzodiazepines and 5-HT. Laurent et al. (1983), recording multiunit activity in the *encephale isole* rat found that intravenous chlordiazepoxide and midazolam depressed neuronal firing in the dorsal raphe nucleus at doses which are known to be anxiolytic. Further, these effects were blocked by the benzodiazepine antagonist flumazenil but the depressent effects of pentobarbitone on raphe neurones was not affected. It is now known that the benzodiazepine receptor is part of a receptor complex including a $GABA_A$ receptor and a $Cl^-$ ion channel in all areas of the brain (Unnerstall et al., 1981). Furthermore, benzodiazepines interact at the receptor complex and enhance GABAergic transmission. In the CNS, sites such as the amygdala and hippocampus, which contain 5-HT and are implicated in anxiety, also have high densities of GABA-containing neurones. Iontophoretic application of GABA in the dorsal raphe, amygdala and hippocampus results in the inhibition of firing of 5-HT neurones, an effect enhanced by benzodiazepines (Gallager, 1978).

The $5\text{-HT}_{1A}$ partial agonists buspirone, gepirone and ipsapirone have been shown to produce similar results to the benzodiazepines on 5-HT release,

turnover, and cell firing but differ in their behavioural profile (see Chapter 3). Buspirone, gepirone and ipsapirone all inhibit dorsal raphe neurone firing, mimicking the effect of 5-HT (Dourish et al., 1986; Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Wilkinson et al., 1987). In the hippocampus all three compounds produced only the inhibitory component of the action of 5-HT (Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Martin and Mason, 1987). Measurement of 5-HT *in vivo* with either microdialysis or differential pulse voltammetry, the 5-$HT_{1A}$ agonists have been found to decrease 5-HT release (Crespi et al., 1990; Hjorth and Magnusson; 1988; Hutson et al., 1989; Sharp et al., 1989a). This effect is thought to be predominantly as a result of action at the somatodendritic autoreceptor in the dorsal raphe nucleus (Hjorth and Magnusson, 1988; Hutson et al., 1989; Sharp et al., 1989c). In behavioural studies the picture is somewhat confusing with the 5-$HT_{1A}$ partial agonists having either anxiolytic, anxiogenic or no effect depending on the behavioural model used (for review see Introduction to this thesis and Traber and Glaser, 1987).

A new potential anxiolytic compound, known as F 2692 (chemical name: 1-(3'-trifluoro-methylphenyl) 1,4-dihydro 3-amino 4-oxo 6-methyl pyridazine), has been shown to have an anxiolytic profile in the elevated X-maze (Assie et al., 1991) but only has weak affinity for the benzodiazepine binding site (Assie et al., 1991). Also no tolerance has been seen after chronic treatment (Chopin et al., 1991). At present the mechanism of action of this compound is unknown but the effects of F 2692 in the elevated X-maze can be reversed with flumazenil, a benzodiazepine antagonist, but only at very high doses (20mg/kg i.p.) (Assie et al., 1991) suggesting that it may be having part of its effect via the benzodiazepine-$GABA_A$ receptor complex.

The aim of this study was to first determine the effects of placing a rat on the elevated X-maze upon the levels of extracellular 5-HT and 5-HIAA in the ventral hippocampus, and to determine whether there is any difference between restricting the animal to either the closed or open arms on the changes in levels of extracellular 5-HT and 5-HIAA. Second, to determine the effects of an established anxiolytic, diazepam, a 5-$HT_{1A}$ partial agonist, ipsapirone, and an anxiolytic which as yet does not have a known mechanism of action, F 2692, on the changes in extracellular 5-HT concurrent with the animals' behaviour when they are placed on the elevated X-maze.

## METHOD

### Animals

Naive male Lister hooded rats (240-300g) (Nottingham University Medical School Animal Unit) were housed in groups of four on a 12 hour light/dark cycle (lights on 0700-1900), with food and water available ad libitum.

### Procedure

Dialysis probes were prepared and implanted under halothane anaesthesia as described in Chapter 4. After implantation rats were housed singly in a high-sided open top cage for 24 hours to recover. After this period of time the animal's inlet and outlet were connected and the probe perfused with artificial CSF at a flow rate of 1µl/min as described in Chapter 4. Dialysis samples were collected every 20 minutes and analysed for 5-HT and 5-HIAA via HPLC-ECD as described in Chapter 4.

### Restriction to either Closed or Open Arms

30 Minutes after perfusion started four 20 minute dialysis samples were taken. After this time the animal was placed on the X-maze with either the closed or open arms sectioned off with pieces of matt black perspex (15cm x 10cm) (during the time the animal was on the X-maze the liquid swivel was moved from a fixed position above the cage to one above the centre of the X-maze where it could freely rotate). The animal was on the X-maze for a period of 20 minutes (four groups of 5 minutes) during which time its behaviour was monitored as described in Chapter 2 and one dialysis sample collected. The animal was then returned to its home cage and dialysis samples taken for a further two hours.

### Effect of Established and Putative Anxiolytics

30 minutes after perfusion started four 20 minutes dialysis samples were taken. After this time the animal received an intraperitoneal injection of either vehicle (1% methyl cellulose), diazepam (2.5mg/kg), ipsapirone (1mg/kg) or F 2692 (10mg/kg) and dialysis samples were taken for a further 40 minutes. The animal was then placed on the X-maze and its behaviour monitored for a period of 20 minutes (four groups of 5 minutes) (one dialysis sample) as described in Chapter 2. After this time the animal was returned to its home cage and dialysis samples taken for a further two hours.

**Drugs**

All drugs were suspended or dissolved in 1% v/v methyl cellulose (in 0.9% w/v saline), by sonication. All drugs were prepared immediately prior to use and injected i.p. in a volume of 1 ml/kg.

Compounds were obtained from the following sources: Methyl cellulose (BDH Chemicals Ltd.); diazepam (Courtin and Warner Ltd.); ipsapirone (Troponwerk); F 2692 (Pierre Fabre).

**Data Analysis**

All the results for the experiments were expressed as the mean ± S.E.M.. All behavioural data were analysed using a 2-tailed Mann-Whitney U-test and all neurochemical data were analysed using a one-way ANOVA with repeated measures followed by a Dunnett's t-test to identify any significant differences between vehicle control and drug treated groups. Vehicle controls were assigned to each of the series of experiments and grouped together for statistical analysis compared to the drug treated groups.

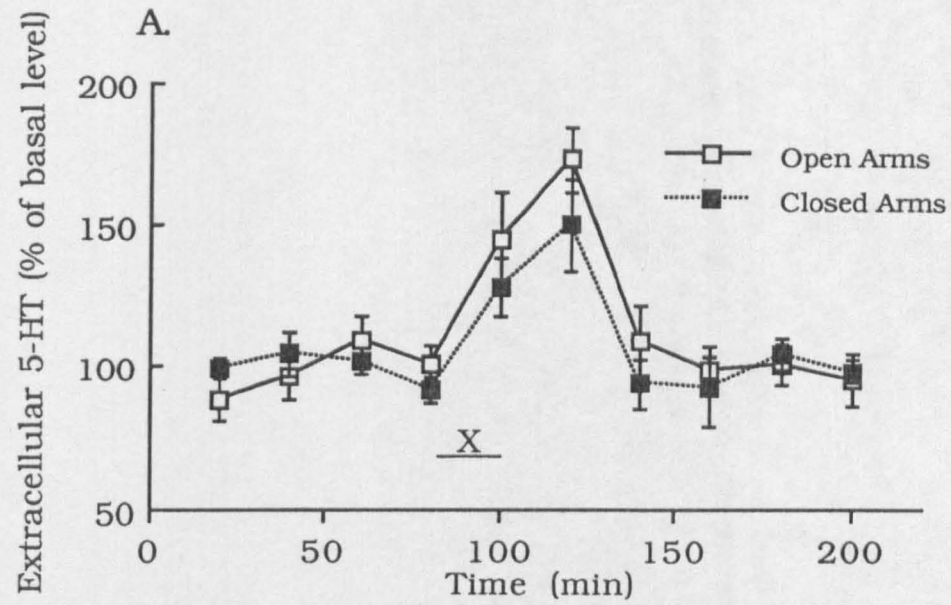
**RESULTS**

**Restriction to either Closed or Open Arms**

Restriction to either closed or open arms resulted in a significant increase in extracellular levels of 5-HT whether the animal was restricted to either the closed or open arms (Fig. 1A); there was no significant change in the extracellular levels of 5-HIAA (Fig. 1B). Exposure to the X-maze resulted in an increase in the levels of 5-HT which peaked 20 minutes after being returned to the home cage (a result of a deadspace of approximately 15 minutes and picking up the rat to return it to the home cage) and returned back to basal levels 40 minutes after being exposed to the X-maze (Fig. 1A). The increase in extracellular 5-HT appeared to be greater when the animal was restricted to the open arms compared to the closed arms but this did not reach significance (Fig. 1). When restricted to the open arms the rats' spent a decreasing amount of time on the open and end of open arms with each successive 5 minute time period (Fig. 2A and 2B), and hence an increasing amount of time was spent in the central square of the X-maze (data not shown).

**Figure 1**

Effect of restricting the rat to either the open or closed arms of the elevated X-maze for a 20 minute period (marked by X) on the extracellular levels of (A) 5-HT and (B) 5-HIAA measured using intracerebral microdialysis with HPLC-ECD. The results are expressed as the mean extracellular levels of 5-HT and 5-HIAA compared to the basal samples ± S.E.M., n=6. Basal levels of 5-HT and 5-HIAA before being restricted to either the open or closed arms were: 5-HT: open = 71 ± 10 fmol/20µl, closed = 64 ± 11 fmol/20µl; 5-HIAA: open = 1.17 ± 0.22 pmol/20µl, closed = 1.24 ± 0.31 pmol/20µl. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures followed by a Dunnett's t-test to identify any significant differences between open and closed arm values. There were no significant differences at the $p<0.05$ level.


A.
Extracellular 5-HT (% of basal level)
200
150
100
50
0
50
100
150
200
Time (min)
X
Open Arms
Closed Arms

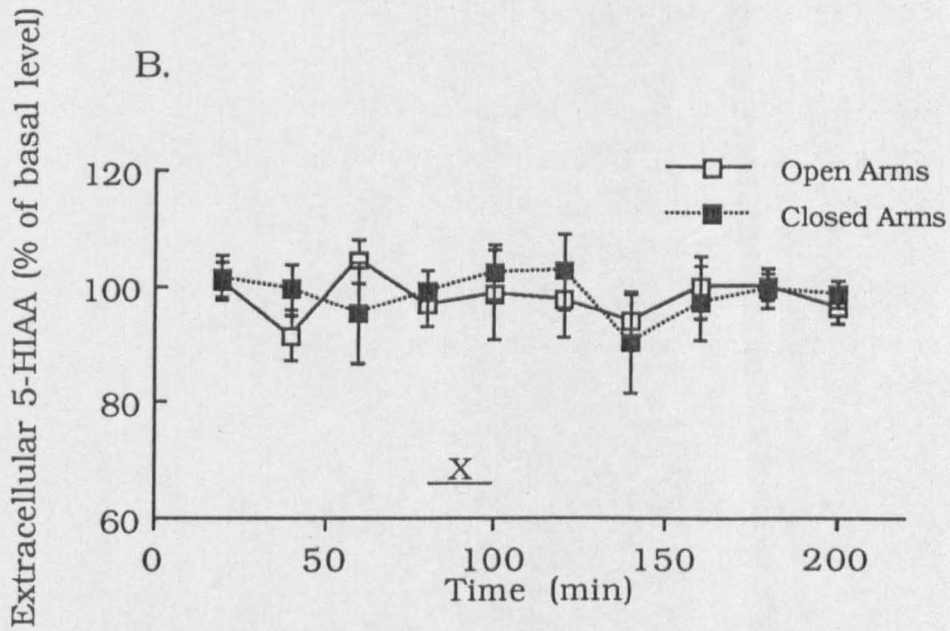
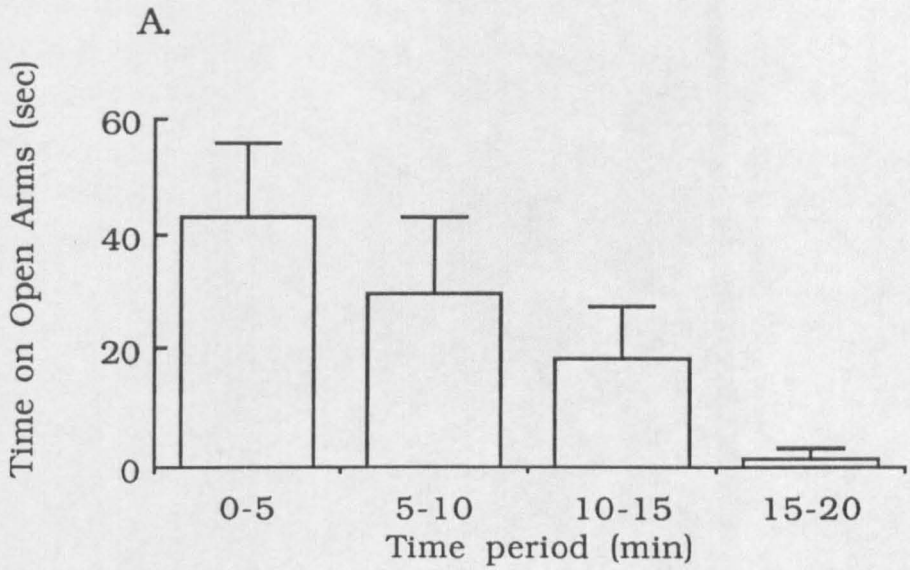
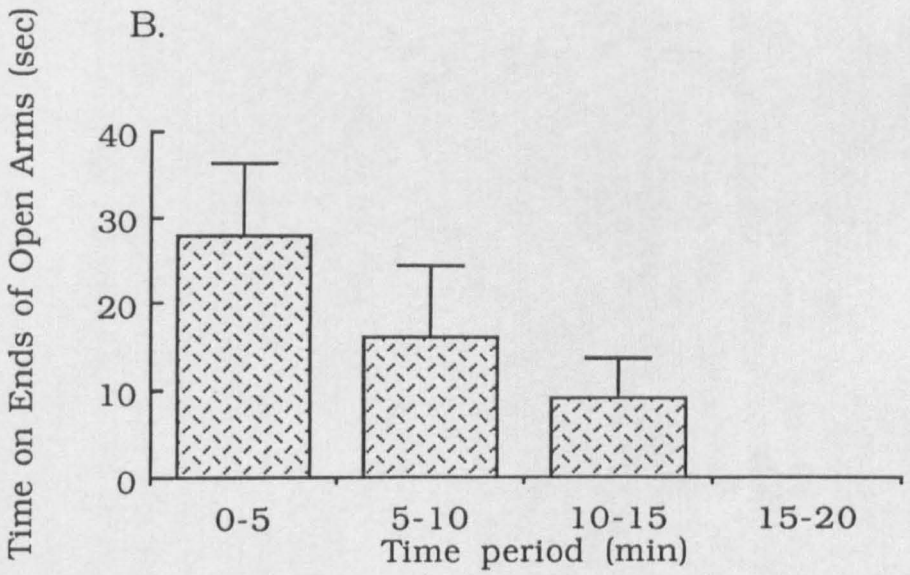

B.
Extracellular 5-HIAA (% of basal level)
120
100
80
60
0
50
100
150
200
Time (min)
X
Open Arms
Closed Arms

**Figure 2**

(A) The time spent on the open and (B) ends of open arms of rats restricted to the open arms on the elevated X-maze for a period of 20 minutes expressed in successive 5 minute intervals. The results are expressed as the mean ± S.E.M. of 6 observations.


A.
Time on Open Arms (sec)
60
40
20
0
0-5
5-10
10-15
15-20
Time period (min)


B.
Time on Ends of Open Arms (sec)
40
30
20
10
0
0-5
5-10
10-15
15-20
Time period (min)

**Effects of Pharmacological Agents on Extracellular Levels of 5-HT and 5-HIAA and Behaviour**

**a) Neurochemistry**

Giving the animal free access to both the open and closed arms of the X-maze not surprisingly resulted in an increase in the extracellular levels of 5-HT but there was no change in the extracellular levels of 5-HIAA (Fig. 3). Systemic diazepam (2.5mg/kg i.p.) had no effect on 5-HT levels in the home cage but significantly reduced the increase in extracellular 5-HT levels when the rat was on the X-maze (Fig. 3A). Furthermore, diazepam produced a significant decrease in extracellular 5-HT from 60 min after removal from the X-maze until the end of the experiment (Fig. 3A) but had no effect on the extracellular levels of 5-HIAA (Fig. 3B). Similarly, systemic ipsapirone (1mg/kg i.p.) had no effect in the home cage but significantly reduced both the increase in extracellular 5-HT when the animal was placed on the X-maze and the basal levels of extracellular 5-HT reaching a maximum 80-100 minutes after being placed on the X-maze (Fig. 4A). F 2692 (10mg/kg i.p.) significantly reduced basal levels of 5-HT in the home cage before exposure to the X-maze (Fig. 5A) and then significantly reduced both the increase in extracellular 5-HT when the animal was exposed to the X-maze and the basal levels of extracellular 5-HT having a maximum effect 100 minutes after being placed on the X-maze (Fig. 5A). Ipsapirone and F 2692 had no significant effect on the extracellular levels of 5-HIAA throughout this group of experiments (Fig. 4B and 5B).

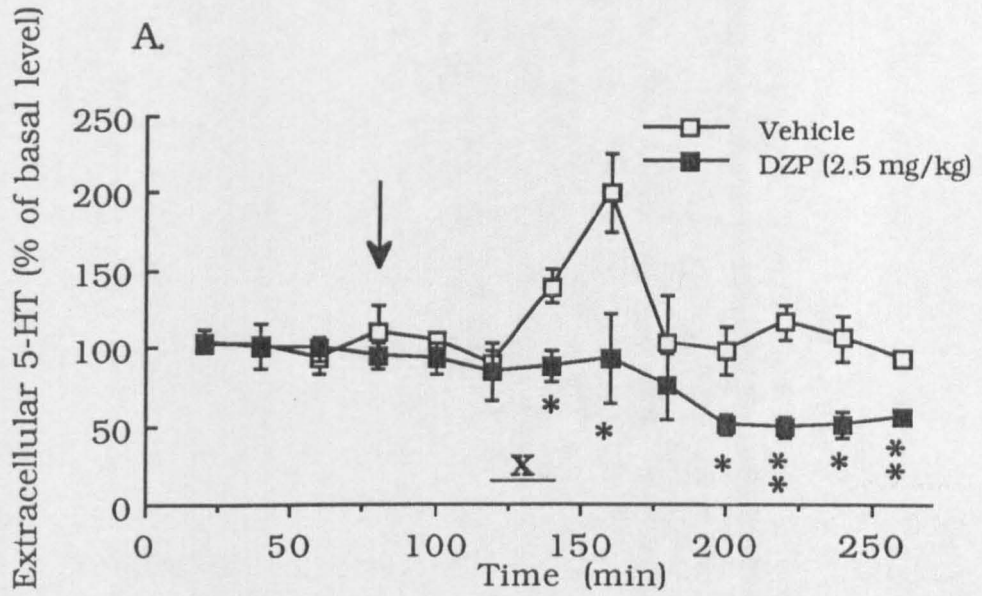
**b) Behaviour**

During the first 5 minutes on the X-maze (standard exposure time during a behavioural experiment) diazepam (2.5mg/kg i.p.) significantly increased both the time spent on the open and ends of open arms (Fig. 6A) and the percentage open:total entries and time (Fig. 6B). Over the full period of exposure to the X-maze (20 minutes; one dialysis sample) diazepam significantly increased the time spent on the open arms (Fig. 7A) and the percentage open:total entries and time (Fig. 7B). There were no significant differences in the number of arm and end of arm entries (an index of locomotor activity) between the vehicle control and the diazepam treated group for both the 5 minute and 20 minute data (Table 1). Ipsapirone (1mg/kg i.p.) had no effect on any of the behavioural parameters measured both during the first 5 minutes (Fig. 8) and the whole 20 minute exposure on the X-maze (Fig. 9). F 2692 significantly increased both the time spent on the open

**Figure 3**

Effect of diazepam (DZP, 2.5mg/kg i.p.) (arrow) on the extracellular levels of (A) 5-HT and (B) 5-HIAA when placed on the elevated X-maze for a 20 minute period (marked by X) and then returned to the home cage. Extracellular levels of 5-HT and 5-HIAA were measured using intracerebral microdialysis with HPLC-ECD. The results are expressed as the mean extracellular levels of 5-HT and 5-HIAA compared to the basal samples ± S.E.M., n=10 for vehicle controls and n=6 for diazepam treated rats. Basal levels of 5-HT and 5-HIAA before being placed on the X-maze were: 5-HT: vehicle = 64 ± 10 fmol/20µl, DZP = 69 ± 11 fmol/20µl; 5-HIAA: vehicle = 1.30 ± 0.32 pmol/20µl, DZP = 1.25 ± 0.36 pmol/20µl. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures (5-HT: $F = 17.85$, $p<0.001$; 5-HIAA: $F = 0.813$, $p<0.39$) followed by a Dunnett's t-test to identify any significant differences between vehicle and drug treated groups.

*$p<0.05$, **$p<0.01$ significantly different from vehicle treated animals. One-way ANOVA with repeated measures with post-hoc Dunnett's t-test.


A.
Extracellular 5-HT (% of basal level)
250
200
150
100
50
0
Vehicle
DZP (2.5 mg/kg)
X
0
50
100
150
200
250
Time (min)

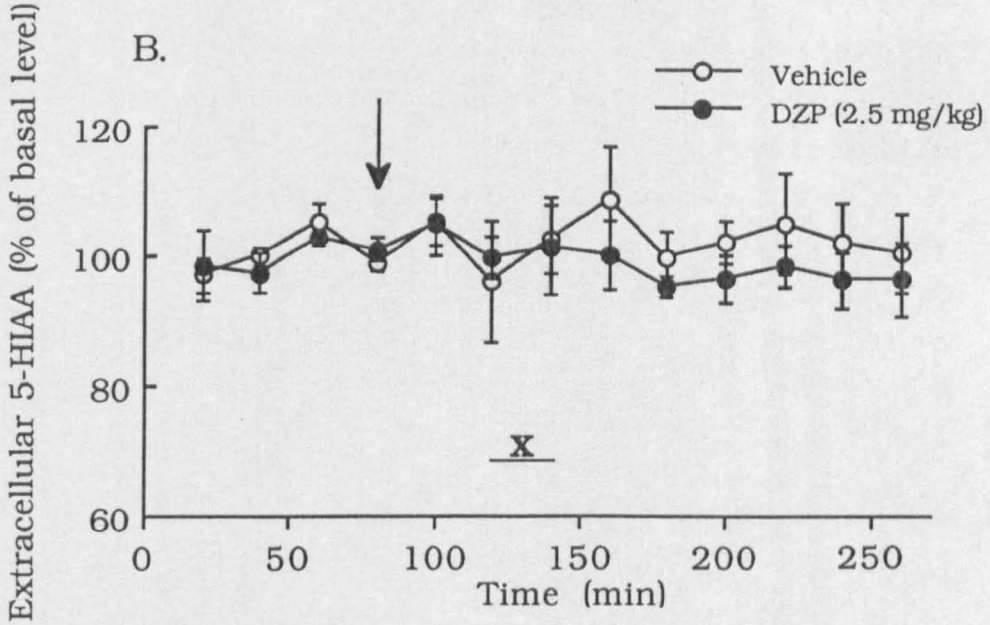

B.
Extracellular 5-HIAA (% of basal level)
120
100
80
60
Vehicle
DZP (2.5 mg/kg)
X
0
50
100
150
200
250
Time (min)

**Figure 4**

Effect of ipsapirone (1mg/kg i.p.) (arrow) on the extracellular levels of (A) 5-HT and (B) 5-HIAA when placed on the elevated X-maze for a 20 minute period (marked by X) and then returned to the home cage. Extracellular levels of 5-HT and 5-HIAA were measured using intracerebral microdialysis with HPLC-ECD. The results are expressed as the mean extracellular levels of 5-HT and 5-HIAA compared to the basal samples ± S.E.M., n=10 for vehicle controls and n=6 for ipsapirone treated rats. Basal levels of 5-HT and 5-HIAA before being placed on the X-maze were: 5-HT: vehicle = 64 ± 10 fmol/20µl, ipsapirone = 71 ± 12 fmol/20µl; 5-HIAA: vehicle = 1.30 ± 0.32 pmol/20µl, ipsapirone = 1.14 ± 0.20 pmol/20µl. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures (5-HT: $F = 10.15$, $p<0.001$; 5-HIAA: $F = 0.486$, $p<0.57$) followed by a Dunnett's t-test to identify any significant differences between vehicle and drug treated groups.

*$p<0.05$, **$p<0.01$ significantly different from vehicle treated animals. One-way ANOVA with repeated measures with post-hoc Dunnett's t-test.

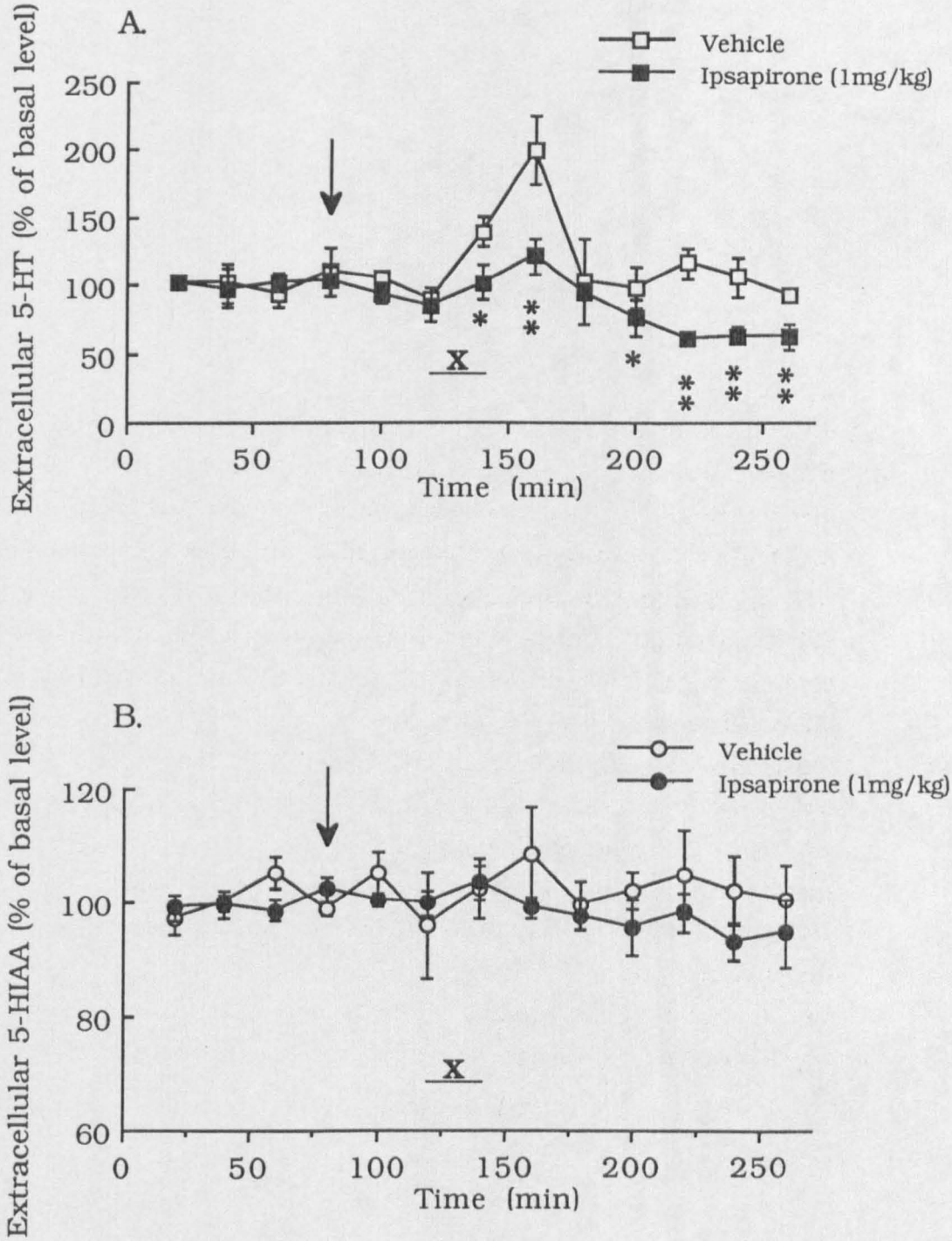
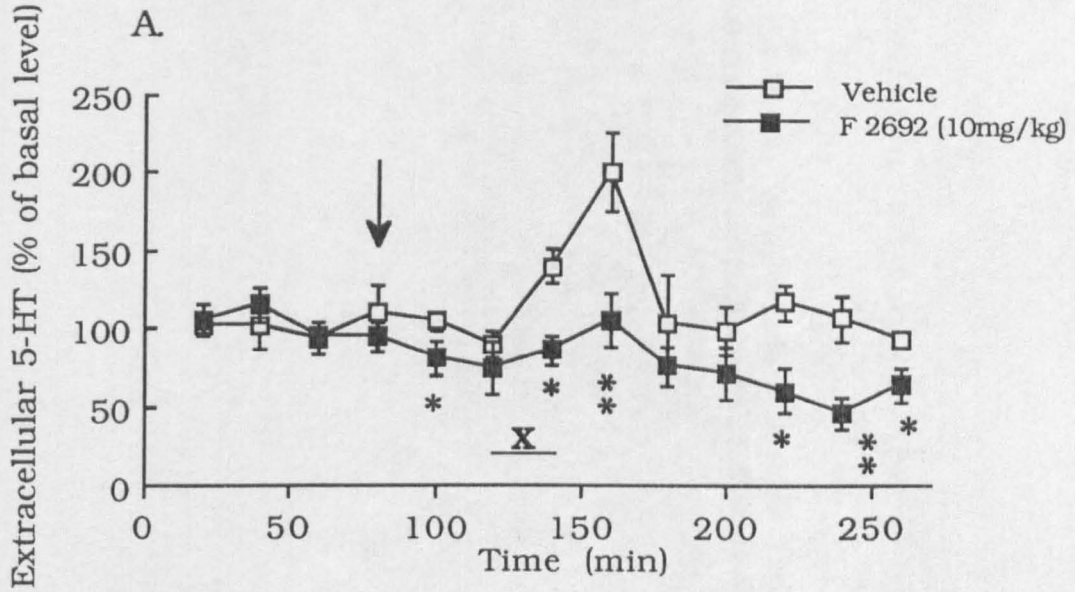

A.
Vehicle
Ipsapirone (1mg/kg)
Extracellular 5-HT (% of basal level)
250
200
150
100
50
0
X
0
50
100
150
200
250
Time (min)
B.
Vehicle
Ipsapirone (1mg/kg)
Extracellular 5-HIAA (% of basal level)
120
100
80
60
X
0
50
100
150
200
250
Time (min)

**Figure 5**

Effect of F 2692 (10mg/kg i.p.) (arrow) on the extracellular levels of (A) 5-HT and (B) 5-HIAA when placed on the elevated X-maze for a 20 minute period (marked by X) and then returned to the home cage. Extracellular levels of 5-HT and 5-HIAA were measured using intracerebral microdialysis with HPLC-ECD. The results are expressed as the mean extracellular levels of 5-HT and 5-HIAA compared to the basal samples ± S.E.M., n=10 for vehicle controls and n=6 for F 2692 treated rats. Basal levels of 5-HT and 5-HIAA before being placed on the X-maze were: 5-HT: vehicle = 64 ± 10 fmol/20µl, F2692 = 68 ± 9 fmol/20µl; 5-HIAA: vehicle = 1.30 ± 0.32 pmol/20µl, F2692 = 1.41 ± 0.36 pmol/20µl. Changes in the levels of 5-HT and 5-HIAA were analysed separately using a one-way ANOVA with repeated measures (5-HT: $F = 9.21$, $p<0.001$; 5-HIAA: $F = 0.462$, $p<0.51$) followed by a Dunnett's t-test to identify any significant differences between vehicle and drug treated groups. *$p<0.05$, **$p<0.01$ significantly different from vehicle treated animals. One-way ANOVA with repeated measures with post-hoc Dunnett's t-test.


A.
Extracellular 5-HT (% of basal level)
250
200
150
100
50
0
Vehicle
F 2692 (10mg/kg)
X
0
50
100
150
200
250
Time (min)

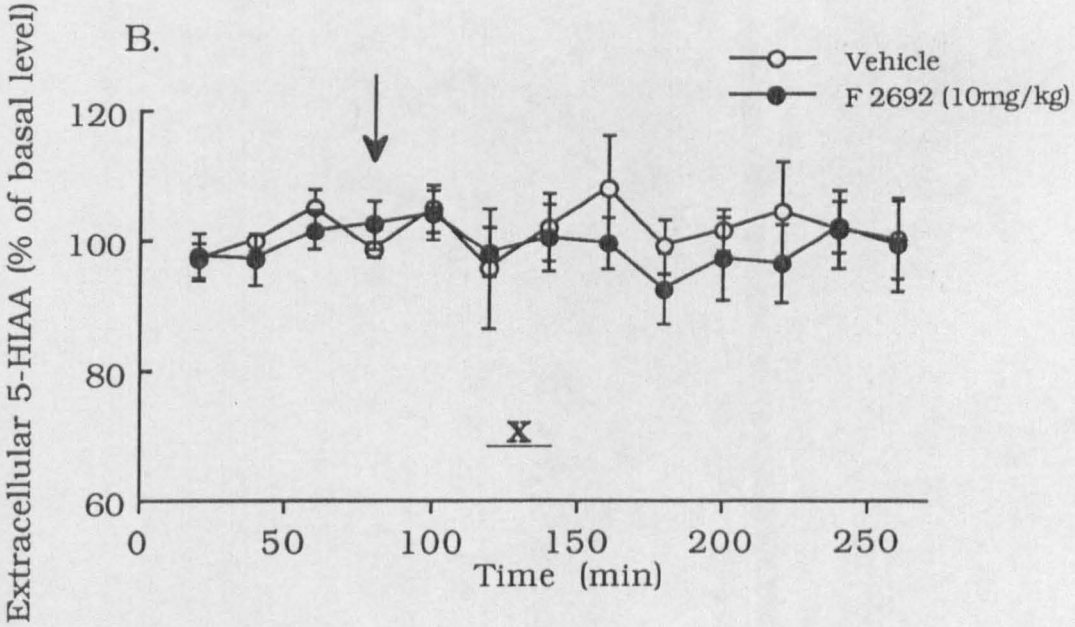
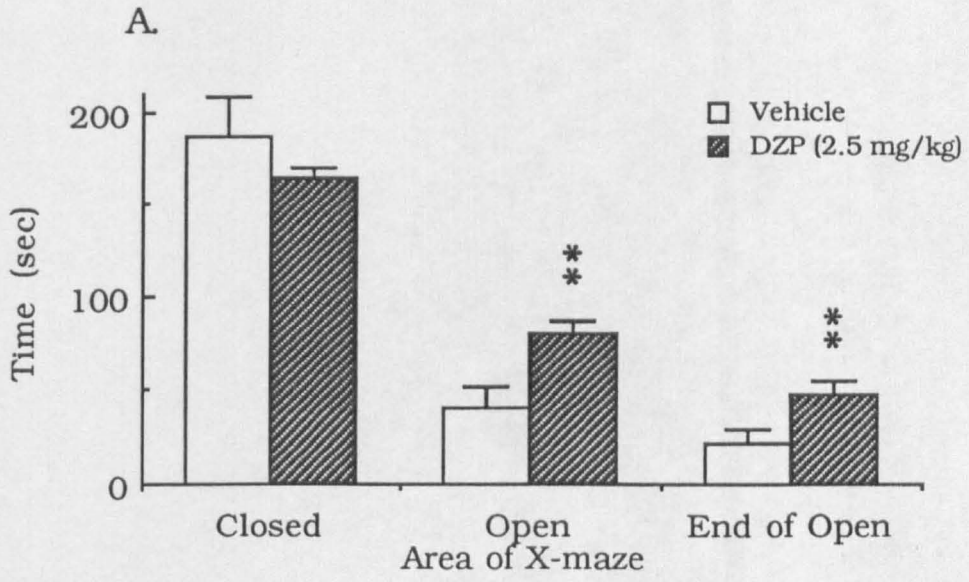

B.
Extracellular 5-HIAA (% of basal level)
120
100
80
60
Vehicle
F 2692 (10mg/kg)
X
0
50
100
150
200
250
Time (min)

**Figure 6**

Effects of acute administration of diazepam (DZP, 2.5mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 5 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for diazepam.

*$p<0.05$, **$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
Vehicle
DZP (2.5 mg/kg)
Time (sec)
200
100
0
**
**
Closed
Open
End of Open
Area of X-maze

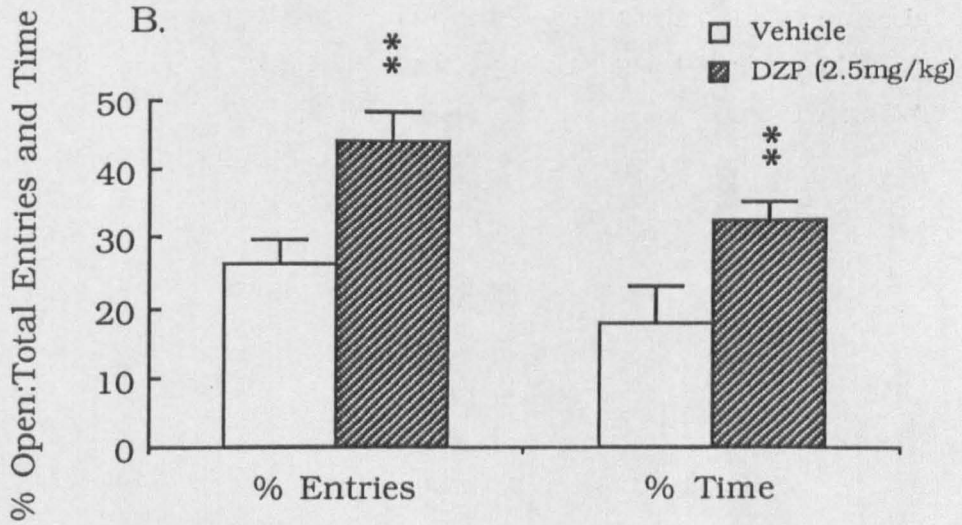
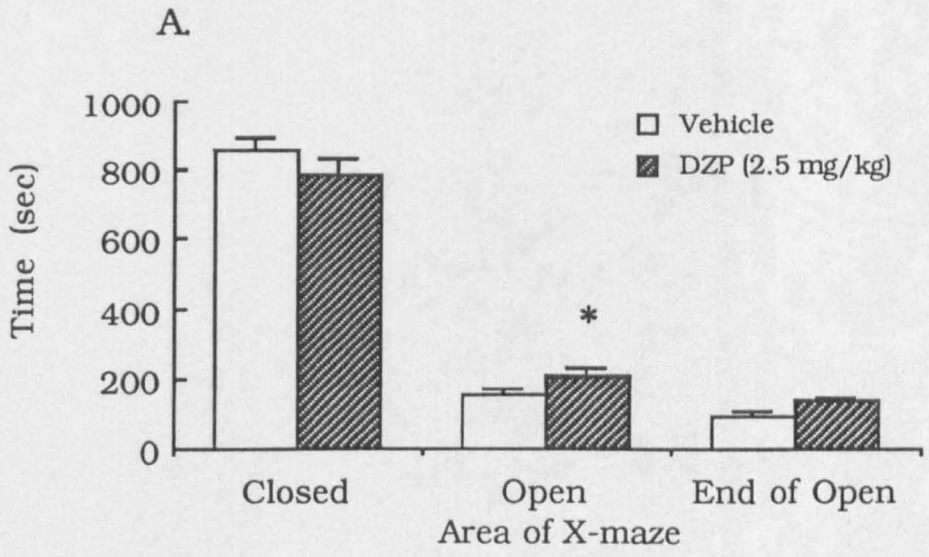

B.
Vehicle
DZP (2.5mg/kg)
% Open:Total Entries and Time
50
40
30
20
10
0
**
**
% Entries
% Time

**Figure 7**

Effects of acute administration of diazepam (DZP, 2.5mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 20 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for diazepam.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
1000
800
600
400
200
0
Time (sec)
Vehicle
DZP (2.5 mg/kg)
*
Closed
Open
End of Open
Area of X-maze

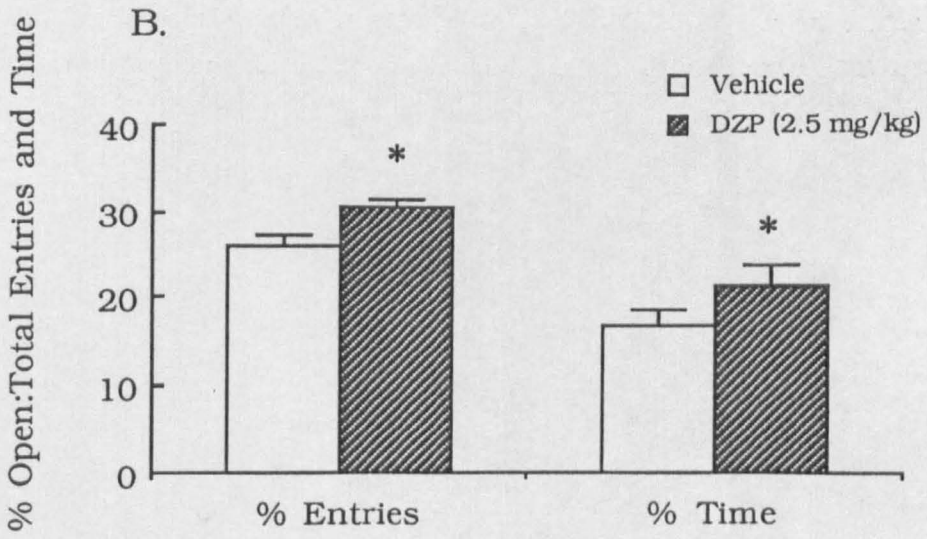

B.
40
30
20
10
0
% Open:Total Entries and Time
Vehicle
DZP (2.5 mg/kg)
*
*
% Entries
% Time

**Figure 8**

Effects of acute administration of ipsapirone (1mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 5 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for ipsapirone.

There was no significant difference from controls. 2-tailed Mann-Whitney U-test.

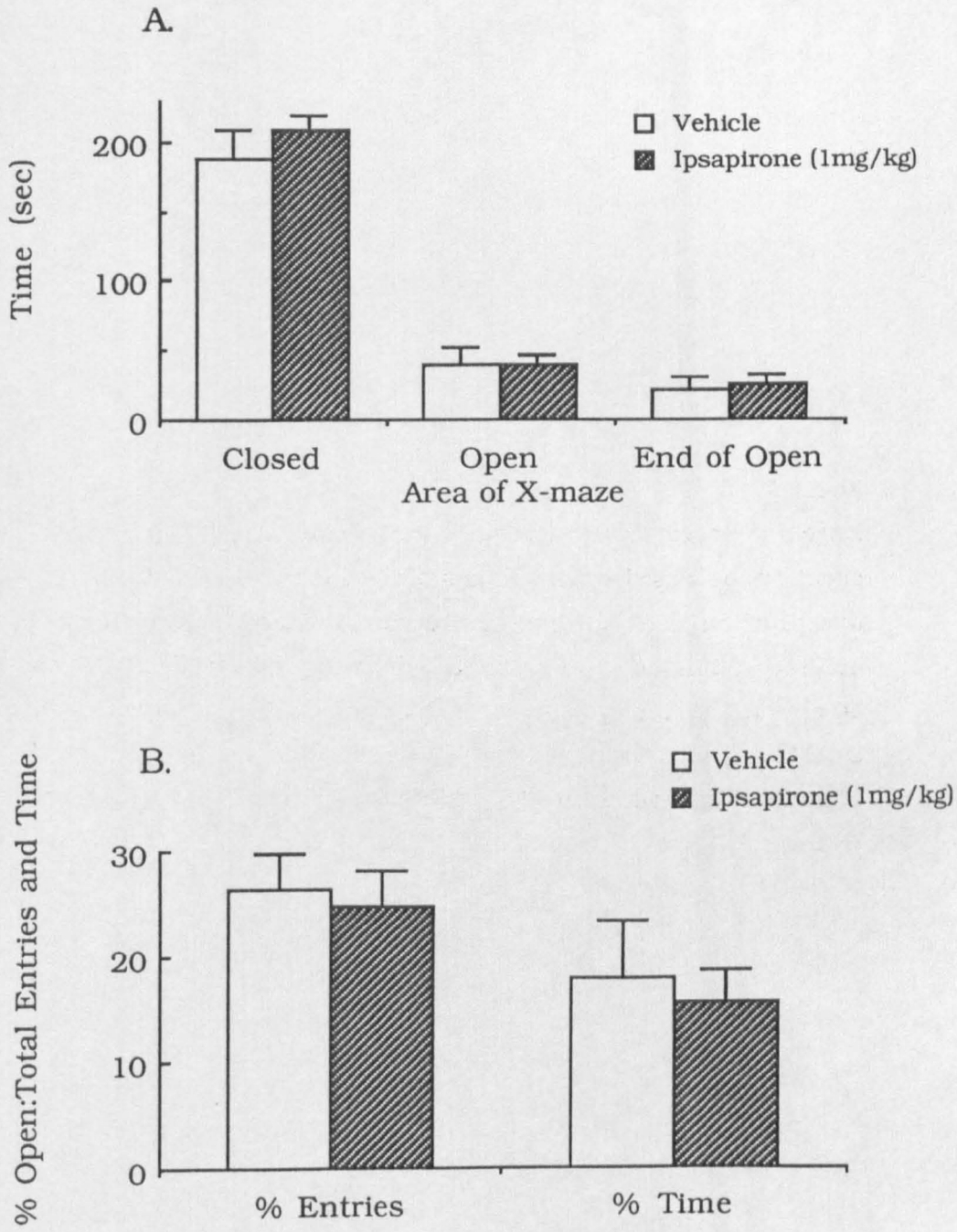
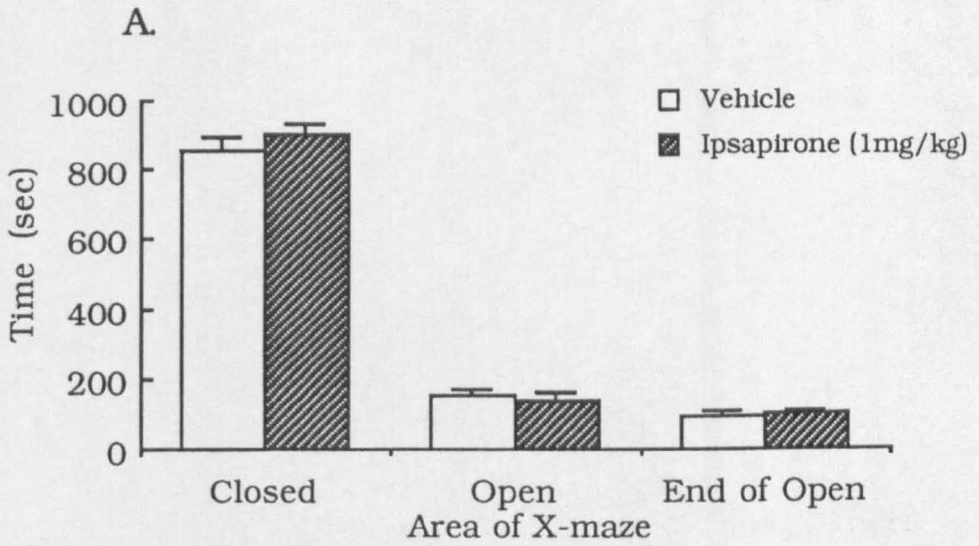
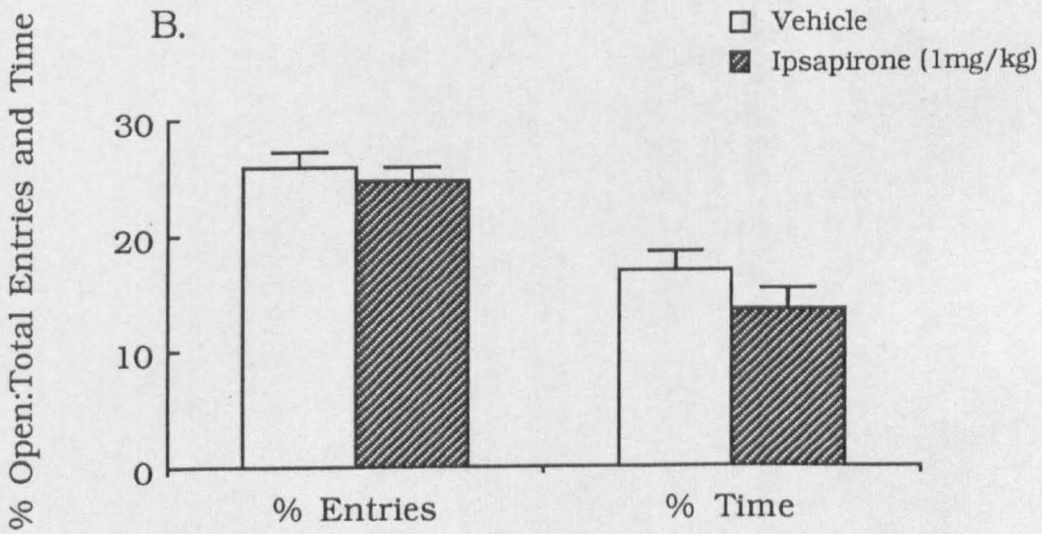

A.
Time (sec)
200
100
0
Vehicle
Ipsapirone (1mg/kg)
Closed
Open
End of Open
Area of X-maze
B.
% Open:Total Entries and Time
30
20
10
0
Vehicle
Ipsapirone (1mg/kg)
% Entries
% Time

**Figure 9**

Effects of acute administration of ipsapirone (1mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 20 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for ipsapirone.

There was no significant difference from controls. 2-tailed Mann-Whitney U-test.


A.
Vehicle
Ipsapirone (1mg/kg)
Time (sec)
1000
800
600
400
200
0
Closed
Open
End of Open
Area of X-maze


B.
Vehicle
Ipsapirone (1mg/kg)
% Open:Total Entries and Time
30
20
10
0
% Entries
% Time

and ends of open arms (Fig. 10A) and the percentage open:total entries and time (Fig. 10B) during the first 5 minutes on the X-maze. F 2692 also significantly increased the number of arm and end of arm entries compared to vehicle control (Table 1) during the first 5 minutes. After the full 20 minutes on the X-maze F 2692 had no significant effect upon any of the parameters scored (Fig. 11) although the percentage open:total entries approached significance ($p<0.1$) (Fig. 11B).

**Correlations**

For the vehicle control data correlations were carried out between the 'peak increase' in extracellular 5-HT (percentage increase above basal level when placed on the X-maze) and either:- (a) the percentage open:total entries (F test, $p=0.1037$, correlation coefficient = 0.554) (Fig. 12 A); (b) the percentage open:total time (F test, $p=0.3874$, correlation coefficient = 0.308) (Fig. 12B); (c) time spent on open arms (F test, $p=0.2647$, correlation coefficient = 0.390) (Fig. 13A); (d) time spent on the ends of the open arms (F test, $p= 0.241$, correlation coefficient = 0.409) (Fig. 13B) over the 20 minute exposure period. None of these showed any significant correlation.

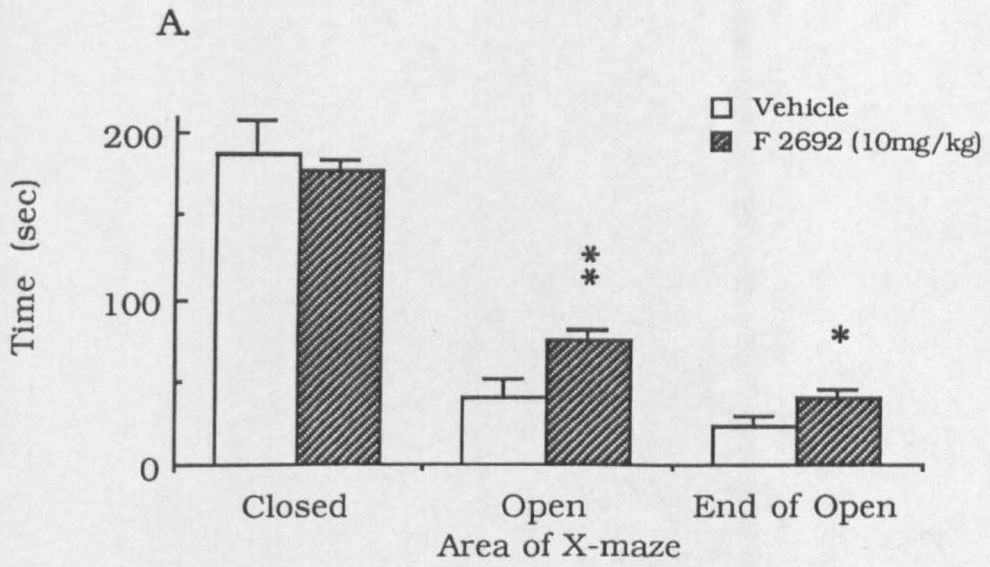
**DISCUSSION**

The present study, using *in vivo* microdialysis, demonstrated that 20 minute exposure on the X-maze results in increased levels of extracellular 5-HT which return back to basal levels 40 minutes after returning the animal to its home cage. Exposure to the X-maze had no effect on the extracellular 5-HIAA levels, supporting data from Chapter 4 where it was suggested that the extracellular 5-HIAA measured is not the product of the released 5-HT but is from the intraneuronal metabolism of unreleased 5-HT which then 'spills over' into the synaptic cleft (Grahame-Smith, 1971, 1974; Kuhn et al., 1986; Kalen et al., 1988; Auerbach et al., 1989; Sharp et al., 1989c; Crespi et al., 1990). Thus changes in 5-HIAA levels are not a good indication of corresponding changes in 5-HT levels. Generally, exposure to 'stress' occurring in a variety of different forms results in an increase in neurotransmitter release. Using microdialysis, increases in hippocampal 5-HT have been observed after handling and applying a tail-pinch (Kalen et al., 1989), and increases in dopamine in the nucleus accumbens and acetylcholine in the hippocampus observed after restraining the animals (Imperato et al., 1991). Thus the increase in 5-HT observed when the animal

**Figure 10**

Effects of acute administration of F 2692 (10mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 5 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for F 2692.

*$p<0.05$, **$p<0.01$ significantly different from controls. 2-tailed Mann-Whitney U-test.


A.
Vehicle
F 2692 (10mg/kg)
Time (sec)
200
100
0
**
*
Closed
Open
End of Open
Area of X-maze

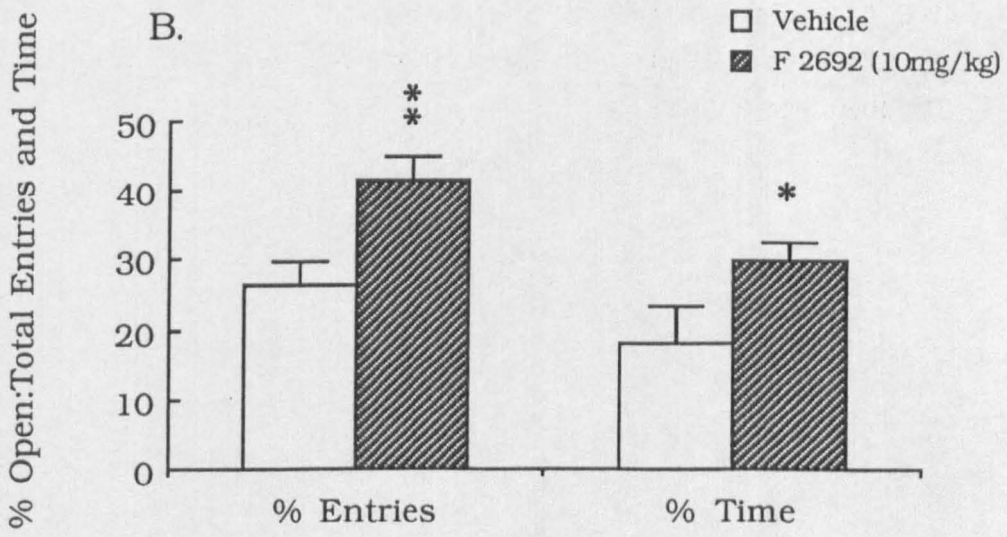
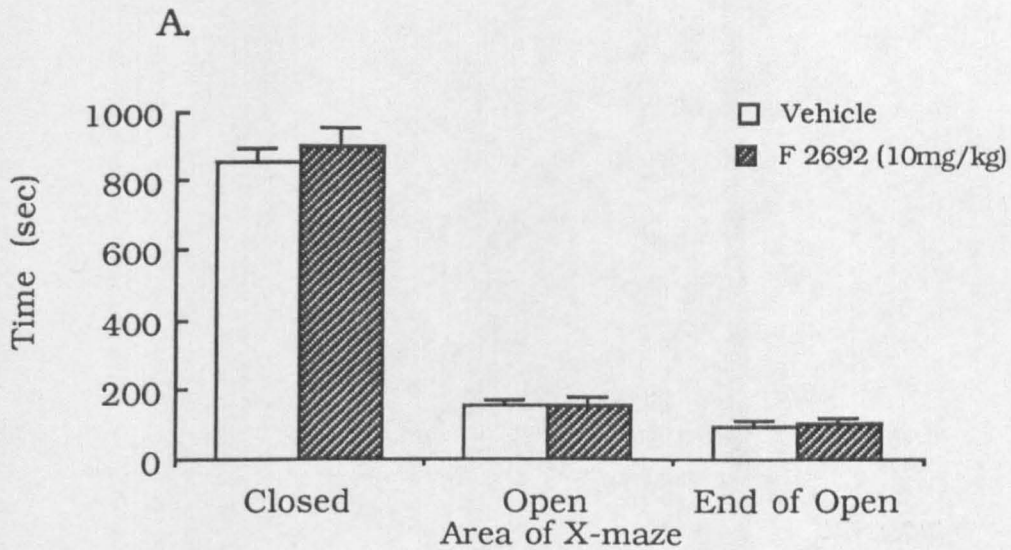

B.
Vehicle
F 2692 (10mg/kg)
% Open:Total Entries and Time
50
40
30
20
10
0
**
*
% Entries
% Time

**Figure 11**

Effects of acute administration of F 2692 (10mg/kg i.p.) on (A) the time spent on the closed, open and end of the open arms, and (B) the percentage open:total entries and time. Behaviour was monitored in the elevated X-maze for a 20 minute period (during which time microdialysis was continued) 40 minutes after drug administration. Data are the expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for F 2692.

There were no significant differences at the $p<0.05$ level. 2-tailed Mann-Whitney U-test.


A.
Vehicle
F 2692 (10mg/kg)
Time (sec)
1000
800
600
400
200
0
Closed
Open
End of Open
Area of X-maze

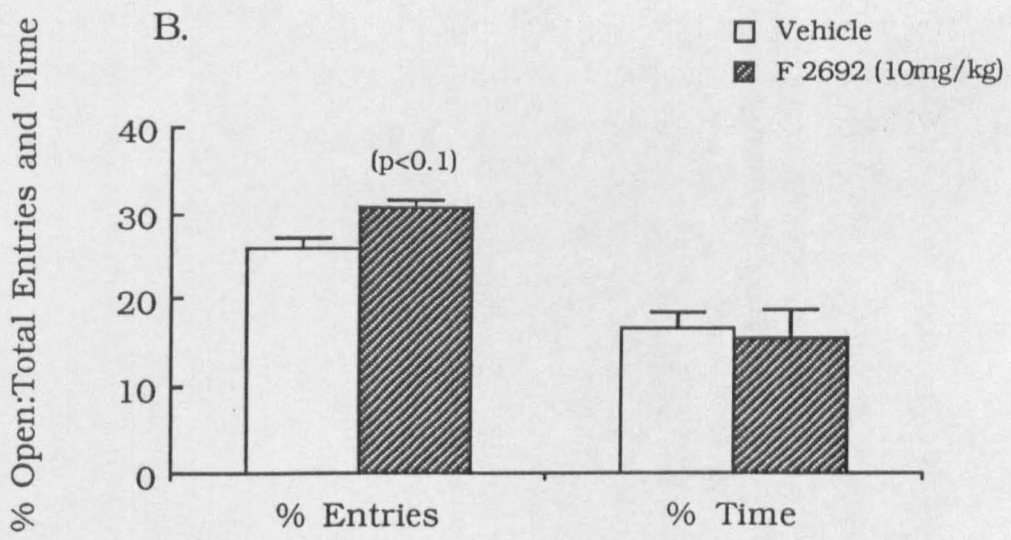

B.
Vehicle
F 2692 (10mg/kg)
% Open:Total Entries and Time
40
30
20
10
0
(p<0.1)
% Entries
% Time

**Table 1**

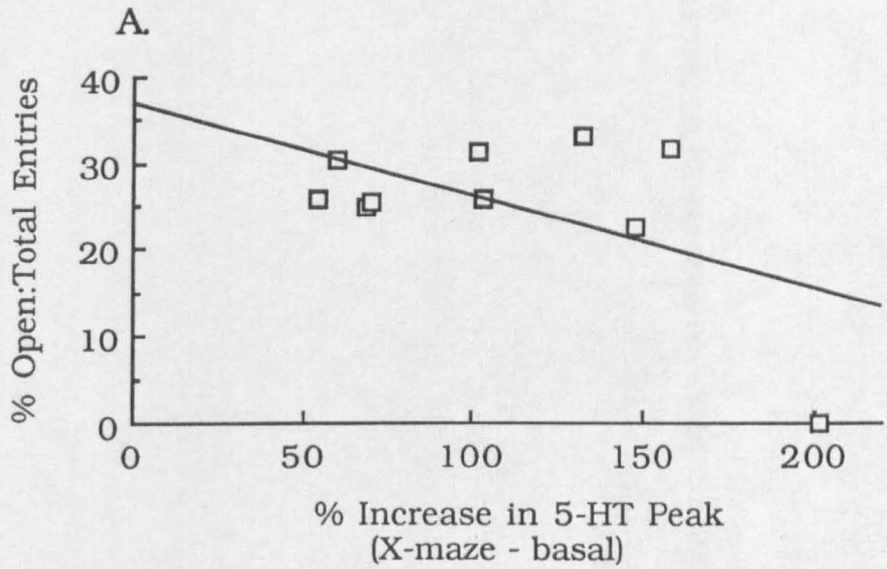
Effect of diazepam (2.5mg/kg i.p.), ipsapirone (1mg/kg i.p.) and F 2692 (10mg/kg i.p.) on the total number of arm and end of arm entries during 5 and 20 minutes exposure to the X-maze 40 minutes after injection. Results are expressed as the mean ± S.E.M., n=10 for vehicle and n=6 for drug.

*$p<0.05$ significantly different from controls. 2-tailed Mann-Whitney U-test.

| Treatment | No. of Arm and End of Arm Entries | |
|---|---|---|
| | 5 min | 20 min |
| Vehicle | 26.5 ± 1.3 | 92.7 ± 4.8 |
| Diazepam | 30.5 ± 3.3 | 93.5 ± 8.4 |
| Ipsapirone | 24.3 ± 1.9 | 83.5 ± 6.2 |
| F 2692 | 35.0 ± 4.4 * | 82.5 ± 9.9 |

**Figure 12**

Graph of increase in 5-HT peak (expressed as a percentage of the basal level) (x axis) verses (A) percentage open:total entries (y axis) and (B) percentage open:total time (y axis) over a 20 minute period on the X-maze. The line on each graph represents a simple regression analysis of 10 data points from the vehicle treated group only; (A) slope = -.106, correlation coefficient = .554, F test: p = .1037; (B) slope = -.05, correlation coefficient = .308, F test: p = .3874.


A.
% Open:Total Entries
40
30
20
10
0
0
50
100
150
200
% Increase in 5-HT Peak
(X-maze - basal)

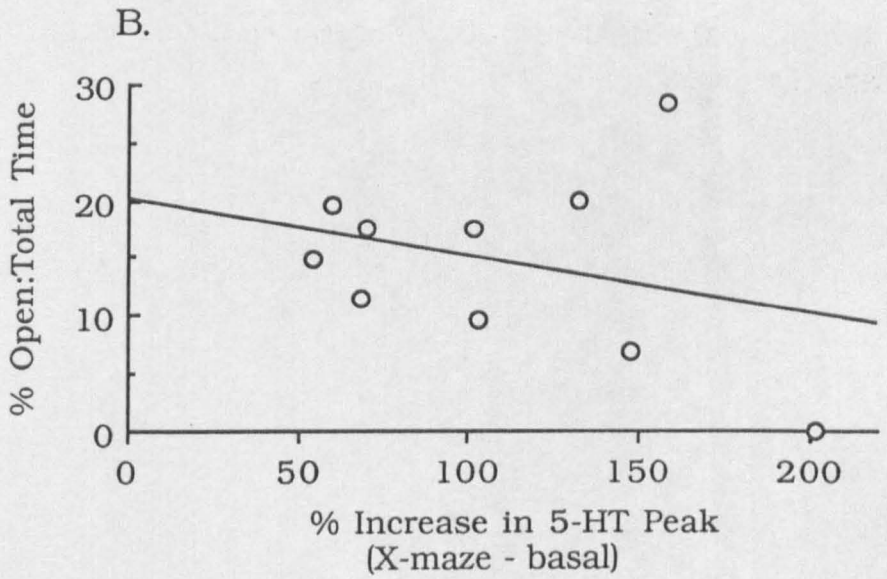
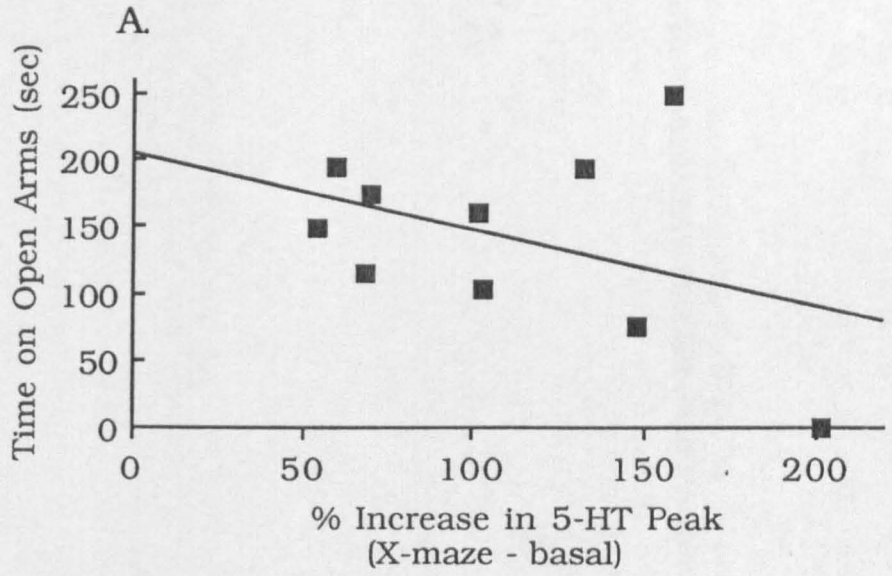
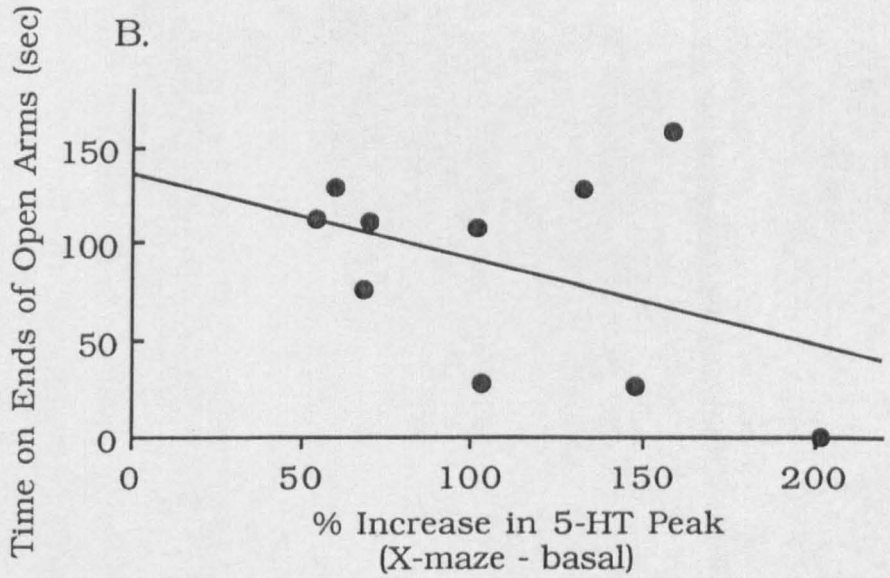

B.
% Open:Total Time
30
20
10
0
0
50
100
150
200
% Increase in 5-HT Peak
(X-maze - basal)

**Figure 13**

Graph of increase in 5-HT peak (expressed as a percentage of the basal level) (x axis) verses (A) time on the open arms (y axis) and (B) time spent on the ends of the open arms (y axis) over a 20 minute period on the X-maze. The line on each graph represents a simple regression analysis of 10 data points from the vehicle treated group only; (A) slope = -.565, correlation coefficient = .39, F test: p = .2647; (B) slope = -.441, correlation coefficient = .409, F test: p = .241.


A.
Time on Open Arms (sec)
250
200
150
100
50
0
0
50
100
150
200
% Increase in 5-HT Peak
(X-maze - basal)


B.
Time on Ends of Open Arms (sec)
150
100
50
0
0
50
100
150
200
% Increase in 5-HT Peak
(X-maze - basal)

is placed on the X-maze may occur as a result of 'stress' in a novel environment. It is also possible that the increased 5-HT levels may be a result of increased locomotor activity since it has been demonstrated that during the night (or dark phase) rats have greater locomotor activity and also greater 5-HT and noradrenaline release (Kalen et al., 1989). Thus, placing the rat on the X-maze results in exploration and increased activity compared to the home cage, and could contribute to the increased 5-HT levels. Using two different designs of X-maze Copland and Balfour (1987) were able to show a correlation between locomotor activity and 5-HT turnover in several brain regions, giving support for the hypothesis that 5-HT turnover in the hippocampus may be directly related to the level of spontaneous activity. However, in this study the number of arm and end of arm entries were recorded as an index of general locomotor activity and no significant differences were found between the vehicle controls and any of the drug treated groups after the 20 minute exposure period, suggesting that the drug-induced alterations in extracellular 5-HT seen in this study are not the result of changes in locomotor activity.

The increase in 5-HT observed when animals were restricted to the open arms appeared to be greater than that observed by isolation in the closed arms but this did not reach significance. It is thought that the open arms of the elevated X-maze cause greater 'fear' in the rat and hence it spends less time exploring this area, and this effect is reversed by the benzodiazepines. Thus it would be expected that restricting the animals to the open arms would result in a greater increase in 5-HT release compared to the closed arms, if 5-HT is involved in 'fear/anxiety'. A possible explanation as to why no significant difference was observed between the two different types of arm is that when the rat is restricted to the open arms it spends approximately 60 seconds exploring the open arms in the first two 5 minute periods and for the last 5 minute observation period (15 -20 minutes on the X-maze) it only ventures out onto the open arms for an average period of 3 seconds, i.e. the animal spends most of its time sat in the central square of the X-maze and not on the open arms. This demonstrates the aversion to the open arms upon which the model is based, and a greater increase in extracellular 5-HT may be observed if the animal was placed on an elevated runway which had no central square to act as a 'closed environment'. This is a critical experiment to determine whether changes in 5-HT levels are graded with anxiety.

In the vehicle control rats the increase in extracellular 5-HT was analysed for correlations with a range of behavioural measures of anxiety on the X-maze. For all of the behaviours scored except the percentage open:total entries there is no significant correlation between that behaviour and the increase in 5-HT when the animal is placed on the X-maze. For the percentage open:total entries the correlation reaches 10.37%, indicating that there may be some relationship between this parameter and 5-HT levels in the ventral hippocampus. It is possible that the greater the increase in 5-HT when the animal is placed on the X-maze the more 'anxious' it is and less likely to enter the open arms, thus reducing the percentage open:total entries. However, it is very difficult to say if this theory is correct, as it should be pointed out that the other parameters were not changed. Also a larger group of animals is required for a more accurate correlation.

Diazepam markedly inhibited the increase in extracellular 5-HT in the ventral hippocampus and had an anxiolytic profile over the 20 minute period when the rat was placed on the X-maze. The ability of diazepam to inhibit the increase in 5-HT when the animal was placed on the X-maze may be associated with the anxiolytic profile it produces in the same animal. Diazepam treated animals have significantly reduced increases in extracellular 5-HT when placed on the X-maze and significantly greater percentage open:total entries and time and spent more time on the open arms compared to vehicle controls. Diazepam also inhibited 5-HT release from the ventral hippocampus when the rat was returned to its home cage. It cannot be determined from this study whether the decrease in basal 5-HT levels when the animal is returned to the home cage is a direct result of diazepam, agreeing with a similar microdialysis study carried out by Pei et al. (1989b), or a result of exposure to the X-maze as diazepam produced a very marked reduction in increased 5-HT when on the X-maze and did not inhibit 5-HT levels in the home cage before exposure. The major innervation of the ventral hippocampus originates from the dorsal raphe (Azmitia and Segal, 1978) and using autoradiography and light-microscopic immuno-cytochemistry it has been demonstrated that there is a high density of the $GABA_A$/benzodiazepine receptor complex in both the dorsal raphe and ventral hippocampus (Squires and Braestrup, 1977; De Blas et al., 1988). In agreement with this finding and the one by Pei et al. (1989b) that systemic diazepam decreased extracellular 5-HT in the ventral hippocampus, iontophoretic or systemic administration of benzodiazepines inhibit 5-HT

cell firing in the dorsal raphe and potentiate the inhibitory action of GABA agonists (Gallager, 1978; Trulson et al., 1982). It has been shown using synaptosomal preparation of hippocampus that GABA and diazepam decrease $K^+$-evoked release of [$^3$H]-5-HT (Balfour, 1980), and with microdialysis that local perfusion of flurazepam decreases extracellular 5-HT levels (Pei et al., 1989b). Thus in this study diazepam may be having its inhibitory effect by acting at the dorsal raphe and also the 5-HT terminal region in the hippocampus. The data from this part of the study suggest that reducing 5-HT release may be one of the mechanisms of action of the benzodiazepines which is important for their anxiolytic activity.

Ipsapirone treated animals did not differ in their behaviour from vehicle controls over the 20 minute period on the X-maze. Similar results were observed with this dose of ipsapirone (1 mg/kg) when placed on the maze for a 5 minute period, and the possible reasons for this behavioural profile are discussed in Chapter 3. However, ipsapirone was seen to significantly reduce the increase in extracellular 5-HT when the animals were placed on the X-maze and to reduce basal levels when the animal was returned to its home cage. Ipsapirone and two structural analogues, buspirone and gepirone, all inhibit dorsal raphe neurone firing, mimicking the effect of 5-HT (Dourish et al., 1986; Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Wilkinson et al., 1987). In the hippocampus all three compounds produced only the inhibitory component of the action of 5-HT (Rowan and Anwyl, 1987; Van der Maelen et al., 1986; Martin and Mason, 1987). This group of 5-$HT_{1A}$ partial agonists have been found to decrease 5-HT release using either *in vivo* microdialysis or differential pulse voltammetry (Crespi et al., 1990; Hjorth and Magnusson; 1988; Hutson et al., 1989; Sharp et al., 1989a). This effect is thought to be predominantly as a result of action at the somatodendritic autoreceptor in the dorsal raphe nucleus (Hjorth and Magnusson, 1988; Hutson et al., 1989; Sharp et al., 1989c). The ability of ipsapirone to mimic the effects of diazepam on 5-HT release would suggest that the inhibition of the increase in extracellular 5-HT observed with diazepam is not important for its anxiolytic activity. However, 5-$HT_{1A}$ receptors are found not only on the cell bodies/dendrites of 5-HT neurones in the raphe nuclei but also at postsynaptic sites in the limbic terminal regions (Hoyer et al. 1986a). Thus, even though ipsapirone is inhibiting 5-HT release in the hippocampus via an action at the raphe nuclei it may also be stimulating postsynaptic 5-$HT_{1A}$ receptors in the hippocampus to have an overall effect of not altering 5-HT

neuronal activity, resulting in a behavioural profile that it not different from the vehicle treated animals.

F 2692 had an anxiolytic profile in the X-maze when placed on it for a 5 minute period, agreeing with the results of Assie et al. (1991). However, the anxiolytic profile was lost when the animals were placed on the X-maze for a 20 minute period. F 2692 was synthesized as a non-benzodiazepine which acts at the benzodiazepine-$GABA_A$ receptor complex (in the hope that the benzodiazepine side effects would be absent), and a possible reason for the absence of an anxiolytic profile after 20 minutes is that F 2692 has been reported to have sedative effects at 30 mg/kg i.p. (Marsden, unpublished observations) using the same X-maze and protocol as this study for a 5 minute time period, and hence sedative effects may become apparent on the X-maze at 10 mg/kg when the animal is exposed for a 20 minute period. Looking at the number of arm and end of arm entries, it can be seen that F 2692 significantly increased the number of entries after 5 minutes but not 20 minutes, indicating the onset of sedation in the remaining 15 minutes. F 2692 reduces both the increase in extracellular 5-HT when the animal is placed on the X-maze and the basal levels when the animal is returned to its home cage. The mechanism by which it does this is at present unknown as it has low affinity for the benzodiazepine site (Assie et al., 1991) and the effect of F 2692 on the X-maze can be reversed with flumazenil but only at high doses (20 mg/kg i.p.) (Assie et al., 1991) suggesting that it is only having a weak effect at the benzodiazepine site. The side effects of this compound are very similar to those of the benzodiazepines, suggesting that F 2692 may be acting through the benzodiazepine-$GABA_A$ receptor complex but not necessarily at the benzodiazepine site. Thus, the decrease in extracellular 5-HT, both on the X-maze and when returned to the home cage, seen with F 2692 may be a result of its action somewhere on the benzodiazepine-$GABA_A$ receptor complex. The ability of F 2692 to reduce the increase in extracellular 5-HT observed when the rat is placed on the X-maze may be associated with its anxiolytic activity provided that sedative effects were predominant after 20 minutes.

In summary, placing a rat on the X-maze increased the extracellular levels of 5-HT in the ventral hippocampus but the correlation between 'increased 5-HT' and 'anxious behaviour' was not good. Thus it is important to determine whether there is a significant difference in the increased 5-HT levels when

animals are restricted to either the open or the closed arms. It is possible that the lack of correlation may be a result of the 20 minute time period, and small discrete effects occurring within the first 5 minutes may be missed, so it is necessary to try to increase the sensitivity of the assay so that 5 minutes samples can be analysed. Diazepam, ipsapirone and F 2692 all reduced this increase in extracellular 5-HT and decreased basal 5-HT levels when the animal was returned to its home cage. Diazepam and F 2692 produced an anxiolytic profile when on the X-maze for 5 minutes but only diazepam produced an anxiolytic profile after 20 minutes exposure. It is possible that the reduced increase in extracellular 5-HT after diazepam pretreatment may be one of the mechanisms by which diazepam has its anxiolytic action. The decrease in 5-HT release being important for anxiolytic activity is supported by F 2692, an anxiolytic with weak activity at the benzodiazepine-$GABA_A$ site, also reducing extracellular 5-HT and having an anxiolytic profile. It would be useful to try lower doses of F 2692 where the sedative effects may not be as prenounced. However, against this theory is the ability of ipsapirone to reduce extracellular 5-HT and not to have any anxiolytic activity in this study, but it is possible that post-synaptic 5-$HT_{1A}$ receptor stimulation by ipsapirone in the hippocampus and other limbic regions may have the net effect of not changing 5-HT function in these animals. It may be useful to try lower doses ipsapirone which are more selective for the somatodendritic autoreceptor in an attempt ot eliminate any postsynaptic 5-$HT_{1A}$ receptor stimulation.

# CHAPTER 6

# THE EFFECTS OF ISOLATION-REARING AND SUBSEQUENT RESOCIALISATION ON THE BEHAVIOUR OF RATS ON THE ELEVATED X-MAZE MODEL OF ANXIETY

## INTRODUCTION

The rearing conditions (social environment) to which an animal is exposed in the early stages of life have been shown to influence its subsequent behaviour. One of the most prominent and reproducible findings following social isolation is increased locomotor activity, and this is found in many species including rats (Syme, 1973; Morley and Worsham, 1978), mice (Essman, 1966; Wilmot et al., 1986), monkeys (Mason et al., 1968), cats (Seitz, 1959) and dogs (Fox and Stelzner, 1966). Isolation-reared rats show increases in locomotor activity when placed in a novel environment with no change in the spontaneous activity (when habituated to the test environment) (Sahakian et al., 1975, 1977). In the rat, social isolation has been reported to produce other behavioural disturbances such as reduced (Parker and Morinan, 1986) or enhanced (Sahakian et al., 1977; Einon and Morgan, 1977) exploratory behaviour, and a possible anxiogenic profile in the elevated X-maze model of anxiety (Morinan and Parker, 1985).

Few studies have examined the relative permanence of isolation-induced changes. Resocialisation can be effective in reversing many, but not all, of the effects of social isolation. Gentsch et al. (1988) demonstrated that the hyperactivity and greater tail-flick latencies in isolation-reared rats could be reversed by resocialisation, as can maze learning deficits (Wood and Greenough, 1974). Other experiments have failed to detect recovery from isolation-induced impairments, particularly if isolation was introduced at weaning age (Einon and Morgan, 1977; File, 1978; Kraemer et al., 1984). This might be expected as brain maturation processes would be more susceptible to permanent change at an early age. Hypertension (Naranjo and Fuentes, 1985) and neurochemical changes (Blanc et al., 1980) induced by isolation of mature rats are completely reversed by resocialisation, supporting this theory.

The initial aim of this study was to investigate the effects of social isolation of rats at weaning on their behaviour in the elevated X-maze model of anxiety (Handley and Mithani, 1984). Furthermore, to then determine whether resocialisation of the rats reared in isolation from weaning reversed any behavioural changes observed in the X-maze and if isolation of the socially-reared group when adult produced behaviour similar to that seen when isolation was induced at weaning.

## METHODS

48 male Lister hooded rats (Nottingham University Medical School Animal Unit) obtained at weaning (21 days of age) were divided into the two rearing condition groups counterbalanced by weight. Rats were housed either individually or in social groups of four per cage for the duration of the first part of the experiment. All cages were constructed of plastic and were lined with sawdust, so subjects had to be removed from their cages for cleaning. The isolation-reared rats were housed singly in a cage 41cm x 26cm x 20cm high. The social group cages were 52cm x 32cm x 20cm high. Both groups had food and water available ad libitum. All animals were housed in the same holding room with a 12 hr. light:12 hr. dark cycle (0700-1900 lights on). It is important to note that the isolated animals still had auditory, visual, and olfactory contact with the other animals and hence the study is concerned only with the effects of social isolation.

30 days post-weaning the animals were monitored in locomotor activity cages (described below) and 3 days later monitored on the elevated X-maze (described below). After testing, half of the isolation-reared animals remained isolated (n=12) (isolation-reared) and the other half were socially housed in groups of four (n=12) (isolation-reared and then socially-housed), and half of the socially-reared animals remained in groups of four (n=12) (socially-reared) and the others isolated (n=12) (socially-reared and then isolated); the regrouping being counterbalanced by the rats behaviour on the elevated X-maze. 30 days post-rehousing the animals were retested on the elevated X-maze.

### Spontaneous Locomotor Activity

#### Procedure

Animals were placed in Perspex cages (25cm x 18cm x 18cm)with wire mesh tops, and each cage was located between two photocells. An infrared beam ran between the photocells and movement of the animal from one half of the cage to the other resulted in this beam being broken and an incremental count being recorded. Counts were recorded every 5 min for a period of 1 hr immediately after the animal was placed in the cage. Activity was monitored between 1030 and 1430 hrs.

**Elevated X-maze**

**Apparatus and Procedure**

The elevated X-maze apparatus and protocol was identical to that described in Chapter 2 with the exception that the X-maze was only elevated to a height of 50cm and the animals were not isolated for a 5 minute period before being exposed to the X-maze because the effects of isolation are being studied and thus socially-housed animals should not be isolated.

**Statistics**

All results are expressed as the mean ± standard error of the mean (S.E.M.). The ratio of open:total arm entries and open to total time was calculated for each rat individually and the mean ± S.E.M. for each treatment group presented.

All data after 1 month (2 groups) were analysed using 2-tailed Mann-Whitney U-test.

All data after 1 month rehousing (2 months from original housing) (4 groups) were analysed using Kruskal Wallis one way analysis of variance by ranks test with post-hoc Dunn's test.

**RESULTS**

**Spontaneous Locomotor Activity**

Rats reared in isolation were spontaneously hyperactive when placed in the novel environment compared to the socially reared controls but the hyperactivity only occurred between 5 and 35 min in the activity cages (Fig. 1). After this time there was no significant difference in the number of counts between the isolation- and socially-reared animals.

**X-maze**

Isolation-reared rats spent significantly less time on the open and the end of the open arms (Fig. 2A) and had significantly reduced percentage open/total entries and time (Fig. 2B) but no difference was observed in the time spent on the enclosed arms (Fig. 2A) or the total number of arm and end of arm entries (Fig. 2B).

**Figure 1**

Locomotor activity measured in 5 minute intervals for a period of 60 minutes in isolation- and social-reared rats, expressed as photobeam interruptions. Results are expressed as the mean number of interruptions ± S.E.M.; n=24 in each group.

*$p<0.05$ significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.

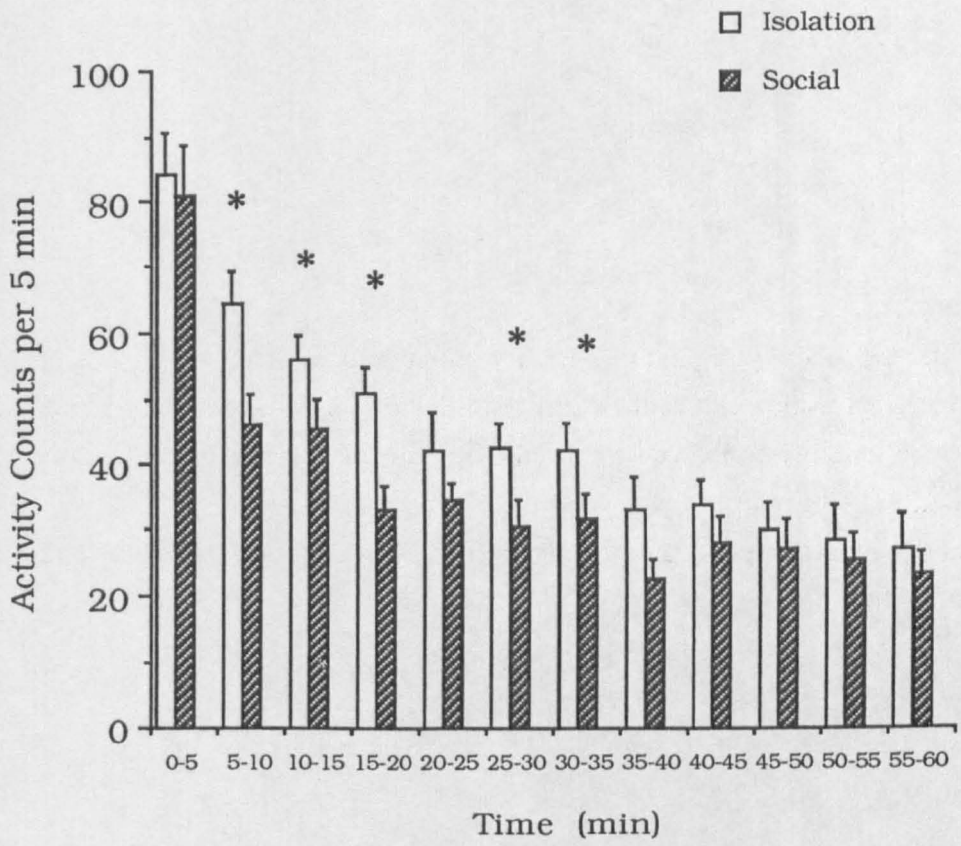
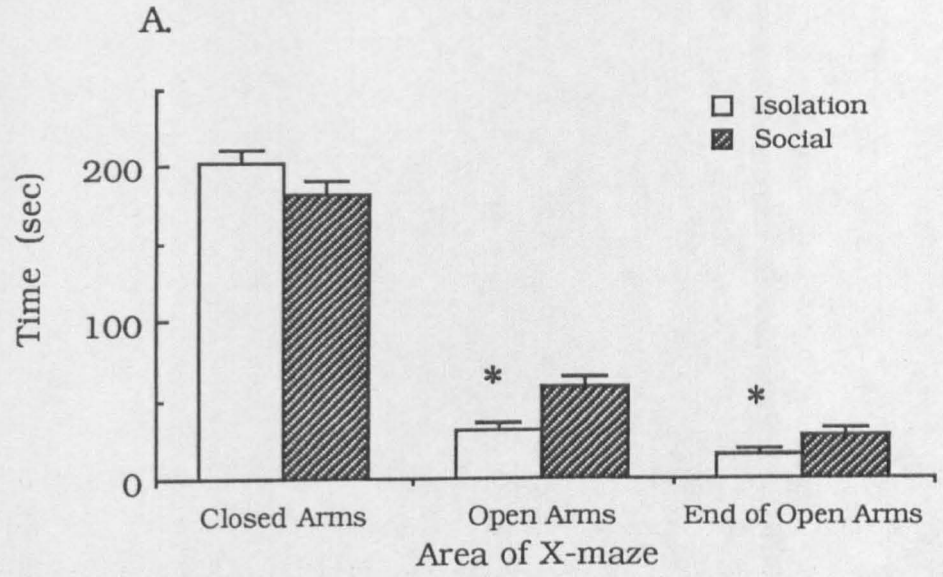
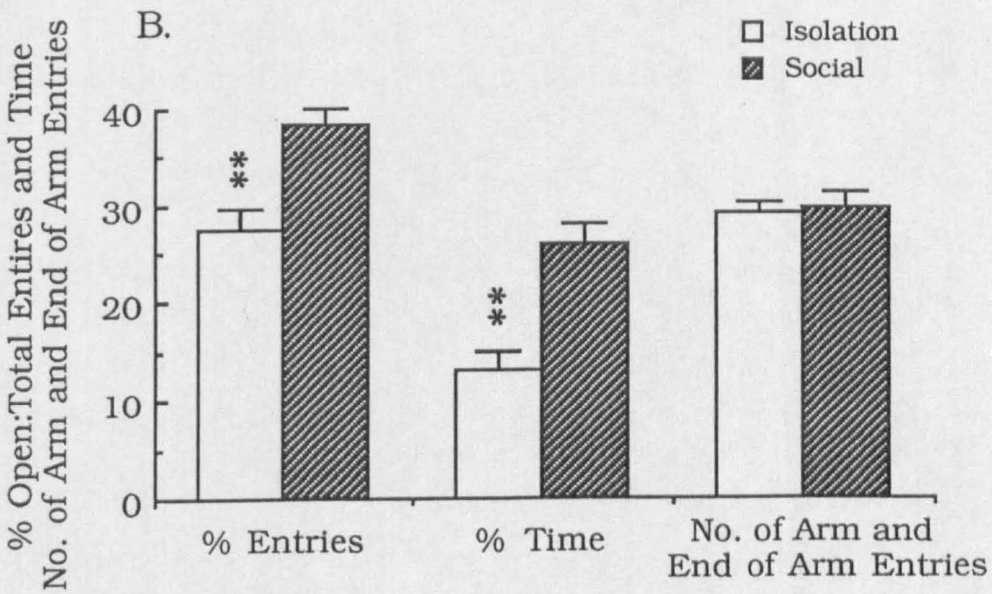

Isolation
Social
100
80
60
40
20
0
Activity Counts per 5 min
*
*
*
*
*
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
Time (min)

**Figure 2**

(A) Time spent in the closed arms, open arms and end of open arms, and (B) percentage open:total entries and time and total number of arm and end of arm entries in isolation- and socially-reared rats during 5 minute exposure on the elevated X-maze. Results are expressed as the mean ± S.E.M.; n=24 in each group.

*$p<0.05$ significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.


A.
Isolation
Social
Time (sec)
200
100
0
*
*
Closed Arms
Open Arms
End of Open Arms
Area of X-maze


B.
Isolation
Social
% Open:Total Entires and Time
No. of Arm and End of Arm Entries
40
30
20
10
0
**
**
% Entries
% Time
No. of Arm and
End of Arm Entries

**Resocialisation**

**X-maze**

There were no significant differences between any of the 4 groups of animals in the time spent on the enclosed arms of the X-maze (Fig. 3A) or in the total number of arm and end of arm entries (Fig. 3B). However, animals reared in isolation throughout the experiment and those isolated at weaning and then socially housed spent significantly less time on the open and ends of open arms compared to the socially-reared throughout group (Fig. 3A). The behaviour in the X-maze of rats kept in isolation throughout the experiment was not significantly different from those kept in isolation then rehoused in groups. This was also true for rats housed in social groups throughout and those housed in groups then isolated (Fig. 3A and 3B).

**DISCUSSION**

In agreement with previous results (Morley and Worsham, 1978; Sahakian et al., 1975) isolation-reared animals were hyperactive compared to socially reared controls when placed in a novel environment. After a period of about 35 min the animals had habituated to the environment and no difference in locomotor activity was observed between the two groups, again agreeing with work by Sahakian at al. (1975; 1977). This part of the study confirms that the initial rearing conditions used in our laboratory produced effects comparable with those reported in the literature.

In the elevated X-maze, isolation-reared rats showed a significant reduction in the time on the open and ends of open arms and in the percentage open:total entries and time compared to socially-reared controls. There was no significant difference in the total number of arm and end of arm entries, indicating that reduced exploration of the open arms is not a result of alterations in locomotor activity. The anxiolytic activity of benzodiazepine-like compounds can be detected on the X-maze as an increase in time spent exploring the open arms (Chapter 2; Pellow et al., 1985), and conversely, compounds with anxiogenic activity, such as $\alpha_2$-adrenoceptor antagonists, decrease the time spent in the open arms (Chapter 2; Handley and Mithani, 1984). This suggests that isolation-reared rats display an anxiogenic profile compared to socially-housed controls when placed on the X-maze, and that this is not as a result of the increased locomotor activity observed in these animals. These results are in agreement with a previous

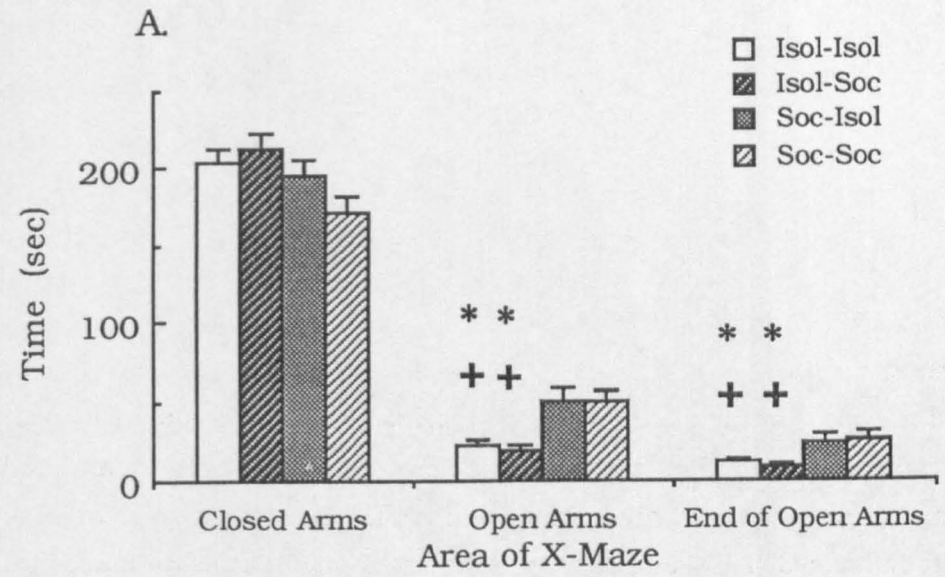
**Figure 3**

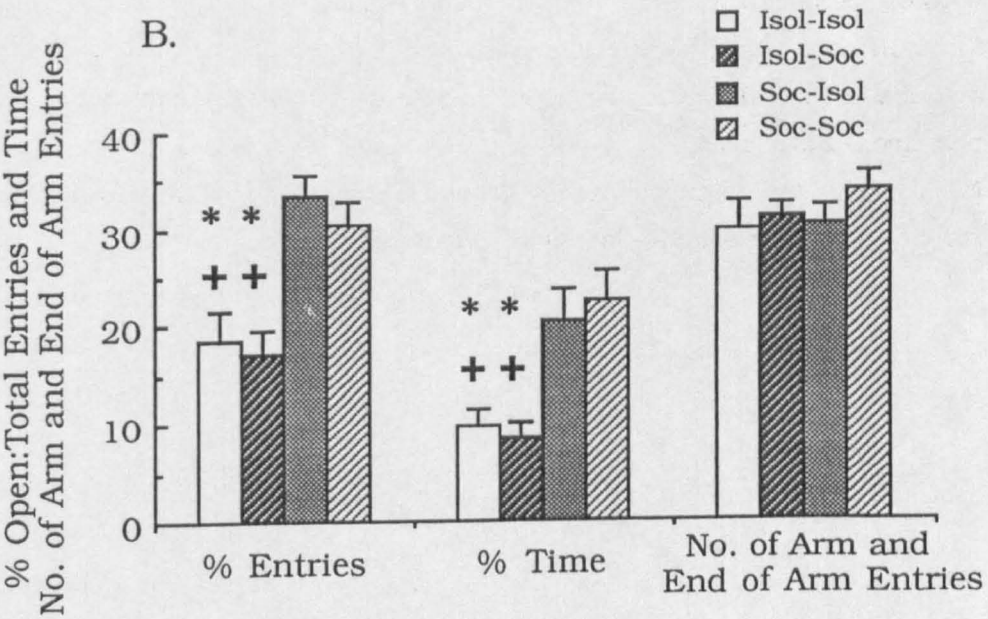
(A) Time spent in the closed arms, open arms and end of open arms, and (B) percentage open:total entries and time and total number of arm and end of arm entries in isolation-reared (Isol-Isol), isolation-reared and then socially-housed (Isol-Soc), socially-reared and then isolation-housed (Soc-Isol), and socially-reared (Soc-Soc) rats during 5 minute exposure on the elevated X-maze. Results are expressed as the mean ± S.E.M.; n=12 in each group.

*$p<0.05$ significantly different from socially-reared animals.

$^{+}p<0.05$ significantly different from socially-reared and then isolated animals. Kruskal Wallis one way analysis of variance by ranks test with post-hoc Dunn's test.

No significant differences were observed between either the Isol-Isol and Isol-Soc groups or the Soc-Soc and Soc-Isol groups.


A.
Isol-Isol
Isol-Soc
Soc-Isol
Soc-Soc
Time (sec)
200
100
0
* *
+ +
* *
+ +
Closed Arms
Open Arms
End of Open Arms
Area of X-Maze


B.
Isol-Isol
Isol-Soc
Soc-Isol
Soc-Soc
% Open:Total Entries and Time
No. of Arm and End of Arm Entries
40
30
20
10
0
* *
+ +
* *
+ +
% Entries
% Time
No. of Arm and
End of Arm Entries

study (Parker and Morinan, 1986), and indicate that the changes seen in locomotor activity and behaviour on the X-maze are via two different mechanisms.

Resocialisation of isolation-reared rats did not alter the behaviour of these animals on the X-maze. There was no significant difference between the rats reared and kept in isolation throughout and the rats reared in isolation then socially-housed. Both groups explored the open arms significantly less than the socially-reared throughout group. Thus, after 30 days resocialisation the animals that were isolation-reared and then socially-housed displayed a statistically significant anxiogenic profile compared to socially-reared throughout controls. Socially-reared rats that were isolated when adult displayed no significantly different behaviour on the X-maze compared to the socially-reared throughout group. This indicates that isolation of adult rats does not produce the anxiogenic profile observed on the X-maze in rats isolated at weaning. Further, resocialisation of the isolation-reared animals does not reverse this anxiogenic profile, thus the changes observed may indicate a permanent developmental change. The behaviour of rats after 30 (initial isolation) and 60 days (isolation-reared throughout animals) isolation from weaning was very similar, suggesting that the effects observed are a result of developmental changes from weaning which do not appear to markedly increase after a period of 30 days isolation. It has yet to be determined the time after weaning at which isolation can be introduced and these behavioural differences are no longer observed. Also, handling during the development of the isolation-reared animals, which has been shown to reverse some changes such as hyperactivity and tail-flick latency (Gentsch et al., 1988), may reverse the behavioural changes observed in this study.

In summary, rats reared in isolation from weaning display an anxiogenic profile on the elevated X-maze compared to socially-reared controls, and this anxiogenic profile is not observed when adult rats are isolated. Further, resocialisation of the isolation-reared rats does not reverse the anxiogenic profile. Thus the changes observed in the isolation-reared rats appear to be developmental and long lasting as they are not reversed by resocialisation. Usually in the elevated X-maze compounds such as FG7142 or idazoxan are normally used to induce an anxiogenic profile; however, with isolation-reared rats the same profile is observed and it would be of interest to determine whether there are any neurochemical changes in pre- and

postsynaptic 5-HT function and if these are related to the behavioural changes reported here.

# CHAPTER 7

# CHANGES IN PRE- AND POSTSYNAPTIC RECEPTOR FUNCTION IN ISOLATION-REARED RATS.

## INTRODUCTION

As discussed in the Introduction to the thesis isolated rats demonstrate many behavioural disturbances such as increased locomotor activity (Syme, 1973; Morley and Worsham, 1978), reduced (Parker and Morinan, 1985) or enhanced (Einon and Morgan, 1977; Sahakian et al., 1977) exploratory behaviour and deficits in both learning and spatial memory tasks (Juraska et al., 1984). It has been demonstrated in Chapter 6 that isolation-reared animals have an anxiogenic profile on the elevated X-maze compared to socially housed controls and that this effect is not reversed by resocialisation. These and other disturbances define the "isolation syndrome" (Morgan et al., 1975; Morinan and Parker, 1985; Sahakian et al., 1977). The central dopaminergic system has been a focus of research on the biochemical effects of isolation rearing for some years now. For example, isolates show greater increases in frontal cortex dopamine (DA) metabolism in response to footshock (Blanc et al., 1980) and DA release stimulated by amphetamine is increased (Jones et al., 1988, 1990) as well as other indices of dopaminergic function (Weinstock et al 1978; Guisado et al., 1980). However limited data are available on the "isolation syndrome" and central 5-HT systems and contrasting reports have suggested either an increase (File and Vellucci, 1978) or a decrease (Morinan and Parker, 1985) in the turnover of central 5-HT.

Isolation-reared animals have an anxiogenic profile compared to socially-housed controls and with the implications of the involvement of 5-HT in anxiety the aim of this study was to determine whether there are changes in pre- and post-synaptic 5-HT function in isolation-reared animals. Presynaptic changes were investigated by studying the effects of pharmacological stimulation on 5-HT release measured in the frontal cortex of isolated rats in comparison with socially reared controls using intracerebral microdialysis. The functional state of the post-synaptic 5-$HT_{1A}$ and 5-$HT_2$ receptors was investigated using the '5-HT behavioural syndrome'. This was observed following administration of 5-HT precursors such as L-tryptophan or 5-hydroxytryptophan (5-HTP) in the presence of a peripheral decarboxylase inhibitor (Grahame-Smith, 1971; Jacobs, 1976; Green et al., 1983b). Components of the 5-HT syndrome are also observed after administration of the 5-$HT_{1A}$ full agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT; Hjorth et al., 1982; Tricklebank et al.,

1985a; Goodwin and Green, 1985) and consists of hyperlocomotion, hindlimb abduction, head weaving, flat body posture and reciprocal forepaw treading (Hjorth et al., 1982; Tricklebank et al., 1985a). It is thought that the flat body posture and reciprocal forepaw treading are mediated by post-synaptic 5-$HT_{1A}$ receptors (Tricklebank et al., 1985a). The main behavioural response seen after 5-$HT_2$ receptor agonists is the head-twitch response in mice (Corne et al., 1963; Corne and Pickering, 1967; Goodwin and Green, 1985; Heaton et al., 1988) and the 'wet-dog shake' (WDS) response in rats (Bedard and Pycock, 1977; Mathews and Smith, 1980; Goodwin and Green, 1985). Intrathecal administration of the selective 5-$HT_2$ agonists 2,5-dimethoxy-α,4-dimethyl-2-aminopropane (DOM) and 1-(2,5-dimethoxy-4-iodomethyl)-2-amino-propane (DOI) produces WDS and also back muscle contractions; both behaviours can be antagonised by ketanserin, a 5-$HT_2$ antagonist (Fone et al., 1989, 1990). Thus the functional states of the post-synaptic 5-$HT_{1A}$ and 5-$HT_2$ receptors were studied by observing the '5-HT behavioural syndrome' induced by the non-selective 5-HT receptor agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), the relatively selective 5-$HT_{1A}$ full agonist 8-OH-DPAT and the 5-$HT_{2/1C}$ agonist DOI.

## METHODS

### a) *In Vivo* Microdialysis

#### Animals

15 male Lister hooded rats (Nottingham University Medical School Animal Unit) obtained at weaning (21 days of age) were divided into the two rearing condition groups counterbalanced by weight. Rats were housed either individually (n=7) or in social groups of four per cage (two groups of n=4) for the duration of the experiment. All cages were constructed of plastic and were lined with sawdust, so subjects had to be removed from their cages for cleaning. The isolation-reared rats were housed singly in a cage 41cm x 26cm x 20cm high. The social group cages were 52cm x 32cm x 20cm high. Both groups had food and water available *ad libitum*. All animals were housed in the same holding room with a 12 hr. light:12 hr. dark cycle (0700-1900 lights on). It is important to note that the isolated animals still had auditory, visual, and olfactory contact with the other animals and hence the study is concerned only with the effects of social isolation.

**Procedure**

**i) Spontaneous Locomotor Activity**

30 days post-weaning animals were placed in Perspex cages (25cm x 18cm x 18cm) with wire mesh tops, and each cage was located between two photocells. An infrared beam ran between the photocells and movement of the animal from one half of the cage to the other resulted in this beam being broken and an incremental count being recorded. Counts were recorded every 5 min for a period of 1 hour immediately after the animal was placed in the cage. Activity was monitored between 1000 and 1430 hrs.

**ii) Neurochemical Studies**

2 days after the locomotor activity had been determined the neurochemical study commenced. Animals were anaesthetised with chloral hydrate (500 mg/kg i.p.) and positioned in a stereotaxic frame (anaesthesia was maintained throughout the study). A microdialysis probe (CMA/11, Carnegie Medicin) was stereotaxically implanted into one frontal cortex (anterior-posterior 3.6mm, lateral, 1.5mm, ventral 2mm; Paxinos and Watson, 1986) and perfused with artificial CSF (described in Chapter 4) at a flow rate of 1µl/min. Two hours after implantation into the frontal cortex baseline measurements were made for 80 minutes with dialysis samples collected every 20 minutes. The dialysis samples were analysed for 5-HT and 5-HIAA as described in Chapter 4. During the first minute of each of the subsequent 3 dialysate samples KCl (1µl, 1mM) was administered locally via a cannula (100µm diameter) implanted alongside the dialysis probe. It has been shown using *in vivo* voltammetry that the extracellular levels of 5-HT have returned to baseline 10 minutes after each KCl injection (Crespi, personal communication). After a further 40 mins fenfluramine (25 mg/kg i.p.) was administered and samples collected for the next 80 minutes.

**b) 5-HT Induced Behaviours**

**Animals**

40 male Lister hooded rats (Nottingham University Medical School Animal Unit) obtained at weaning (21 days of age) were divided into the two rearing condition groups counterbalanced by weight. Housing and isolation conditions were exactly the same as the microdialysis study.

**Procedure**

**i) Spontaneous Locomotor Activity**

30 days post-weaning the animals locomotor activity was studied as described in the microdialysis study.

**ii) 5-HT Behavioural Syndrome**

Approximately 7 days after studying the spontaneous locomotor activity the animals were selected to receive one of the following drugs (counterbalanced according to their locomotor activity):

a) 5-MeODMT (2 mg/kg i.p.). The forepaw treading, flat body posture and headweaving components of the '5-HT behavioural syndrome' were scored during 30 seconds every 2 minutes for 14 minutes immediately after injection, using a rank scale similar to that employed by Tricklebank et al. (1985a) where 0=absent, 1=equivocal, 2=present and 3=intense.

b) 8-OH-DPAT (0.32 mg/kg s.c.). Observations commenced immediately after injection and forepaw treading and flat body posture scored using the above rank scale for 30 seconds every 2 minutes for a total of 30 minutes.

c) DOI (2.5 mg/kg i.p.). Observations commenced immediately after injection and wet-dog shakes (defined as multiple rotatory movements of head and shoulders and/or body about the long axis typical of a dog emerging from water) and back muscle contractions counted in 2 minute periods for a total of 14 minutes.

Behavioural observations on these rats occurred in Perspex cages (25cm x 18cm x 18cm) with wire mesh tops. The animals had no prior experience of this test environment and drugs were not administered using a blind protocol.

Preliminary dose response studies were carried out using group reared animals to determine a dose of each agonist which did not produce a maximal behavioural response (data not shown). This was to ensure that differences in responsiveness (either an increase or decrease) between the two groups of animals could be observed.

**Drugs**

All drugs, dissolved in 0.9% saline, were prepared immediately before administration and injected either subcutaneously (s.c.) or intraperitoneally (i.p.) in a volume of 1 ml/kg. KCl was dissolved in artificial CSF (composition described in Chapter 4). The compounds were obtained from

the following sources: KCl (BDH Chemicals Ltd.); fenfluramine hydrochloride and 5-MeODMT (Sigma); 8-OH-DPAT and DOI (RBI Chemicals).

**Analysis of Data**

The behavioural data were analysed using a 2-tailed Mann-Whitney U-test but are displayed as mean ± standard error of the mean (S.E.M.) in order to illustrate the group variation. All microdialysis data were analysed using a one-way ANOVA with repeated measures followed by a Dunnett's t-test to identify any significant differences between the two groups.

## RESULTS

**a) Locomotor activity**

Both groups of rats reared in isolation were spontaneously hyperactive compared to their respective socially reared controls but the hyperactivity only occurred between 10 and 35 minutes in the activity cages in the first group of isolated rats (microdialysis study) (Fig. 1A) and between 10 and 40 minutes in the second group of isolated rats (5-HT behavioural syndrome study) (Fig. 1B). After this time there was no significant difference in the number of counts between the isolation- and socially-reared animals. The activity counts per 5 minute period were higher in the second part of this study compared to the first part.

**b) Microdialysis**

Basal 5-HT levels determined by microdialysis were lower in the isolation-reared animals compared to the socially-reared ones but not to a significant extent (Table 1). There was no difference in the levels of 5-HIAA between the two groups (Table 1). The 5-HIAA : 5-HT ratio (used as a measure of 5-HT turnover) was greater in isolation-reared rats but this did not reach significance (Table 1).

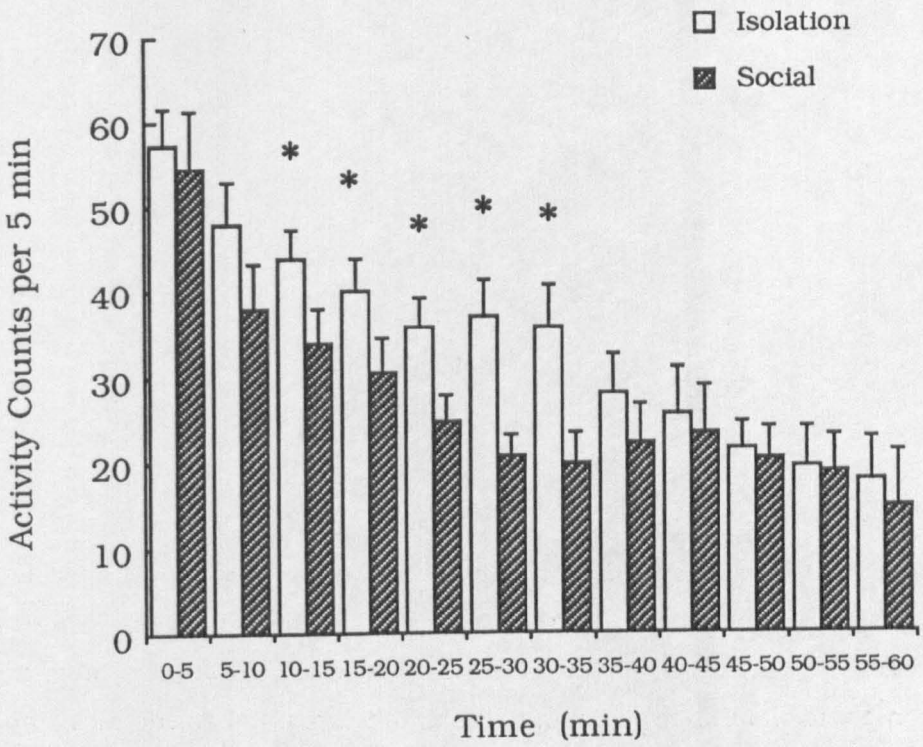
Local injections of KCl stimulated 5-HT release as shown in Fig. 2. Measurement by microdialysis shows that stimulated 5-HT release is reduced in isolation-reared rats but this only reached significance on the first depolarisation (Fig. 2A). All of the three KCl injections slightly

**Figure 1**

Locomotor activity measured in 5 minute intervals for a period of 60 minutes in isolation- and social-reared rats used in either (A) the microdialysis study, or (B) the 5-HT behavioural syndrome study, expressed as photobeam interruptions. Results are expressed as the mean number of interruptions ± S.E.M.; n=7 in each group for the microdialysis study and n=18 in each group for the 5-HT behavioural syndrome study.

*$p<0.05$ significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.

A.


Isolation
Social
Activity Counts per 5 min
70
60
50
40
30
20
10
0
*
*
*
*
*
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
Time (min)

B.

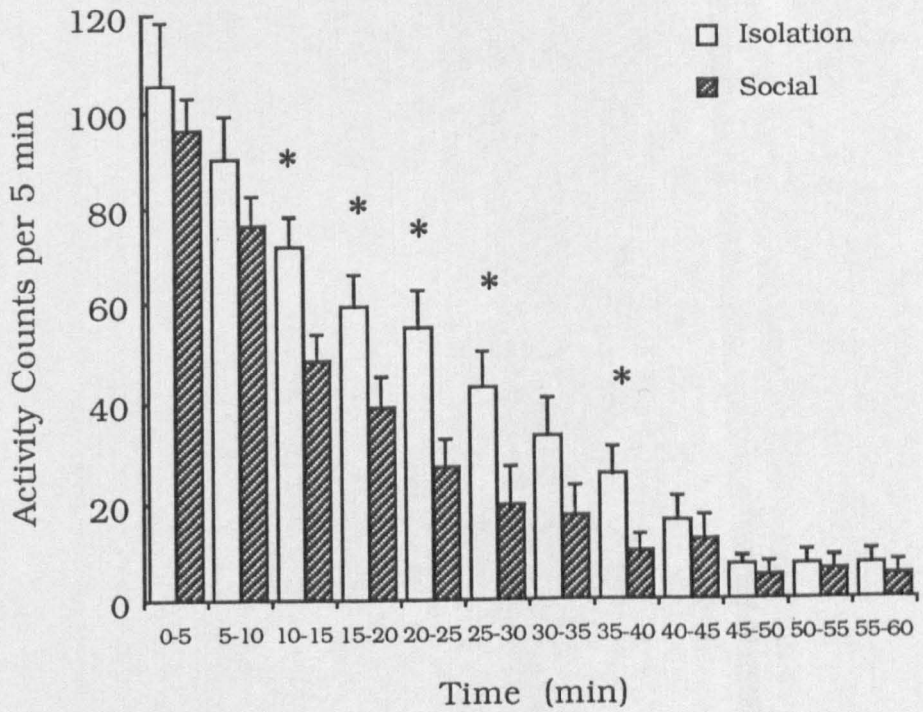

Isolation
Social
Activity Counts per 5 min
120
100
80
60
40
20
0
*
*
*
*
*
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
Time (min)

**Table 1**

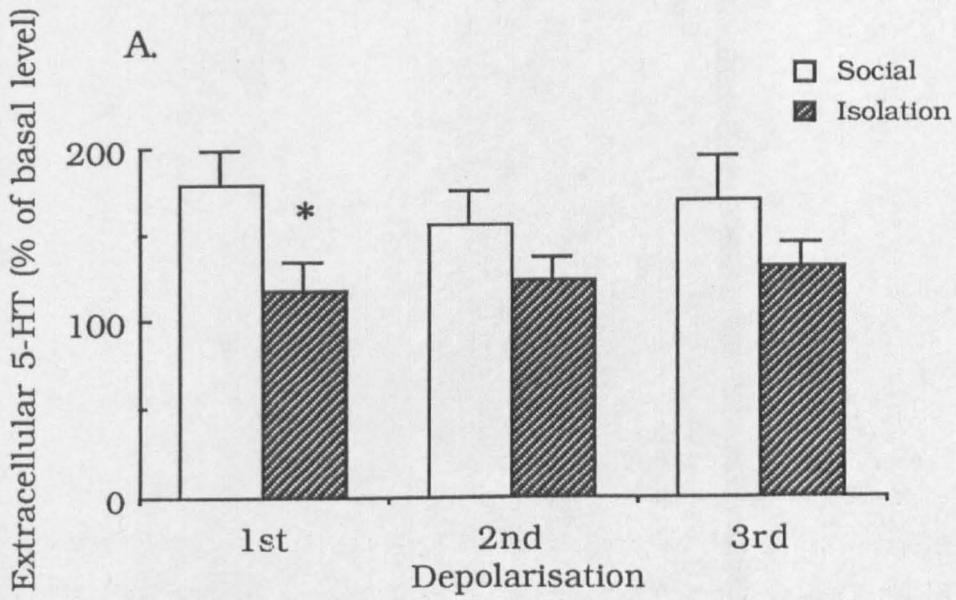
Extracellular levels of 5-HT and 5-HIAA in the frontal cortex of isolation- and socially-reared rats measured using microdialysis. 5-HT and 5-HIAA were analysed using HPLC-ECD. The ratio of 5-HIAA:5-HT was calculated and is shown. Results are expressed as the mean ± S.E.M., n=7 in each group.

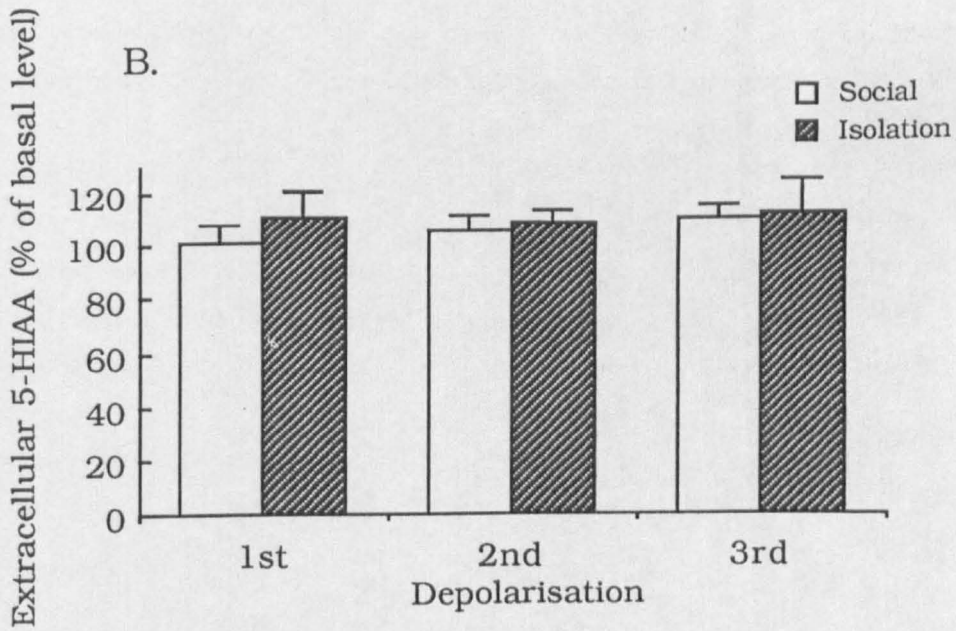
| | Rearing conditions | |
|---|---|---|
| | Social | Isolation |
| 5-HT levels (fmol/20µl) | 177 ± 23 | 132 ± 18 |
| 5-HIAA levels (pmol/20µl) | 1.33 ± 0.06 | 1.48 ± 0.08 |
| 5-HIAA/5-HT | 7.90 ± 0.97 | 11.52 ± 1.21 |

**Figure 2**

Effect of three local injections of KCl (1µl, 1mM) (named depolarisations 1 - 3) on the extracellular levels of (A) 5-HT and (B) 5-HIAA as measured by microdialysis in the frontal cortex of either isolation- or socially-reared rats. 5-HT and 5-HIAA were analysed using HPLC-ECD. Results are expressed as the mean ± S.E.M. percentage effects compared to basal levels for each group; n=7 in each group.

*$p<0.05$ significantly different from socially-reared animals. One-way ANOVA with repeated measures with post-hoc Dunnett's t-test.


A.
Social
Isolation
Extracellular 5-HT (% of basal level)
200
100
0
*
1st
2nd
3rd
Depolarisation


B.
Social
Isolation
Extracellular 5-HIAA (% of basal level)
120
100
80
60
40
20
0
1st
2nd
3rd
Depolarisation

increased the levels of 5-HIAA in the dialysate but no significant difference was observed between the two groups (Fig. 2B).

Fenfluramine increased the extracellular levels of 5-HT as detected by microdialysis (Fig. 3A). Measurement by microdialysis found no significant difference between the two groups but it was observed that the 5-HT release appeared to occur earlier in the socially-reared animals (Fig. 3A). Fenfluramine had no effect on the levels of 5-HIAA in either isolation or socially reared animals as measured by microdialysis (Fig. 3B).

**c) 5-HT Behavioural Syndrome**

a) 5-MeODMT

5-MeODMT (2 mg/kg i.p.) induced body tremor, circling behaviour, hindlimb abduction, head weaving, forepaw treading and flat body posture. These were observed in both groups of animals but the body tremor, circling behaviour and hindlimb abduction were to infrequent to be scored. Of the behaviours scored, 5-MeODMT produced a significantly greater degree of forepaw treading and flat body posture in the isolation-reared animals compared to the socially-reared controls (Fig. 4A and 4B respectively). There was no significant difference in the amount of head weaving between the two groups of rats (Fig. 4C).

b) 8-OH-DPAT

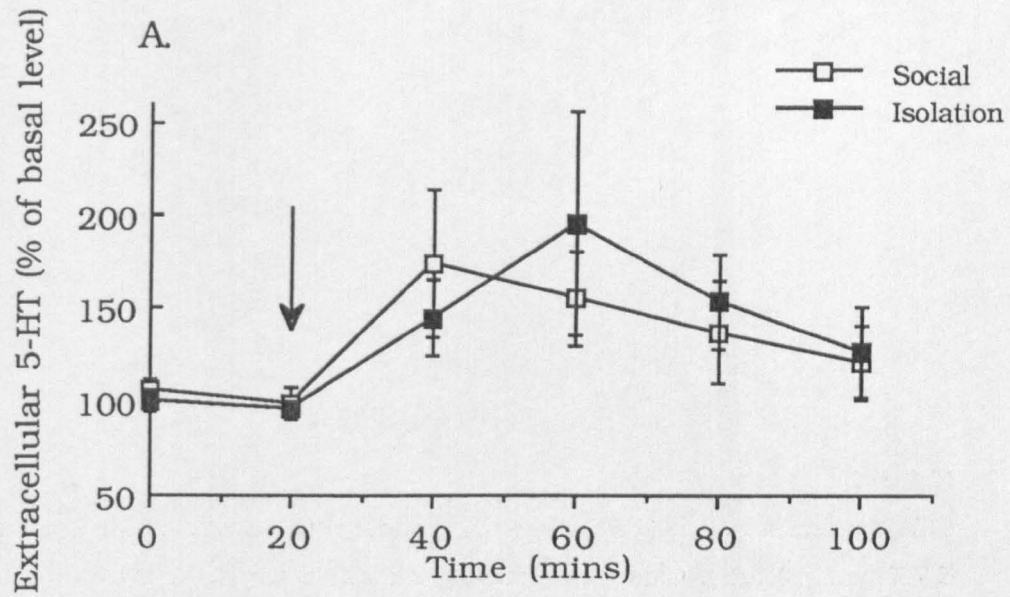
8-OH-DPAT (0.32 mg/kg s.c.) produced mastication, some head weaving, forepaw treading and flat body posture. All these behaviours were observed in both groups of animals but the mastication and head weaving were too infrequent to be included in the scoring list. 8-OH-DPAT produced a significantly greater cumulative score in isolation-reared animals compared with the socially housed controls for forepaw treading (Fig. 5A) and flat body posture (Fig. 5B) with the enhanced degree of forepaw treading being most marked.

c) DOI

DOI (2.5 mg/kg i.p.) produced wet-dog shakes, back muscle contractions, hyperactivity, slight forepaw treading, some flat body posture, mastication and circling. Wet-dog shakes and back muscle contractions were the most marked of these responses while hyperactivity, forepaw treading, flat body posture, mastication and circling were infrequent. The number of DOI-

**Figure 3**

Effect of fenfluramine (25mg/kg i.p.) (arrow) on the extracellular levels of (A) 5-HT and (B) 5-HIAA as measured by microdialysis in the frontal cortex of either isolation- or socially-reared rats. 5-HT and 5-HIAA were analysed using HPLC-ECD. Results are expressed as the mean ± S.E.M. percentage effects compared to basal levels for each group; n=7 in each group.


A.
Social
Isolation
Extracellular 5-HT (% of basal level)
250
200
150
100
50
0
20
40
60
80
100
Time (mins)

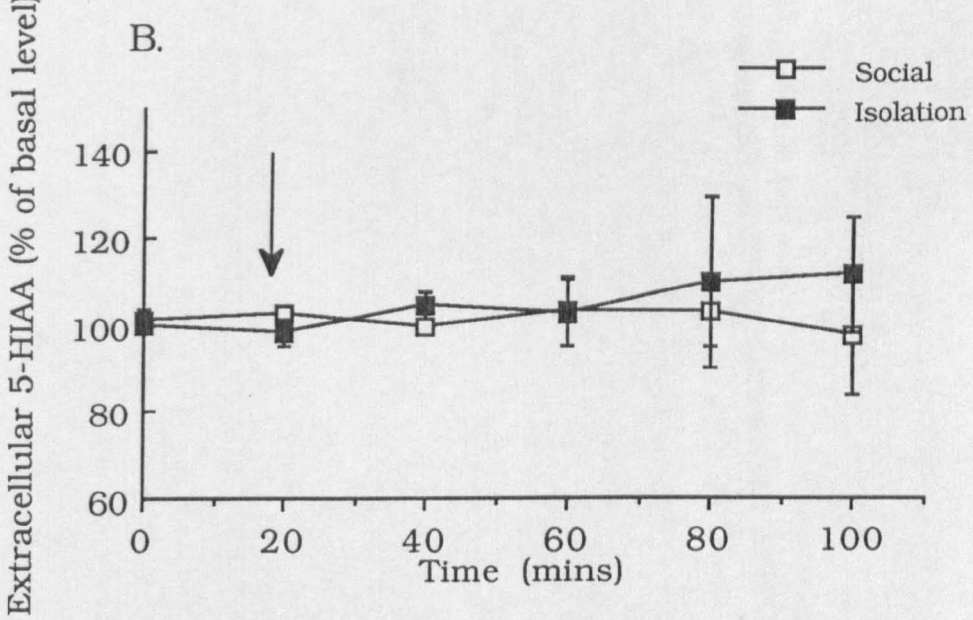

B.
Social
Isolation
Extracellular 5-HIAA (% of basal level)
140
120
100
80
60
0
20
40
60
80
100
Time (mins)

**Figure 4**

Effect of 5-MeODMT (2mg/kg i.p.) on forepaw treading (A), flat body posture (B) and head weaving (C) behaviour in isolation- and socially-reared rats over a 14 min time period. Separate components of the '5-HT behavioural syndrome' were scored by a rank scale (see Materials and Methods) for 30 sec every 2 min for 14 min immediately after injection. Each column represents the mean cumulative behavioural score ± SEM obtained; n=6 rats in each group.

*$p<0.05$, **$p<0.01$ significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.

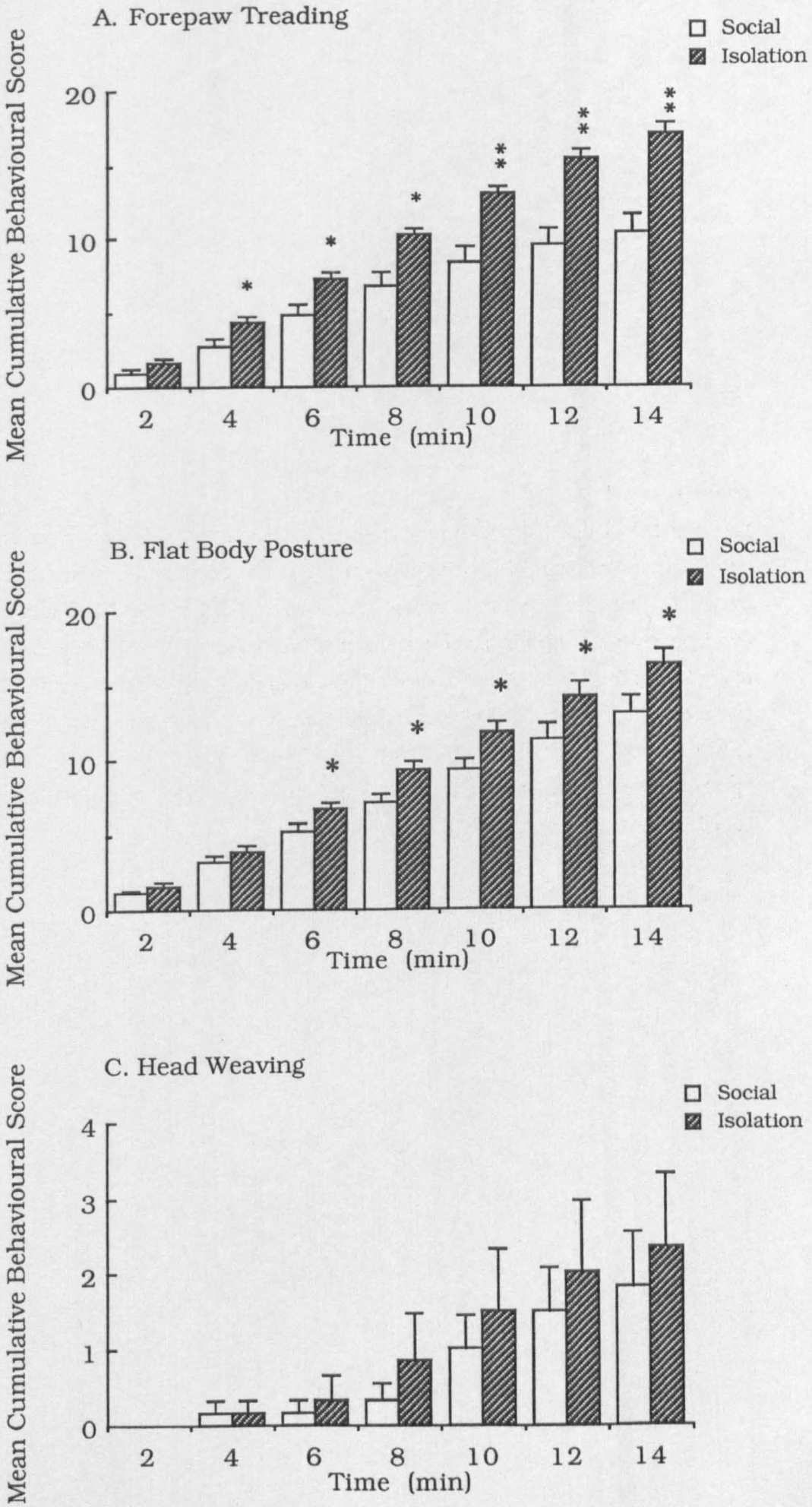
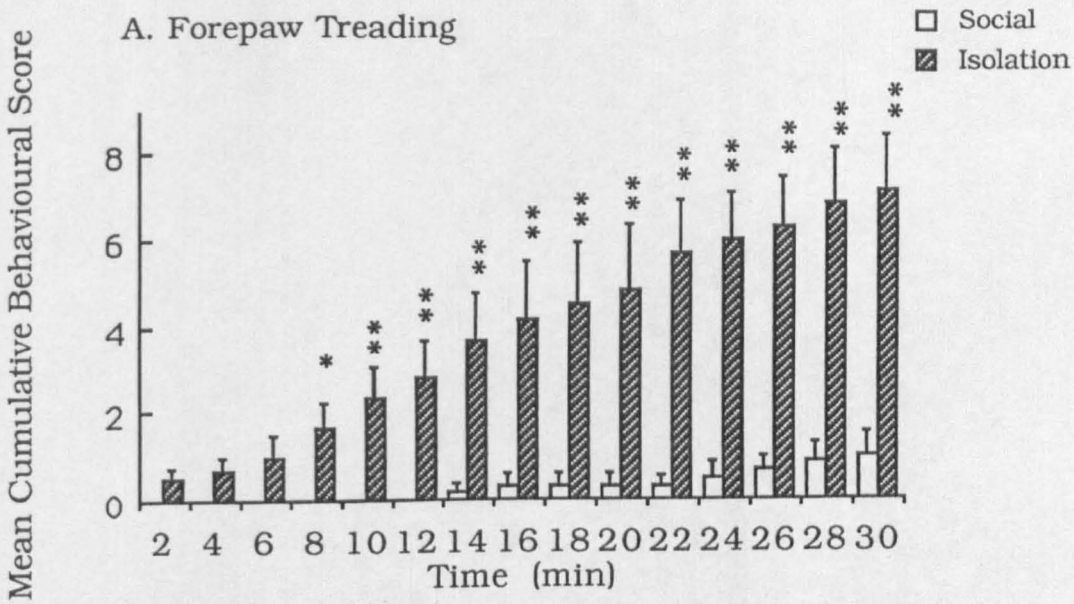

A. Forepaw Treading
Social
Isolation
Mean Cumulative Behavioural Score
20
10
0
2
4
6
8
10
12
14
Time (min)
B. Flat Body Posture
Social
Isolation
Mean Cumulative Behavioural Score
20
10
0
2
4
6
8
10
12
14
Time (min)
C. Head Weaving
Social
Isolation
Mean Cumulative Behavioural Score
4
3
2
1
0
2
4
6
8
10
12
14
Time (min)

**Figure 5**

Effect of 8-OH-DPAT (0.32 mg/kg s.c.) on forepaw treading (A) and flat body posture (B) behaviour in isolation- and socially-reared rats over a 30 min time period. Separate components of the '5-HT behavioural syndrome' were scored by a rank scale (see Materials and Methods) for 30 sec every 2 min for 30 min immediately after injection. Each column represents the mean cumulative behavioural score ± SEM obtained; n=6 rats in each group.
*p<0.05, **p<0.01 significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.


A. Forepaw Treading
Social
Isolation
Mean Cumulative Behavioural Score
0
2
4
6
8
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (min)

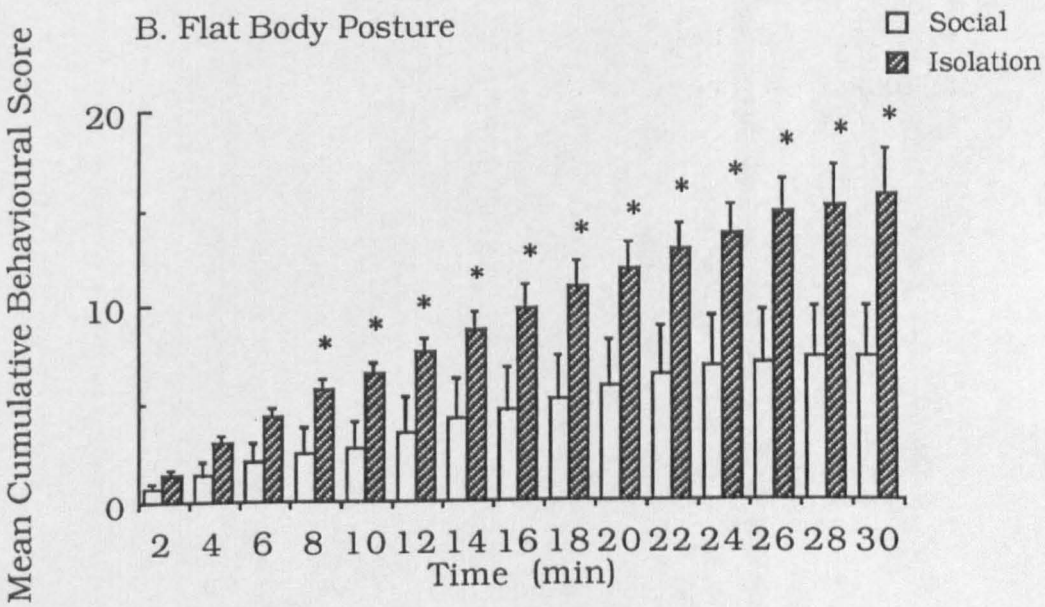
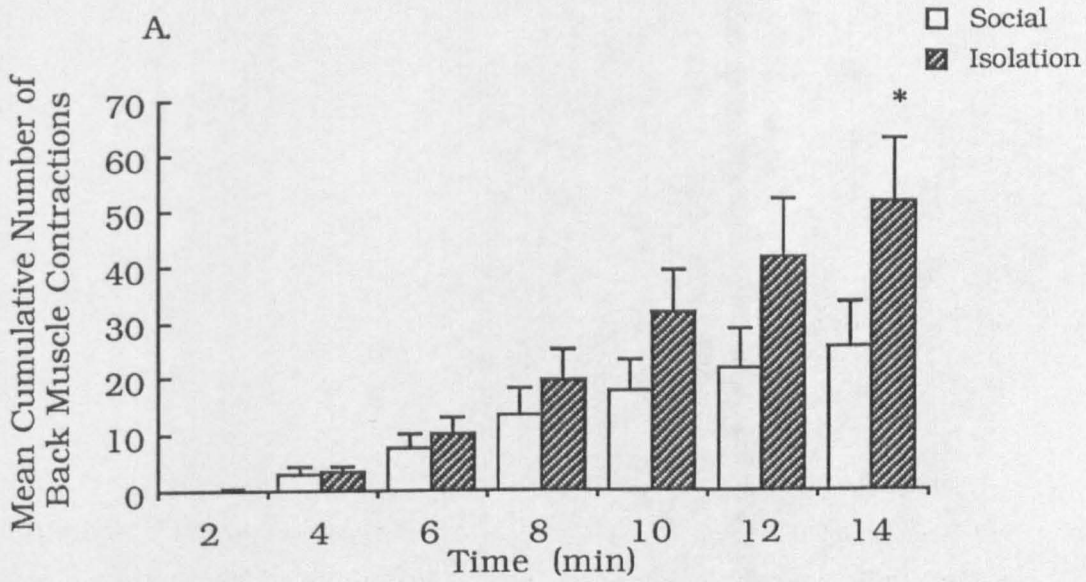
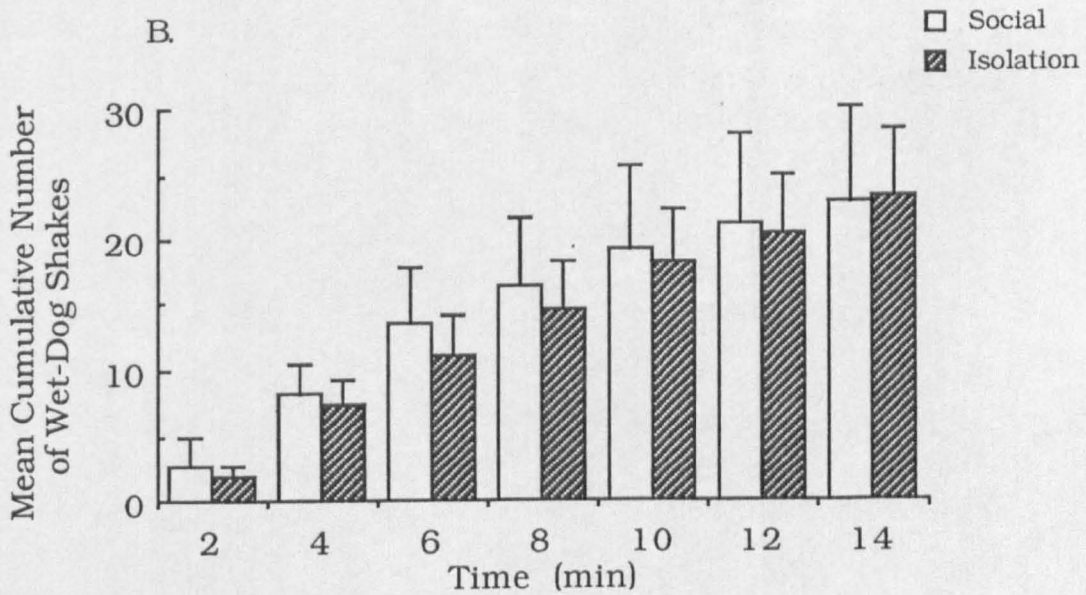

B. Flat Body Posture
Social
Isolation
Mean Cumulative Behavioural Score
0
10
20
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (min)

**Figure 6**

Effect of DOI (1mg/kg i.p.) on back muscle contractions (A) and wet-dog shakes (B) in isolation- and socially-reared rats over a 14 min time period. Back muscle contractions and wet-dog shakes were counted during 2 min periods for a total of 14 min immediately after injection. Each column represents the mean cumulative behavioural score ± SEM obtained; n=6 rats in each group.

*$p<0.05$ significantly different from socially-reared animals. 2-tailed Mann-Whitney U-test.


A.
Social
Isolation
Mean Cumulative Number of Back Muscle Contractions
70
60
50
40
30
20
10
0
*
2
4
6
8
10
12
14
Time (min)


B.
Social
Isolation
Mean Cumulative Number of Wet-Dog Shakes
30
20
10
0
2
4
6
8
10
12
14
Time (min)

induced back muscle contractions at the end of the 14 min period was significantly greater in the isolation-reared rats compared with the socially-housed controls (Fig. 6A). However, over the 14 min observation period there was no significant difference in the number of wet-dog shakes between the isolation- and socially-reared animals (Fig. 6B).

**DISCUSSION**

In agreement with previous results (Chapter 6; Sahakian at al., 1975) isolation-reared animals were hyperactive compared to socially reared controls when placed in a novel environment. After a period of about 40 min the animals had habituated to the environment and no difference in spontaneous locomotor activity was observed between the two groups, again agreeing with work by Sahakian at al. (1975). The reason for the difference in the activity counts per 5 minute period observed between the two different parts of the study is not clear. This part of the study was carried out to confirm that the rearing conditions used in our laboratory produced effects comparable with those reported in the literature and that the same behavioural effects as those studied in Chapter 6 were present in these animals in the hope that neurochemical changes would be similar.

The microdialysis data obtained suggest that there was a small, but non-significant, increase in 5-HT turnover in isolation-reared rats as the 5-HIAA:5-HT ratio was greater. In contrast, the increase in extracellular 5-HT when stimulated with KCl was significantly less in isolation-reared animals suggesting that KCl stimulated release of 5-HT was reduced in these animals. There was no significant change in extracellular 5-HIAA indicating that it is a poor correlate of 5-HT neuronal function, possibly because it is not the product of the released 5-HT but arises from the intraneuronal metabolism of un-released 5-HT (Grahame-Smith, 1971, 1974; Kuhn et al., 1986; Kalen et al., 1988; Auerbach et al., 1989; Sharp et al., 1989c; Crespi et al., 1990).

Fenfluramine increased the levels of extracellular 5-HT in both the isolation- and socially-reared groups. There was no significant difference in the effect of fenfluramine between these two groups. A very similar effect on both extracellular 5-HT and 5-HIAA levels monitored by *in vivo* microdialysis in the frontal cortex of naive rats treated with fenfluramine has been reported (Crespi et al., 1990), thus supporting the feasibility of

performing experiments with fenfluramine even after several injections of KCl. The reduced stimulated release of 5-HT, together with the possible increase in 5-HT turnover and delayed effects of fenfluramine in the frontal cortex suggest that the "functional pool" (Glowinski et al., 1979) may be reduced and the higher turnover of 5-HT preferentially stored in the "reserve pool" (Green and Grahame-Smith, 1975), with prolonged stimulation possibly leading to 5-HT 'spilling over' into the "functional pool". However, further experiments are required to determine whether this is true, possibly using a technique such as voltammetry with which more frequent sampling of 5-HT levels can occur.

The agonists used in the second part of this study produced various '5-HT mediated responses' via either 5-$HT_{1A}$ and/or 5-$HT_{2/1C}$ postsynaptic receptor stimulation. The ability of 8-OH-DPAT and 5-MeODMT to produce the full 5-HT behavioural syndrome has been well established (Tricklebank et al., 1985a & b). After reserpine and p-chlorophenylalanine pretreatment the forepaw treading and flat body posture components of the behavioural syndrome are still induced by 8-OH-DPAT (Tricklebank et al., 1985a) suggesting that indirect catecholamine mechanisms are not involved in the production of these two components and that the response to 8-OH-DPAT is mediated by a post-synaptic receptor. Since 8-OH-DPAT is a potent and selective 5-$HT_{1A}$ agonist (Hjorth et al., 1982) it was concluded that forepaw treading and flat body posture occurred as a result of 5-$HT_{1A}$ receptor activation (Tricklebank et al., 1985a). Stimulation of the 5-$HT_{1A}$ receptor by either the non-selective receptor agonist 5-MeODMT (Tricklebank et al., 1985b) or the 5-$HT_{1A}$ selective agonist 8-OH-DPAT (Hjorth *et al.*, 1982) produced a significantly greater response in the isolation-reared animals, indicating postsynaptic 5-$HT_{1A}$ supersensitivity. We have shown earlier in this chapter that stimulated release of 5-HT is reduced in isolation-reared animals, suggesting reduced presynaptic function. It is possible that the reduced presynaptic function may result in compensatory postsynaptic receptor up-regulation. In work investigating isolation-induced behavioural deficits in mice Frances et al. (1990) also found enhanced effects of 8-OH-DPAT in isolated animals compared to the controls suggesting hyperreactivity to 5-$HT_{1A}$ agonists. However, an electrophysiological study looking at the sensitivity of the somatodendritic 5-$HT_{1A}$ autoreceptor in the dorsal raphe nucleus found greater inhibition in response to 8-OH-DPAT in isolation-reared rats but this did not reach significance (Mundey et al.,

1991). The use of a different strain of rat and absence of reserpine pretreatment may explain the reduced forepaw treading behaviour observed in this study in the socially-reared group after 8-OH-DPAT treatment compared with published data (e.g. Tricklebank et al., 1985a). The head weaving component of the 5-HT behavioural syndrome induced by both 5-MeODMT and 8-OH-DPAT is abolished by reserpine pretreatment (Tricklebank, 1984), suggesting that this component is not a direct result of post-synaptic receptor activation. This may explain why no difference was observed in 5-MeODMT-induced head weaving between social- and isolation-reared animals in the present study.

In alcohol-preferring rats it has been shown that there is a reduction in the tissue levels of 5-HT in the cortex, hippocampus and striatum (Murphy et al., 1982). In this same strain of rats it has been shown that there is a higher density of $5\text{-HT}_{1A}$ receptors in the hippocampus and cerebral cortex (Wong et al., 1990) and they suggest that this results from reduced levels of 5-HT. This is indirect supportive evidence for the effects observed in this study, i.e. reduced presynaptic 5-HT function may lead to the enhanced post-synaptic responsivity observed in the present study following agonist induced post-synaptic receptor stimulation.

Back muscle contractions have been shown to be mediated by $5\text{-HT}_2$ receptors (Fone et al., 1989; Fone et al., 1990) whilst wet-dog shakes are thought to involve $5\text{-HT}_2$ and other (non-5-HT) receptors (Bedard and Pycock, 1977; Fone et al., 1987; Yap and Taylor, 1983; Yocca et al., 1990). DOI, a $5\text{-HT}_2$ agonist (Glennon, 1987), produced some forepaw treading and slight flat body posture, indicating some $5\text{-HT}_{1A}$ activity, and using a lower dose may have removed all activity at this site. DOI produced a greater number of back muscle contractions in isolation-reared rats indicating that the $5\text{-HT}_2$ receptor responsiveness is also enhanced in the socially-isolated rats. In contrast, DOI induced wet-dog shakes were not increased in the isolation-reared animals. However, other neurotransmitters apart from 5-HT have been implicated in this wet-dog shake behaviour. For example, dopamine has an inhibitory effect on 5-HT-mediated wet-dog shake behaviour (Bedard and Pycock, 1977), and a previous study has demonstrated increased dopaminergic function in isolation-reared animals (Jones et al., 1988). This may explain the lack of effect of isolation-rearing on DOI induced wet-dog shakes. The enhanced DOI-induced back muscle

contractions in isolation-reared animals is of interest as few other studies investigating 5-$HT_2$ receptors have shown up-regulation. Fone et al. (1989) showed a significantly greater number of back muscle contractions induced by the 5-$HT_2$ selective agonist 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM) (Glennon, 1987) 10 days after intrathecal administration of 5,7-dihydroxytryptamine but with no change observed in wet-dog shake behaviour. Enhanced 5-$HT_2$ receptor-mediated responses and 5-$HT_2$ binding sites have been observed after electroconvulsive shock treatment in rats (Green et al., 1983a). However, chronic treatment with 5-$HT_2$ antagonists, for example ritanserin, leads to down-regulation and reduced 5-$HT_2$ receptor number in the frontal cortex (Leysen et al., 1986) indicating the difficulty of pharmacologically up-regulating these receptors. The 5-$HT_2$ receptors mediating back muscle contractions are thought to be located post-synaptic to the bulbospinal serotonergic nerve terminals and hence it is possible that these spinal 5-$HT_2$ receptors may have different characteristics to those located supraspinally.

An alternative explanation for the differences in responsiveness of the two groups of animals to 5-HT agonists is a change in metabolism of the drugs, with it being slower in the isolation-reared animals, thus leading to a greater behavioural response. Against this hypothesis is that only certain responses were enhanced (forepaw treading and flat body posture) while others (head weaving and wet-dog shakes) were unchanged, whereas a change in metabolism would be expected to alter all of the responses.

In summary, the results indicate that isolation-rearing produces changes in the releasable pool of 5-HT in the frontal cortex compared to group-reared rats and there is an enhanced postsynaptic responsiveness of the 5-$HT_{1A}$ and 5-$HT_2$ receptors in these animals. However, the present study has not determined whether the changes in responsiveness of the 5-$HT_{1A}$ and 5-$HT_2$ receptors are as a result of increased receptor number or an enhanced response by the receptor-coupling mechanism system, questions which are currently under investigation. Further studies are also needed to determine whether the effects observed with the 5-HT agonists in the isolation-reared animals occur as a result of a long-term developmental change caused by social isolation.

# CHAPTER 8

# GENERAL DISCUSSION

The overall aim of this thesis was to study 5-HT neuronal function, using *in vivo* microdialysis to monitor 5-HT release, and to correlate this with ongoing behaviour thought to reflect anxiety. After studying two different models of anxiety, the elevated X-maze and the fear-potentiated acoustic startle paradigm, the elevated X-maze was used to study the effects of various serotonergic agents thought to be putative anxiolytics after both acute and chronic treatment. Using the X-maze it was possible to combine behaviour with microdialysis and monitor neurochemical and behavioural changes in the same animal, and then to study the effects of established (BDZ) and putative (5-HT) anxiolytics on these changes. Using *in vivo* techniques allowed the study of both pre- and postsynaptic 5-HT function in the socially-isolated rats which displayed 'anxious' behaviour on the X-maze.

**Pharmacological Validation of the Elevated X-Maze and Fear-Potentiated Acoustic Startle Behavioural Paradigms**

Both the elevated X-maze and the fear-potentiated acoustic startle paradigms are well established behavioural models of anxiety. In order to look at the effects of putative anxiolytics, such as the 5-$HT_{1A}$ receptor partial agonist ipsapirone, it is first necessary to demonstrate that the paradigms used in this thesis could detect the activity of established anxiolytic and anxiogenic compounds, and also the suitability of these models for combined studies with microdialysis. In the elevated X-maze, diazepam, a benzodiazepine agonist, was found to dose-dependently reduce the indices of anxiety but had no effect on the general locomotor activity. In the fear-potentiated acoustic startle paradigm diazepam was found to have an anxiolytic effect but at doses which also caused some sedation. Conversely, the partial inverse agonist at the benzodiazepine/$GABA_A$ receptor complex, FG 7142, which has been found to induce anxiety in man (Dorow et al., 1983), dose-dependently increased the indices of anxiety in the X-maze. In the potentiated startle paradigm, FG 7142 significantly increased both the potentiated startle and the basal startle. As a result of the increased basal startle it cannot be determined whether the raised potentiated startle is a result of an anxiogenic effect of FG 7142 or a result of increased arousal caused by the drug. Idazoxan, an $\alpha_2$-adrenoceptor antagonist, significantly increased the indices of anxiety in the X-maze, but as a result of variations between individual animals this only approached significance in the potentiated

startle paradigm. The anxiogenic effects of idazoxan in the X-maze are in agreement with clinical studies in which yohimbine, another $\alpha_2$-adrenoceptor antagonist, has been reported to produce anxiety and induce panic attacks (Charney et al., 1984), and clonidine, an $\alpha_2$-adrenoceptor agonist, to reduce anxiety (Redmond, 1982).

Although both behavioural paradigms have been pharmacologically validated, the elevated X-maze produced more reproducible and dose-dependent results in comparison to the potentiated startle. For this reason, and that the model has been well validated physiologically (Pellow et al., 1985), together with the simplicity of procedure, the elevated X-maze was chosen as the behavioural model of anxiety for use in the studies in this thesis. Having established the behavioural responses to standard anxiolytic and anxiogenic compounds it was possible to look at the effects of acute and chronic treatments of various agents that directly interact with 5-HT receptors on behaviour in the X-maze. Further, with the X-maze being open-roofed it was possible to combine this test with microdialysis, thus allowing simultaneous neurochemical and behavioural changes to be measured in the same animal.

**Effects of Acute and Chronic Treatment of Various Serotonergic Agents in the Elevated X-Maze**

Acute diazepam produced a significant anxiolytic profile on the elevated X-maze. Chronic treatment with diazepam produced an anxiolytic profile, indicating no tolerance to its anxiolytic effects. This may be due to the selected dose of diazepam not being large enough to induce tolerance using the X-maze, or that the dose produced a supramaximal effect on the X-maze and hence even if some tolerance developed it was still possible to observe a maximal effect on the X-maze. 24 hours after the last dose of diazepam no anxiolytic activity was observed and behaviour did not differ significantly from controls. However, using a different design of elevated X-maze File and Baldwin (1989) have been able to detect anxiogenic withdrawal effects using chlordiazepoxide at doses of 5 and 20 mg/kg i.p.. It may be necessary to use higher (i.e. 10 or 20 mg/kg) doses of diazepam to cause significant withdrawal behaviour as indicated by previous studies (Costall et al., 1990a). Idazoxan given both acutely and chronically resulted in an anxiogenic profile, indicating a lack of tolerance to the drug. This is supported by electrophysiological data where acute idazoxan after 14 days chronic

treatment with idazoxan increased dorsal raphe nucleus 5-HT neuronal firing to the same level as that of acute idazoxan (Garratt et al., 1991). Acute administration of the 5-$HT_{1A}$ partial agonist ipsapirone had no effect on behaviour on the X-maze at doses of 0.01 and 1mg/kg i.p., but 0.1mg/kg i.p. produced a significant anxiogenic profile. 5-$HT_{1A}$ receptors are located both presynaptically on the cell bodies/dendrites of 5-HT neurones in the raphe nuclei and also postsynaptically in the limbic terminal regions (Hoyer et al. 1986a). It is possible that the balance of receptor stimulation of these two sites varies depending on the dose of ipsapirone, with low doses acting predominantly at pre-synaptic sites and higher doses acting at a mixture of pre- and postsynaptic sites. From the results of this study two possible explanations for the effects of ipsapirone are that 0.1 mg/kg i.p. is a high enough dose to cause postsynaptic receptor stimulation and produce an anxiogenic profile on the X-maze (with the reason for no activity with the 1 mg/kg dose being unknown), or stimulation of the presynaptic receptors at the 0.1 mg/kg dose produces an anxiogenic profile which is blocked by increasing the dose to 1 mg/kg and stimulating both pre- and postsynaptic 5-$HT_{1A}$ sites. Overall, the effects seen with ipsapirone do not fit well with those in the literature. Chronic treatment with ipsapirone at these three doses had no significant effect on rat behaviour on the X-maze. A similar study has shown chronic administration of another 5-$HT_{1A}$ partial agonist, buspirone (1 mg/kg i.p. twice daily for 14 days), to have no effect in the X-maze (Moser, 1989) although the drug has activity in the clinic (Goldberg and Finnerty, 1979; Goa and Ward, 1984). However, 24 hours after withdrawing chronic treatment, ipsapirone at 1mg/kg produced a significant anxiogenic profile, indicating a withdrawal effect which may be the result of changes in 5-$HT_{1A}$ receptor function after chronic treatment or to some unexplained withdrawal effect of that specific dose. Ritanserin, a 5-$HT_2$ receptor antagonist, given acutely had no effect on any of the behavioural parameters, but chronic treatment produced a significant anxiolytic effect which was still present 24 hours after cessation of treatment, correlating with effects observed in the clinic (Ceulemans et al., 1985). Further studies are required to determine whether there is any causal relationship between ritanserin induced down-regulation of 5-$HT_2$ receptors (Leysen et al., 1986) and the anxiolytic profile observed in the present study. Acute ondansetron, a 5-$HT_3$ receptor antagonist, at all of the doses studied had no effect on behaviour on the X-maze. However, chronic ondansetron treatment produced a significant anxiolytic profile. At present prelimiary clinical

trial data has been published but the necessary statistical analysis on this data has not been carried out (Abuzzahab, 1991). Thus, the results demonstrate that the X-maze can detect anxiolytic activity in non-benzodiazepine drugs, with the 5-$HT_2$ and 5-$HT_3$ receptor antagonists, ritanserin and ondansetron respectively, showing anxiolytic profiles but only after chronic treatment. In contrast, the X-maze failed to detect anxiolytic activity with the 5-$HT_{1A}$ partial agonist ipsapirone after either acute or chronic treatment. In this study we were not able to detect any possible anxiolytic activity of ipsapirone, and a greater range of doses may be necessary to observe some activity. Ipsapirone displays a different clinical profile compared to ritanserin and ondansetron and it is possible that the X-maze is unable to easily detect compounds with 5-$HT_{1A}$ receptor partial agonist activity.

**Intracerebral Microdialysis and Neuronal Release**

Intracerebral microdialysis was chosen as the technique to examine *in vivo* serotonergic function. Before microdialysis can be used in conjunction with behaviour on the X-maze or to study stimulated release in isolation-reared rats it was necessary to demonstrate that the 5-HT being measured was of neuronal origin and not from platelets, mast cells or glia (Sneddon, 1973; Austen, 1986; Kimelberg, 1986). Chapter 4 describes the validation procedure used for the technique and these experiments led to the conclusion that the release of 5-HT measured in the hippocampus was indeed neuronal. In Chapter 7 dialysis is carried out in the frontal cortex of anaesthetised animals, and validation of the origin of 5-HT in this procedure comes from data of Kidd (1990) who used this technique extensively and validated the neuronal origin of 5-HT. The neuronal nature of 5-HT release in the freely moving and anaesthetised animals was first examined using tetrodotoxin (TTX), which blocks action potential conduction in neurones (Hille, 1968), which was included in the perfusate and inhibited 5-HT release, showing that it was dependent upon action potentials. Second, perfusion with $Ca^{2+}$-free CSF also decreased the release of 5-HT in both the conscious and anaesthetised experiments suggesting that a $Ca^{2+}$-dependent mechanism was involved. In both conscious and anaesthetised animals there was a small residual component of 5-HT release which was not sensitive to TTX or dependent upon $Ca^{2+}$ and was probably, therefore, not neuronal. In the anaesthetised animals electrical stimulation of the dorsal raphe nucleus which sends projections to the cortex (Azmitia and Segal, 1978; Parent et al.,

1981) resulted in an increase in 5-HT release. None of these three manipulations altered 5-HIAA levels greatly, confirming previous reports that extracellular 5-HIAA is a product of intraneuronal metabolism of unreleased 5-HT and not of the released 5-HT (Grahame-Smith, 1971, 1974; Kuhn et al., 1986; Kalen et al., 1988; Auerbach et al., 1989; Sharp et al., 1989c; Crespi et al., 1990). Therefore, 5-HIAA levels are not a good indicator of 5-HT release. In anaesthetised animals the $5\text{-HT}_{1A}$ receptor agonist, 8-OH-DPAT, inhibited cortical 5-HT release probably as a consequence of stimulation of the somatodendritic $5\text{-HT}_{1A}$ autoreceptors in the dorsal raphe nucleus which inhibit 5-HT neuronal firing (Sprouse and Aghajanian, 1986; Crespi et al., 1990; VanderMaelen et al., 1986) and thus decrease 5-HT release. Having validated the dialysis technique for measuring neuronal release of 5-HT, it could then be used to determine changes in 5-HT levels upon exposure to the X-maze and the effects of established and putative anxiolytic compounds on these changes. Second, to examine pre-synaptic function in isolation-reared rats.

**Microdialysis and Behaviour on the Elevated X-Maze**

Placing the rats on the X-maze for a 20 minute period resulted in increased extracellular 5-HT which returned to basal levels 40 minutes after the animals were returned to the home cage. The increase in 5-HT is not likely to be as a result of increased locomotor activity as drug-induced changes in extracellular 5-HT levels had no effect on general locomotor activity. It is thought that the open arms produce a greater 'fear' drive in rats, and thus more avoidance, compared to the closed arms and restricting the rats to either the open or closed arms of the X-maze resulted in increased extracellular 5-HT, with the increase being greater when restricted to the open arms, but not to a significant extent. This is possibly due to the rats spending a large proportion of the time in the central square when they were placed on the open arms, and an open elevated runway may have proved a better as this would not have a central square to act as a 'closed environment'. Having established possible reasons for the lack of a significant graded response in increased 5-HT on the X-maze it should also be noted that when studying behaviour on all of the X-maze there was a lack of correlation between the peak increase in 5-HT levels and behaviour, except for the percentage open:total entries which only approached significance, but this may be a result of relatively small numbers for the correlations.

Diazepam inhibited the increase in extracellular 5-HT in the ventral hippocampus and had an anxiolytic profile over the 20 minute period when the rat was placed on the X-maze. The ability of diazepam to inhibit the increase in 5-HT when the animal was placed on the X-maze may be associated with the anxiolytic profile it produces in the same animal. Diazepam also inhibited 5-HT release from the ventral hippocampus when the rat was returned to its home cage. Because no reduction in 5-HT release was observed with diazepam before exposure to the X-maze it cannot be determined from this study whether the decrease in basal 5-HT levels when the animal is returned to the home cage after exposure to the maze is a result of diazepam on basal release, agreeing with a similar microdialysis study carried out by Pei et al. (1989b), or a 'hangover' effect occurring due to exposure to the X-maze where diazepam produced a very marked reduction in increased 5-HT. The data from this part of the study suggest that reducing 5-HT release may be one of the mechanisms of action of the benzodiazepines which is important for their anxiolytic activity.

Ipsapirone treated animals did not differ in their behaviour from vehicle controls over the 20 minute period on the X-maze, but like diazepam, ipsapirone significantly reduced the increase in extracellular 5-HT when the animals were placed on the X-maze and also the basal levels when the animal was returned to its home cage. It is thought that ipsapirone inhibits the extracellular levels of 5-HT in the hippocampus via an action at the 5-$HT_{1A}$ somatodendritic autoreceptor in the dorsal raphe nucleus (Hjorth and Magnusson, 1988; Hutson et al., 1989; Sharp et al., 1989c). This would suggest that inhibtion of hippocampal 5-HT release is not important in the mechanism of action of diazepam. However, ipsapirone may also be stimulating postsynaptic 5-$HT_{1A}$ sites in the limbic regions such as the hippocampus to have an overall effect of not altering 5-HT neuronal activity, resulting in a behavioural profile that is not different from the vehicle treated animals.

Further evidence towards the inhibition of hippocampal 5-HT release being important in the anxiolytic activity of anxiolytics comes from the data of F2692. This compound has very little activity at the benzodiazepine/$GABA_A$ complex but reduces the increase in 5-HT when the animal is on the X-maze and has an anxiolytic profile but only during the first 5 minutes of exposure.

The lack of activity after 20 minutes may be a result of sedatory effects of this compound at the dose used.

Overall, all three compounds inhibited the increase in 5-HT when the animals were exposed to the X-maze. Diazepam and F 2692 produced an anxiolytic profile and it is possible that the reduced increase in extracellular 5-HT after diazepam or F2692 treatment may be one of the mechanisms by which they have their anxiolytic actions. However, against this theory is the ability of ipsapirone to reduce extracellular 5-HT and not to have any anxiolytic activity in this study, but it is possible that post-synaptic 5-$HT_{1A}$ receptor stimulation by ipsapirone in the hippocampus and other limbic regions may have the net effect of not changing 5-HT function in these animals.

Reducing the dialysis sample time by increasing the sensitivty of the HPLC-ECD assay would allow changes in 5-HT levels to be studied over shorter periods of time, such as 5 minutes, the standard exposure time to the X-maze when studying behaviour alone. It is possible that over the period of 20 minutes there is some habituation to the X-maze and this may reduce the changes in 5-HT levels and behaviour between drug treated and control animals. Also, providing the practical difficulties could be overcome, carrying out this type of study using *in vivo* voltammetry would prove very informative as monitoring could then occur over periods of seconds rather than minutes and differences in neurotransmitter release between the open and closed arms could be studied during normal behaviour on the X-maze, eliminating the need to restrict the animal to either of the arms.

**Behavioural Effects of Social Isolation in Rats**

Rats reared in isolation from weaning show many behavioural and neurochemical differences from socially-reared controls. In this study rats were reared from weaning, either in isolation or social groups for 30 days and then for the duration of the experiment, were shown to have increased locomotor activity. Isolation-reared rats displayed an anxiogenic profile on the X-maze compared to socially-reared controls, which was not a result of the increased locomotor activity. These results indicate that the changes seen in spontaneous locomotor activity and behaviour on the X-maze are via two different mechanisms. Resocialisation of isolation-reared rats for a further 30 days did not reverse this anxiogenic profile, and isolation of the

socially-reared rats for 30 days did not produce an anxiogenic behavioural profile, indicating that the differences observed may be a result of a permanent developmental change. It remains to be determined what neurochemical events are involved in the sustained effects of rearing in isolation and whether the anxiogenic profile observed in the isolation-reared rats is related to alterations in 5-HT function.

**Alterations in pre- and postsynaptic receptor function in isolation-reared rats**

Identical rearing conditions to those used in Chapter 6 were used and isolation-reared rats again showed increased locomotor activity. Using *in vivo* microdialysis it was demonstrated that 5-HT turnover in isolation-reared rats was slightly increased but the stimulated increase in extracellular 5-HT by local KCl application was significantly less, suggesting that KCl stimulated release of 5-HT is reduced in isolation-reared animals. Also, after systemic administration of fenfluramine the rise in extracellular 5-HT was slightly 'later' in the isolation-reared animals. The reduced stimulated release of 5-HT, together with the possible increase in 5-HT turnover and delayed effects of fenfluramine in the frontal cortex suggest that the "functional pool" (Glowinski et al., 1979) may be reduced and the higher turnover of 5-HT preferentially stored in the "reserve pool" (Green and Grahame-Smith, 1975), with prolonged stimulation possibly leading to 5-HT 'spilling over' into the "functional pool".

The functional state of the post-synaptic 5-$HT_{1A}$ and 5-$HT_2$ receptors was investigated using the "5-HT behavioural syndrome" induced by the non-selective 5-HT receptor agonist, 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT), the relatively selective 5-$HT_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and the 5-$HT_{2/1C}$ receptor agonist 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). It is thought that the flat body posture and forepaw treading components of the syndrome are the result of postsynaptic 5-$HT_{1A}$ receptor activation (Tricklebank et al., 1985a), and 'wet-dog shakes' and back muscle contractions are a result of 5-$HT_2$ receptor stimulation (Fone et al., 1989). 5-MeODMT and 8-OH-DPAT elicited various components of the "5-HT behavioural syndrome" in both groups of animals, with forepaw treading and flat body posture being significantly more pronounced in isolation-reared animals. DOI produced a significantly greater number of back muscle contractions in isolation-reared animals but

there was no difference between the two groups in the number of wet-dog shakes produced. Forepaw treading and flat body posture are thought to be mediated by 5-$HT_{1A}$ receptor activation, and stimulation of this receptor by either 5-MeODMT or 8-OH-DPAT produced greater responding in isolation-reared rats suggesting supersensitivity of the post-synaptic 5-$HT_{1A}$ receptor. Wet-dog shakes are thought to be mediated by 5-$HT_2$ and other (non-5-HT) receptors while back muscle contractions have been shown to be mediated by 5-$HT_2$ receptors, indicating that there is also an increase in 5-$HT_2$ receptor responsiveness in the socially-isolated animals. In general, the results indicate post-synaptic 5-HT receptor supersensitivity in isolation-reared rats (which may be a consequence of reduced presynaptic function) and these receptor changes may be involved in the behavioural profile observed in such rats.

**Future Experiments**

5-$HT_3$ receptor antagonists have been shown to have an anxiolytic profile in the elevated X-maze and to date these compounds have not been reported to have any effects on serotonergic transmission. Thus it would be worthwhile to study the effects of these compounds on behaviour and 5-HT release in the ventral hippocampus while on the X-maze; if an anxiolytic profile was produced and also inhibition of increased 5-HT release occurred it would provide strong evidence for the involvement of 5-HT in anxiety. Now that microdialysis on the elevated X-maze is possible it would be useful to study a variety of brain regions to determine whether the same neurochemical changes occur throughout the brain, and also to go on and study other neurotransmitters, such as noradrenaline, to determine their involvement in 'anxious' behaviour on the X-maze.

It would be interesting to combine *in vivo* microdialysis with other models of anxiety to determine whether any changes in 5-HT release observed, together with the effects of established and potential anxiolytics on these changes, are the same as those seen in the X-maze, or whether changes in 5-HT function vary depending on the test used.

In isolation-reared rats the postsynaptic supersensitivity may be the result of increased receptor number or increased sensitivity in the receptor-coupled transduction system. Using both binding and second messenger studies it would be possible to determine what changes are occurring at the

postsynaptic membrane. It is interesting to speculate whether the changes observed in the serotonergic system are a direct effect of isolation or secondary to alterations in another system, such as that of dopamine. It is known that some aspects of dopamine-induced behaviour are blocked by 5-$HT_3$ receptor antagonists, and that the dopaminergic system is enhanced in isolation-reared rats, and hence studying the dopamine - 5-$HT_3$ receptor antagonist interaction in these animals may provide information on possible alterations in the interactions of these two systems in socially isolated rats.

The 5-$HT_{1A}$ and 5-$HT_2$ receptor postsynaptic supersensitivity observed in isolation-reared rats could alter their behavioural responses to potential anxiolytics that have a direct action on 5-HT receptors. Thus, it would be interesting to determine the effects of these compounds on behaviour of isolation-reared rats in several models of anxiety.

The changes observed in both the serotonergic and dopaminergic systems in isolation-reared rats are robust and it may be possible using immunohistochemical studies to determine whether these changes are the result of alterations in the sprouting of these neurones during development leading to changes in the density of 5-HT and DA fibres, or alterations in the amount of transmitter stored in the vesicles or the number of vesicles releasing the transmitter when the neurone is depolarised.

**Conclusions**

Overall, 5-HT appears to be involved in anxiety, with 'anxious' behaviour either on the X-maze or by isolation-rearing causing changes in 5-HT function. Thus one of the mechanisms of action of established and putative anxiolytic and anxiogenic compounds may be via modulation of the serotonergic system.

# REFERENCES

**Abuzzahab, F.S.** (1991). Ondansetron: a novel anti-anxiety agent. In: *'New Concepts in Anxiety'* (Eds. Briley, M. and File, S.E.), MacMillan Press, London, pp. 185-189.

**Aghajanian, G.K., Sprouse, J.S. and Rasmussen, K.** (1987). Physiology of the midbrain serotonin system. In: *'Psychopharmacology; The Third Generation of Progress'* (Ed. Meltzer, H.Y.), Raven Press, New York, pp. 141-149.

**Ahlenius, S., Larsson, K., Svensson, L., Hjorth, S., Carlsson, A., Lindberg, P., Wikstrom, H. and Sanchez, D.** (1981). Effects of a new type of receptor agonist on male sexual behaviour. *Pharmacol. Biochem. Behav.* **15**, 785-792.

**Akil, H., Madden, J., Patrick, R.L. and Barchas, J.D.** (1976). Stress-induced increase in endogenous opiate peptides; concurrent analgesia and its partial reversal by naloxone. In: *'Opiates and Endogenous Opiate Peptides'* (Ed. Kosterlitz, H.), Elsevier, Amsterdam, pp. 63-70.

**Albert, T.J., Dempsey, C.W. and Sorenson, C.A.** (1985). Anterior cerebellar vermal stimulation: Effect on behavior and basal forebrain neurochemistry in rat. *Biol. Psychiat.* **20**, 1267-1276.

**Alexander, G.M., Grothusen, J.R. and Schwartzman, R.J.** (1988). Flow dependent changes in the effective surface area of microdialysis probes. *Life Sci.* **43**, 595-601.

**American Psychiatric Association** (1987). Work group to revise DSM-III. Diagnostic and Statistical Manual of Mental Disorders, 3rd Ed. revised. American Psychiatric Association: Washington D.C.

**Amin, A.H., Crawford, T.B.B. and Gaddum, J.H.** (1954). The distribution of substance P and 5-HT in the central nervous system of the dog. *J. Physiol.* **126**, 596-618.

**Amir, S. and Amit, Z.** (1978). Endogenous opioid ligands may mediate stress-induced changes in the affective properties of pain related behaviour in rats. *Life Sci.* **23**, 1143-1152.

**Anden, N.E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L. and Ungerstedt, U.** (1966). Ascending monoamine neurons to the telencephalon and diencephalon. *Acta Physiol. Scand.* **67**, 313-326.

**Anderson, D.C., Johnson, D. and Kempton, H.** (1969a). Second-order fear conditioning as revealed through augmentation of a startle response: Part I. *Psychonomic Sci.* **16**, 5-7.

**Anderson, D.C., Johnson, D. and Kempton, H.** (1969b). Second-order fear conditioning as revealed through augmentation of a startle response: Part II. *Psychonomic Sci.* **16**, 7-9.

**Anisman, H.** (1978). Neurochemical changes elicited by stress. In: *'Psychopharmacology of Aversively Motivated Behaviour'* (Eds. Anisman, H. and Bignami, G.), Plenum Press, New York, pp. 119-171.

**Applegate, C.D., Kapp, B.S., Underwood, M.D. and McNall, C.L.** (1983). Autonomic and somatomotor effects of amygdala central nucleus stimulation in awake rabbits. *Physiol. Behav.* **31**, 353-360.

**Arvidsson, L.-E., Hacksell, U., Nilsson, J.L.G., Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D. and Wikstrom, H.** (1981). 8-Hydroxy-2-(di-n-propylamino) tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. *J. Med. Chem.* **24**, 921-923.

**Arriaga, F., Leitao, J., Padua, J., Ruiz, I., Tropa, J., Sousa, M.P., Mills, F.J. and Gelders, Y.** (1986). Ritanserin, an original sleep quality improving agent in generalised anxiety disorder. *Proc. 15th C.I.N.P. Congr. Puerto Rico*, P174.

**Assie, M-B., Chopin, P. and Briley, M.** (1991). F 2692, a new potential anxiolytic with a benzodiazepine-like profile but weak affinity for the benzodiazepine binding sites. *Br. J. Pharmacol.* **102**, 115P.

**Auerbach, S.B., Minzenberg, M.J. and Wilkinson, L.O.** (1989). Extracellular serotonin and 5-hydroxyindoleacetic acid in hypothalamus of the unanaesthetized rat measured by in vivo dialysis coupled to high-performance liquid chromatography with electrochemical detection: dialysate serotonin reflects neuronal release. *Brain Res.* **499**, 281-290.

**Augustine, G.J., Charlton, M.P. and Smith, S.J.** (1987). Calcium action in synaptic transmitter release. *Ann. Rev. Neurosci.* **10**, 633-693.

**Austen, K.F.** (1978). Biological implications of the structural and functional characteristics of the chemical mediators of immediate-type hypersensitivity. *Harvey Lecture* **73**, 93-161.

**Azmitia, E.C. and Segal, M.** (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* **179**, 641-667.

**Balfour, D.J.K.** (1980). Effects of GABA and diazepam on $^{3}$H-serotonin release from hippocampal synaptosomes. *Eur. J. Pharmacol.* **68**, 11-16.

**Barnes, J.M., Barnes, N.M., Costall, B., Ironside, J.W. and Naylor, R.J.** (1989a). Identification and characterisation of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue. *J. Neurochem.* **53**, 1787-1793.

**Barnes, J.M., Barnes, N.M., Costall, B., Naylor, R.J. and Tyers, M.B.** (1989b). 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature* **338**, 762-763.

**Barnes, N.M., Costall, B., Ironside, J.W. and Naylor, R.J.** (1988). Identification of 5-HT$_3$ recognition sites in human brain tissue using [$^{3}$H]-zacopride. *J. Pharm. Pharmacol.* **40**, 668.

**Barofsky, A-L., Grier, H.C. and Pradhan, T.K.** (1980). Evidence for regulation of water intake by median raphe serotonergic neurons. *Physiol. Behav.* **24**, 951-955.

**Beck, S.G. and Goldfarb, J.** (1985). Spiperone differentiates multiple 5-hydroxy-tryptamine responses in rat hippocampal slices *in vitro*. *Eur. J. Pharmacol.* **166**, 195-197.

**Bedard, P. and Pycock, C.J.** (1977). 'Wet-dog' shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology* **16**, 663-670.

**Beitz, A.J.** (1982). The organization of afferent projections to the midbrain periaqueductal gray of the rat. *Neurosci.* **7**, 133-159.

**Benveniste, H.** (1989). Brain Microdialysis. *J. Neurochem.* **52**, 1667-1679.

**Benveniste, H., Drejer, J., Schousboe, A. and Diemer, N.H.** (1987). Regional cerebral glucose phosphorylation and blood flow after insertion of a microdialysis fiber through the dorsal hippocampus in the rat. *J. Neurochem.* **49**, 729-734.

**Benveniste, H., Hansen, A.J. and Ottosen, N.S.** (1989). Determination of brain interstitial concentrations by microdialysis. *J. Neurochem.* **52**, 1741-1750.

**Benveniste, H. and Huttemeier, P.C.** (1990). Microdialysis - theory and application. *Prog. Neurobiol.* **35**, 195-215.

**Berg, W.K. and Davis, M.** (1985). Associative learning modifies startle reflexes at the lateral lemniscus. *Behav. Neurosci.* **99**, 191-199.

**Bianchi, G., Caccia, S., Della Vedova, F. and Garrattini, S.** (1988). The $\alpha_2$-adrenoceptor antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). *Eur. J. Pharmacol.* **151**, 365-371.

**Bito, L., Davson, H., Levin, E., Murray, M. and Snider, N.** (1966). The concentrations of free amino acids and other electrolytes in cerebrospinal fluid, in vivo dialysate of brain, and blood plasma of the dog. *J. Neurochem.* **13**, 1057-1067.

**Blackshear, M.A., Friedman, R.L. and Sanders-Bush, E.** (1983). Acute and chronic effects of serotonin (5-HT) antagonists on serotonin binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **324**, 125-129.

**Blackshear, M.A., Steranka, L.R. and Sanders-Bush, E.** (1981). Multiple serotonin receptors: regional distribution and effect of raphe lesions. *Eur. J. Pharmacol.* **76**, 325-334.

**Blanc, G., Herve, D., Simon, H., Lisoprawski, A., Glowinski, J. and Tassin, J.** (1980). Response to stress of mesocortico-frontal dopaminergic neurones in rats after long-term isolation. *Nature* **284**, 265-267.

**Blumstein, L.K. and Crawley, J.N.** (1983). Further characterization of a simple, automated exploratory model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.* **18**, 37-40.

**Blundell, J.E.** (1977). Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Int. J. Obesity* **1**, 15-26.

**Blundell, J.E. and Latham, C.J.** (1982). Behavioural pharmacology of feeding. In: *'Drugs and Appetite'* (Ed. Silverstone, T.), Academic Press, London, pp. 41-80.

**Boadle-Biber, M.C.** (1982). Biosynthesis of serotonin. In *'Biology of Serotonergic Transmission'* (Ed. Osborne, N.N.), John Wiley and Sons Ltd., Chichester, pp. 63-94.

**Boaventura, A.M., Blaquiere, B., Palou, A.M. and Oberlander, C.** (1986). Possible involvement of 5-HT1A sites in the anxiolytic response to diazepam. *Neurosci. Lett.* **26**, Suppl. S278.

**Bockaert, J., Dumuis, A., Bouhelal, R., Sebben, M. and Cory, R.N.** (1987). 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. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **335**, 588-592.

**Boissier, J.R., Simon, P. and Aron, C.** (1968). A new method for rapid screening of minor tranquillizers in mice. *Eur. J. Pharmacol.* **4**, 145-151.

**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.** (1986a). Proposals for the classification and nomenclature of functional receptors for 5-hydoxytryptamine. *Neuropharmacology* **25**, 563-576.

**Bradley, P.B., Humphrey, P.P.A. and Williams, R.H.** (1986b). Evidence for the existence of 5-hydroxytryptamine receptors, which are not of the 5-$HT_2$ type, mediating contraction of rabbit isolated basilar artery. *Br. J. Pharmacol.* **87**, 3-4.

**Brady, J.V. and Hunt, H.F.** (1955). An experimental approach to the analysis of emotional behavior. *J. Psychol.* **40**, 313-324.

**Braestrup, C., Schmiechen, R., Neef, G., Nielsen, M. and Petersen, E.N.** (1982). Interaction of convulsive ligands with benzodiazepine receptors. *Science* **216**, 1241-1243.

**Brazell, M.P., Marsden, C.A., Nisbet, A.P. and Routledge, C.** (1985). The 5-$HT_1$ receptor agonist RU-24969 decreases 5-hydroxytryptamine (5-HT) release and metabolism in the rat frontal cortex *in vitro* and *in vivo*. *Br. J. Pharmacol.* **86**, 209-216.

**Briley, M, Chopin, P. and Moret, C.** (1990). Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. *Psychopharmacology* **101**, 187-189.

**Broadhurst, A.M. and Briley, M.** (1988). Catecholamine and 5-HT synthesis ex vivo as an index of in-vivo neuronal activity and regulation. *T.I.P.S.* **9**, 349-350.

**Bronfenbrenner, U.** (1968). Early deprivation in mammals: a cross-species analysis. In: *'Early Experience and Behaviour'* (Eds. Newton, G. and Levine S.), Charles C. Thomas, Illinois.

**Brown, J.S., Kalish, H.I. and Farber, I.E.** (1951). Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *J. Exp. Psychol.* **41**, 317-328.

**Buckzo, W., de Gaetano, G. and Garratini, S.** (1975). Effect of fenfluramine on 5-hydroxytryptamine uptake and release by rat blood platelets. *Br. J. Pharmacol.* **53**, 563-568.

**Bunce, K.T., Elswood, C.J. and Ball, M.T.** (1991). Investigation of the 5-hydroxytryptamine receptor mechanism mediating the short-circuit current response in rat colon. *Br. J. Pharmacol.* **102**, 811-816.

**Bungay, P.M., Morrison, P.F., Dedrick, R.L.** (1990). Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sci.* **46**, 105-119.

**Butler, A., Elswood, C.J., Burridge, J., Ireland, S.I., Bunce, K.T., Kilpatrick, G.J. and Tyers, M.B.** (1990). The pharmacological characterization of 5-$HT_3$ receptors in three isolated preparations derived from guinea-pig tissues. *Br. J. Pharmacol.* **101**, 591-598.

**Capell, H., Ginsberg, R. and Webster, C.D.** (1972). Attenuation of conditioned emotional responding by amphetamine. *Br. J. Pharmacol.* **45**, 525-531.

**Carboni, E. and Di Chiara, G.** (1989). Serotonin release estimated by transcortical dialysis in freely-moving rats. *Neurosci.* **32**, 637-646.

**Carmicheal, J., Cantwell, B.M.J., Edwards, C.M., Rapeport, W.G. and Harris, A.L.** (1988). The serotonin type 3 receptor antagonist BRL 43694 and nausea and vomiting induced by cisplatin. *Br. Med. J.* **297**, 110-111.

**Cassella, J.V., Harty, P.T. and Davis, M.** (1986). Fear conditioning, pre-pulse inhibition and drug modulation of a short latency startle response measured electromyographically from neck muscles in the rat. *Physiol. Behav.* **36**, 1187-1191.

**Ceulemans, D.L.S., Hoppenbrouwers, M.L.J.A., Gelders, Y.G., and Reyntjens, A.J.M.** (1985). The influence of ritanserin, a serotonin antagonist, in anxiety disorders: A double-blind placebo-controlled study versus lorazepam. *Pharmacopsychiatry* **18**, 303-305.

**Chapman, W.P., Schroeder, H.R., Guyer, G., Brazier, M.A.B., Fager, C., Poppen, J.L., Solomon, H.C. and Yakolev, P.I.** (1954). Physiological evidence concerning the importance of the amygdaloid nuclear region in the integration of circulating function and emotion in man. *Science* **129**, 949-950.

**Charney, D.S., Heninger, G.R. and Breier A.** (1984). Noradrenergic function in panic anxiety. Effects of yohimbine in healthy subjects and patients with agoraphobia and panic disorder. *Arch. Gen. Psychiat.* **41**, 751-763.

**Charney, D.S., Woods, S.W., Goodman, W.K. and Heninger, G.R.** (1987). Serotonin function in anxiety II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology* **92**, 14-24.

**Chase, T.N., Katz, R.I. and Kopin, I.J.** (1970). Effect of diazepam on fate of intracisternally injected serotonin-C14. *Neuropharmacology* **9**, 103-108.

**Cheetham, S.C., Frankland, A.T.J., Prow, M.R., Butler, S.A. and Heal, D.J.** (1991). RU 24969: characterisation of the locomotor response in mice with isomers of pindolol and propranolol and effects on brain catecholamines and metabolites. *Br. J. Pharmacol.* (in press).

**Chopin, P. and Briley, M.** (1987). Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. *T.I.P.S.* **8**, 383-388.

**Chopin, P., Stenger, A., Assie, M-B. and Briley, M.** (1991). Pharmacological profile of F 2692, a new potential anxiolytic compound with weak affinity for the benzodiazepine binding sites. *Br. J. Pharmacol.* **102**, 114P.

**Clarke, D.E., Craig, D.A. and Fozard, J.R.** (1989). The 5-$HT_4$ receptor: naughty, but nice. *T.I.P.S.* **10**, 385-386.

**Clarke, A. and File, S.E.** (1981). Neurotoxin lesions of the lateral septum and changes in social and aggressive behaviours. *Br. J. Pharmacol.* **74**, 766P.

**Cohen, D.H.** (1975). Involvement of the avian amygdalar homologue (archistriatum posterior and mediale) in defensively conditioned heart rate change. *J. Comp. Neurol.* **160**, 13-16.

**Collinge, J. and Pycock, C.J.** (1982). Differential actions of diazepam on the release of [$^3$H]5-hydroxytryptamine from cortical and midbrain raphe slices in the rat. *Eur. J. Pharmacol.* **85**, 9-14.

**Colpaert, F.C., Meert, T.F., Niemegeers, C.J.E. and Janssen, P.A.J.** (1985). Behavioural and 5-HT antagonist effects of ritanserin: A pure and selective antagonist of LSD discrimination in rat. *Psychopharmacology* **86**, 45-54.

**Commissaris, R.L., Lyness, W.H. and Rech, R.H.** (1981). The effects of d-lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM), pentobarbital and methaqualone on punished responding in control and 5,7-dihydroxytryptamine-treated rats. *Pharmacol. Biochem. Behav.* **14**, 617-623.

**Commissaris, R.L. and Rech, R.H.** (1982). Interactions of metergoline with diazepam, quipazine and hallucinogenic drugs on a conflict behavior in the rat. *Psychopharmacology* **76**, 282-285.

**Conn, P.J. and Sanders-Bush, E.** (1984). Selective 5-HT-2 antagonists inhibit stimulated phosphatidylinositol metabolism in cerebral cortex. *Neuropharmacology* **23**, 993-996.

**Conn, P.J. and Sanders-Bush, E.** (1986). Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT-2 binding site. *J. Neurosci.* **6**, 3669-3675.

**Conn, P.J., Sanders-Bush, E., Hoffman, B.J. and Hartig, P.R.** (1986). A unique serotonin receptor in the choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci. USA* **83**, 4086-4088.

**Consolazine, A. and Cuello, A.C.** (1982). CNS serotonin pathways. In: *'Biology of Serotonergic Transmission'* (Ed. Osborne, N.N.), John Wiley and Sons Ltd., Chichester, pp. 29-61.

**Cook, L. and Sepinwall, J.** (1975). Behavioural analysis of the effects and mechanisms of action of benzodiazepines. In: *'Mechanisms of Actions of Benzodiazepines'* (Eds. Costa, E. and Greengard, P.), Raven Press, New York, pp. 1-28.

**Copland, A.M. and Balfour, D.J.K.** (1987). Spontaneous activity and brain 5-hydroxyindole levels measured in rats tested in two designs of elevated X-maze. *Life Sci.* **41**, 57-64.

**Corda, M.G., Blaker, W.D., Mendelson, W.B., Guidotti, A. and Costa, E.** (1983). ß-carbolines enhance shock-induced suppression of drinking in rats. *Proc. Natl. Acad. Sci. USA* **80**, 2072-2076.

**Corne, S.J. and Pickering, R.W.** (1967). A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. *Psychopharmacologia (Berl.)* **11**, 65-72.

**Corne, S.J., Pickering, R.W. and Warner, B.T.** (1963). A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. *Br. J. Pharmacol.* **20**, 106-120.

**Costall, B., Domeney, A.M., Gerrard, P.A., Kelly, M.E. and Naylor, R.J.** (1988). Zacopride: anxiolytic profile in rodent and primate models of anxiety. *J. Pharm. Pharmacol.* **40**, 302-305.

**Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J. and Tyers, M.B.** (1987a). The antipsychotic potential of GR38032F, a selective antagonist of 5-$HT_3$ receptors in the central nervous system. *Br. J. Pharmacol.* **90**, 89P.

**Costall, B., Domeney, A.M., Naylor, R.J. and Tattersall, M.B.** (1987b). Emesis induced by cisplatin in the ferret as a model for the detection of antiemetic drugs. *Neuropharmacology* **26**, 1321-1326.

**Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J. and Tomkins, D.M.** (1989a). Exploration of mice in a black and white test box: validation as a model of anxiety. *Pharmacol. Biochem. Behav.* **32**, 777-785.

**Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S. and Tyers, M.B.** (1990a). Ondansetron inhibits a behavioural consequence of withdrawing from drugs of abuse. *Pharmacol. Biochem. Behav.* **36**, 339-344.

**Costall, B., Kelly, M.E., Onaivi, E.S. and Naylor, R.J.** (1990b). The effect of ketotifen in rodent models of anxiety and on the behavioural consequences of withdrawing from treatment with drugs of abuse. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **341**, 547-551.

**Costall, B., Kelly, M.E., Tomkins, D.M. and Tyers, M.B.** (1989b). Profile of action of diazepam and 5-$HT_3$ receptors antagonists in the elevated X-maze. *J. Psychopharmacol.* **3**, 10P.

**Craig, D.A. and Clarke, D.E.** (1989). 5-hydroxtryptamine and cholinergic mechanisms in guinea-pig ileum. *Br. J. Pharmacol.* **96**, 247P.

**Craig, D.A. and Clarke, D.E.** (1991). Peristalsis evoked by 5-HT and renzapride: evidence for putative 5-$HT_4$ receptor activation. *Br. J. Pharmacol.* **102**, 563-564.

**Crawley, J. and Goodwin, F.K.** (1980). Preliminary report of a simple animal behaviour model for the anxiolytic effects of benzodiazepines. *Pharmacol. Biochem. Behav.* **13**, 167-170.

**Crawley, J.N.** (1985). A monoamine oxidase inhibitor reverses the 'separation syndrome' in a new hamster model of depression. *Eur. J. Pharmacol.* **112**, 129-133.

**Crespi. F., Garratt, J.C., Sleight, A.J. and Marsden, C.A.** (1990). *In vivo* evidence that 5-hydroxytryptamine (5-HT) neuronal firing and release are not necessarily correlated with 5-HT metabolism. *Neurosci.* **35**, 139-144.

**Critchley, M.A.E. and Handley, S.L.** (1986). 5-$HT_2$ receptor antagonists show anxiolytic-like activity in the X-maze. *Br. J. Pharmacol.* **89**, 646P.

**Critchley, M.A.E. and Handley, S.L.** (1987). Effects in the X-maze anxiety model of agents acting at 5-$HT_1$ and 5-$HT_2$ receptors. *Psychopharmacology* **93**, 502-506.

**Crow, T.J. and Deakin, J.F.W.** (1981). Affective change and the mechanism of reward and punishment: a neurohormonal hypothesis. In: *'Developments in Psychiatry'* (Eds. Perris, C., Struwe, G. and Jansson, B.), Elsevier, Amsterdam, **Vol. 5**, pp. 536-541.

**Cunningham, K.A., Callahan, P.M. and Appel, J.B.** (1985). Similarities in the stimulus effects of 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT), buspirone and TVXQ 7821: implications for understanding the action of novel anxiolytics? *Soc. Neurosci. Abs.* **11**, 45.

**Dahlstrom, A. and Fuxe, K.** (1964). Evidence for the existence of monoamine containing neurones in the CNS I. Demonstration of monoamines in the cell bodies of brain stem neurones. *Acta Physiol. Scand.* **62**, 1-55.

**Damsma, G., Westerink, B.H.C., de Vries, J.B., Van den Berg, C.J. and Horn, A.S.** (1987). Measurement of acetylcholine release in freely moving rats by means of automated intracerebral dialysis. *J. Neurochem.* **48**, 1523-1528.

**Daszuta, A., Kalen, P., Strecker, R.E., Brundin, P. and Bjorklund, A.** (1989). Serotonin neurons grafted to the adult rat hippocampus. II. 5-HT release as studied by intracerebral microdialysis. *Brain Res.* **498**, 323-332.

**Davis, M.** (1979a). Diazepam and flurazepam: Effects on conditioned fear as measured with the potentiated startle paradigm. *Psychopharmacology* **62**, 1-7.

**Davis, M.** (1979b). Morphine and naloxone: Effects on conditioned fear as measured with the potentiated startle paradigm. *Eur. J. Pharmacol.* **54**, 341-347.

**Davis, M.** (1988). The role of the amygdala and its efferent projections in fear and anxiety. In: *'Psychopharmacology of Anxiety'* (Ed. Tyrer, P.), Oxford University Press, Oxford, pp. 52-79.

**Davis, M. and Astrachan, D.I.** (1978). Conditioned fear and startle magnitude: effects of different footshock and backshock intensities used in training. *J. Exp. Psychol.: Animal Behav. Processes* **4**, 95-103.

**Davis, M., Cassella, J.V. and Kehne, J.H.** (1988). Serotonin does not mediate anxiolytic effects of buspirone in the fear-potentiated startle paradigm: comparison with 8-OH-DPAT and ipsapirone. *Psychopharmacology* **94**, 14-20.

**Davis, M., Gendelman, D.S., Tischler, M.D. and Gendelman, P.M.** (1982). A primary acoustic startle circuit: lesion and stimulation studies. *J. Neurosci.* **6**, 791-805.

**Davis, M., Redmond Jr., D.E. and Baraban, J.M.** (1979). Noradrenergic agonists and antagonists: Effects on conditioned fear as measured by the potentiated startle paradigm. *Psychopharmacology* **65**, 111-118.

**De Blas, A.L., Vitorica, J. and Friedrich, P.** (1988). Localization of the $GABA_A$ receptor in the rat brain with a monoclonal antibody to the 57,000 $M_r$ peptide of the $GABA_A$ receptor/benzodiazepine receptor/$Cl^-$ channel complex. *J. Neurosci.* **8**, 602-614.

**De Sarro, G.B., Ascoiti, C., Froio, F., Libri, V. and Nistico, G.** (1987). Evidence that locus coeruleus is the site where clonidine and drugs acting at $\alpha_1$- and $\alpha_2$-adrenoceptors effect sleep and arousal mechanisms. *Br. J. Pharmacol.* **90**, 675-685.

**De Chaffoy de Courcelles, D., Leysen, J.E., de Clerke, F., Van Belle, H. and Janssen, P.A.J.** (1985). Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-$S_2$ receptor sites. *J. Biol. Chem.* **260**, 7603-7608.

**De Vivo, M. and Maayani, S.** (1986). Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.* **238**, 248-253.

**Deacon, R. and Gardner, C.R.** (1986). Benzodiazepine and 5HT ligands in a rat conflict test. *Br. J. Pharmacol.* **88**, 330P.

**Delgado, J.M.R., Defeudis, F.V., Roth, R.H., Ryugo, D.K. and Mitruka, B.M.** (1972). Dialytrode for long term intracerebral perfusion in awake monkeys. *Arch. Int. Pharmacodyn.* **198**, 9-21.

**Deshmukh, P.P., Nelson, D.L. and Yamamura, H.I.** (1982). Localization of 5-$HT_1$ receptor subtypes in the rat brain by autoradiography. *Fed. Proc.* **41**, 1338.

**Di Chiara, G.** (1990). In vivo brain dialysis of neurotransmitters. *T.I.P.S.* **11**, 116-121.

**Domeney, A.M., Costall, B., Gerrard, P.A., Jones, D.N.C., Naylor, R.J. and Tyers, M.B.** (1991). The effect of ondansetron on cognitive performance in the marmoset. *Pharmacol. Biochem. Behav.* **38**, 169-175.

**Dompert, W.U., Glaser, T. and Traber, J.** (1985). $^3$H-TVXQ 7821: identification of 5-$HT_1$ binding sites as a target for a novel putative anxiolytic. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **328**, 467-470.

**Dorow, R., Horowski, R., Paschelke, G., Amin, M and Braestrup, C.** (1983). Severe anxiety induced by FG7142, a $\beta$-carboline ligand for benzodiazepine receptors. *Lancet* **ii**, 98-99.

**Dourish, C.T., Hutson, P.H. and Curzon, G.** (1985). Characteristics of feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). *Brain Res. Bull.* **15**, 377-384.

**Dourish, C.T., Hutson, P.H. and Curzon, G.** (1986). Putative anxiolytics 8-OH-DPAT, buspirone and TVXQ 7821 are agonists at 5-$HT_{1A}$ autoreceptors in the raphe nuclei. *T.I.P.S.* **7**, 212-214.

**Doyle, V.M., Creba, J.A., Ruegg, U.T. and Hoyer, D.** (1986). Serotonin increases the production of inositol phosphates and mobilises calcium via the 5-$HT_2$ receptor in $A_7r_5$ smooth muscle cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**, 98-103.

**Drust, E.G. and Connor, J.D.** (1983). Pharmacological analysis of shaking behaviour induced by enkephalins, thyrotropin-releasing hormone or serotonin in rats: evidence for different mechanisms. *J. Pharmacol. Exp. Ther.* **224**, 148-154.

**Dumuis, A., Bouhelal, R., Sebben, M., Cory, R. and Bockaert, J.** (1988). A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol. Pharmacol.* **34**, 880-887.

**Dumuis, A., Sebben, M. and Bockaert, J.** (1989). BRL 24924: A potent agonist at a non-classical 5-HT receptor possibly coupled with adenylate cyclase in colliculi neurons. *Eur. J. Pharmacol.* **168**, 381-384.

**Edwards, E., Harkins, K., Ashby Jr., C.R. and Wang, R.Y.** (1991). Effect of 5-hydroxtryptamine$_3$ receptor agonists on phosphoinositides hydrolysis in the rat fronto-cingulate and entorhinal cortices. *J. Pharmacol. Exp. Ther.* **256**, 1025-1032.

**Edwards, J.D. and Eckerman, D.A.** (1979). Effects of diazepam and ethanol alone and in combination on conditioned suppression of key-pecking in the pigeon. *Pharmacol. Biochem. Behav.* **10**, 217-221.

**Eglen, R.M., Swank, S.R., Walsh, L.K.M. and Whiting, R.L.** (1990). Characterization of 5-$HT_3$ and 'atypical' 5-HT receptors mediating guinea-pig contractions *in vitro*. *Br. J. Pharmacol.* **101**, 513-520.

**Eglen, R.M., Wong, E.H.F., Lee, J., Loury, D.N. and Whiting, R.L.** (1991). Possible heterogeneity of 5-hydroxytryptamine$_3$ (5-$HT_3$) sites as labelled

by [$^3$H]GR65630 and [$^3$H]quipazine in various tissues. *Serotonin 1991 meeting, Birmingham University, July 14-17*, P19.

**Einon, D.F.** (1980). Spatial memory and response strategies in rats: age, sex and rearing differences in performance. *Q. J. Exp. Psychol.* **32**, 473-489.

**Einon, D.F. and Morgan, M.J.** (1977). A critical period for social isolation in the rat. *Dev. Psychobiol.* **10**, 123-132.

**Einon, D.F. and Sahakian, B.J.** (1979). Environmentally induced differences in susceptibility of rats to CNS stimulants and CNS depressants: evidence against a unitary explanation. *Psychopharmacology* **61**, 299-307.

**Eison, A.S., Eison, M.S., Stantley, M. and Riblet, L.A.** (1986). Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. *Pharmacol. Biochem. Behav.* **24**, 701-707.

**Elks, M.L., Youngblood, W.W. and Kizer, J.S.** (1979). Serotonin synthesis and release in brain slices: independence of tryptophan. *Brain Res.* **172**, 471-486.

**Elliott, L.E., Skingle, M and Kilpatrick, G.J.** (1991). 5-$HT_{1D}$ binding sites: possible subpopulations and species differences. *Serotonin 1991 meeting, Birmingham University, July 14-17*, P7.

**Elswood, C.J., Bunce, K.T. and Humphrey, P.P.A.** (1991). Identification of the putative 5-$HT_4$ receptors in guinea-pig ascending colon. *Br. J. Pharmacol.* **102**, 398P.

**Emmanouil, D.E. and Quock, R.M.** (1990). Effects of benzodiazepine agonist, inverse agonist and antagonist drugs in the mouse staircase test. *Psychopharmacology* **102**, 95-97.

**Engel, G., Gothert, M., Hoyer, D., Schlicker, E. and Hillenbrand, K.** (1986). Identity of the inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-$HT_{1B}$ binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **332**, 1-7.

**Engel, G., Hoyer, D., Kalkman, H. and Wick, M.B.** (1985). Pharmacological similarity between the 5-HT-D-receptor on the guinea pig ileum and the 5-$HT_2$ binding site. *Br. J. Pharmacol.* **84**, 106P.

**Engel, J.A., Hjorth, S., Svensson, K., Carlsson, A. and Liljequist, S.** (1984). Anticonflict effect of the putative serotonin receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). *Eur. J. Pharmacol.* **105**, 365-368.

**Erspamer, V. and Asero, B.** (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature* **169**, 800-801.

**Essman, W.** (1966). The development of activity differences in isolated and aggregated mice. *Anim. Behav.* **14**, 406-409.

**Estes, W.K. and Skinner, B.F.** (1941). Some quantitative properties of anxiety. *J. Exp. Psychol.* **29**, 390-400.

**Falck, A., Hillarp, N.A., Thieme, G. and Thorp, A.** (1962). Fluorescence of catecholamines and related compounds with formaldehyde. *J. Histochem. Cytochem.* **10**, 348-354.

**Fallon, S.L., Kim, H.S. and Welch, J.J.** (1983). Electrophysiological evidence for modulation of dopamine neurons by 8-hydroxy-2-(di-n-propylamino) tetralin. *Neurosci. Abs.* **8**, 716.

**Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K., Caron, M.G. and Lefkowitz, R.J.** (1988). The genomic clone G-21 which resembles a ß-adrenergic receptor sequence encodes the 5-$HT_{1A}$ receptor. *Nature* **355**, 358-360.

**Feniuk, W., Humphrey, P.P.A., Perren, M.J. and Watts, A.D.** (1985). A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. *Br. J. Pharmacol.* **86**, 697-704.

**Fernstrom, J.D. and Hirsh, M.J.** (1974). Brain serotonin synthesis: reduction in corn malnourished rats. *J. Neurochem.* **28**, 877-879.

**Fiala, B.F.M., Snow, F.M. and Greenough, W.T.** (1977). "Impoverished" rats weigh more than "enriched" rats because they eat more. *Dev. Psychobiol.* **10**, 537-541.

**Fibiger, H.C. and Miller, J.J.** (1977). An anatomical and electrophysiological investigation ot the 5-HT projection from the DRN to the SN in the rat. *Neurosci.* **2**, 975-987.

**File, S.E.** (1978). Exploration, distraction, and habituation in rats reared in isolation. *Dev. Psychobiol.* **11**, 73-81.

**File, S.E.** (1980). The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J. Neurosci. Meth.* **2**, 219-238.

**File, S.E.** (1985). What can be learned from the effects of benzodiazepines on exploratory behaviour? *Neurosci. Biobehav. Rev.* **9**, 45-54.

**File, S.E.** (1990). One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. *Psychopharmacology* **100**, 281-282.

**File, S.E. and Baldwin, H.A.** (1989). Changes in anxiety in rats tolerant to, and withdrawn from, benzodiazepines: behavioural and biochemical studies. In: *'Psychopharmacology of Anxiety'* (Ed. Tyrer, P.), Oxford University Press, Oxford, pp. 28-51.

**File, S.E. and Deakin, J.F.W.** (1980). Chemical lesions of both dorsal and median raphe nuclei and changes in social and aggressive behaviour in rats. *Pharmacol. Biochem. Behav.* **12**, 855-863.

**File, S.E. and Hyde, J.R.G.** (1977). The effects of parachlorophenylalanine and ethanolamine-O-sulphate in an animal test of anxiety. *J. Pharm. Pharmacol.* **29**, 735-738.

**File, S.E. and Hyde, J.R.G.** (1978). Can social interaction be used to measure anxiety? *Br. J. Pharmacol.* **62**, 19-24.

**File, S.E., Hyde, J.R.G. and MacLeod, N.K.** (1979). 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. *J. Affect. Disord.* **1**, 115-121.

**File, S.E., James, T.A. and MacLeod, N.K.** (1981). Depletion of amygdaloid 5-hydroxy-tryptamine concentration and changes in social and aggressive behaviour. *J. Neural Transm.* **50**, 1-7.

**File, S.E. and Johnston, A.L.** (1989). Lack of effects of 5-$HT_3$ receptor antagonists in the social interaction and elevated plus-maze tests of anxiety in the rat. *Psychopharmacology* **99**, 248-251.

**File, S.E., Johnston, A.L. and Pellow, S.** (1987). Effects of compounds acting at CNS 5-hydroxytrypamine systems on anxiety in the rat. *Br. J. Pharmacol.* **90**, 265P.

**File, S.E. and Pellow, S.** (1985). The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. *Br. J. Pharmacol.* **86**, 729-735.

**File, S.E. and Pellow, S.** (1986). Behavioural pharmacology of CGS 9896, a novel putative anxiolytic. *Drug Dev. Res.* **7**, 245-253.

**File, S.E. and Vellucci, S.V.** (1978). Studies on the role of ACTH and of 5-HT in anxiety, using an animal model. *J. Pharm. Pharmacol.* **30**, 105-110.

**Fingl, E. and Gaddum, J.H.** (1953). Hydroxytryptamine blockade and dihydroergotamine *in vitro*. *Fed. Proc.* **12**, 320-321.

**Fletcher, P.J.** (1988). Increased food intake in satiated rats induced by the 5-HT antagonists methysergide, metergoline and ritanserin. *Psychopharmacology* **96**, 237-242.

**Fone, K.C.F., Bennett, G.W. and Marsden, C.A.** (1987). Involvement of catecholaminergic neurones and α-adrenoceptors in the wet-dog shake and forepaw licking behaviours produced by the intrathecal injection of an analogue of thyrotrophin-releasing hormone (CG 3509). *Neuropharmacology* **26**, 1147-1155.

**Fone, K.C.F., Johnson, J.V., Bennett, G.W. and Marsden, C.A.** (1989). Involvement of 5-$HT_2$ receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats. *Br. J. Pharmacol.* **96**, 599-608.

**Fone, K.C.F., Robinson, A.J. and Marsden, C.A.** (1991). Characterization of the 5-HT receptor subtypes involved in the motor behaviours produced by intrathecal administration of a variety of 5-HT agonists in rats. *Br. J. Pharmacol.* **103**, 1547-1555.

**Fontana, D.J., Carabary, T.J. and Commissaris, R.L.** (1989). Effects of acute and chronic anti-panic drug administration on conflict behavior in the rat. *Psychopharmacology* **98**, 157-162.

**Fox, M. and Stelzner, D.** (1966). Behavioural effects of differential early experience in the dog. *Anim. Behav.* **14**, 273-281.

**Frances, H., Khidichian, F. and Monier, C.** (1990). Increase in the isolation-induced social behavioural deficit by agonists at 5-$HT_{1A}$ receptors. *Neuropharmacology* **29**, 103-107.

**Frenken, M.** (1989). Evidence for two populations of 5-hydroxytryptamine receptors in dog basilar artery. *J. Pharmacol. Exp. Ther.* **250**, 379-387.

**Fuxe, K.** (1965). The distribution of monoamine terminals in the central nervous system. *Acta. Physiol. Scand.* **64**, 39-85.

**Gaddum, J.H.** (1961). Push-pull cannulae. *J. Physiol.* **155**, 1P.

**Gallager, D.W.** (1978). Benzodiazepines: potentiation of a GABA inhibitory response in the dorsal raphe nucleus. *Eur. J. Pharmacol.* **49**, 133-143.

**Galvani, P.F.** (1970). Air-puff-elicited startle: Habituation over trials and measurement of a hypothetical emotional response. *Behav. Res. Meth. Instrum.* **2**, 232-233.

**Garattini, S., Jori, A. and Samanin, R.** (1975). The mechanism of action of fenfluramine. *Postgrad. Med. J.* **55 (S1)**, 27-35.

**Gardner, C.R.** (1985). Distress vocalisation in rat pups: a simple screening method for anxiolytic drugs. *J. Pharmacol. Meth.* **14**, 181-187.

**Gardner, C.R.** (1986). Recent developments in 5-HT related pharmacology of animal models of anxiety. *Pharmacol. Biochem. Behav.* **24**, 1479-1485.

**Garratt, J.C., Crespi, F., Mason, R. and Marsden, C.A.** (1991). Effects of idazoxan on dorsal raphe 5-hydroxytryptamine neuronal function. *Eur. J. Pharmacol.* **193**, 87-93.

**Garzon, J. and Del Rio, J.** (1981). Hyperactivity induced in rats by long term isolation: further studies on a new animal model for the detection of antidepressants. *Eur. J. Pharmacol.* **74**, 287-294.

**Garzon, J., Fuentes, J.A. and Del Rio, J.** (1979). Antidepressants antagonise the hyperactivity induced in rats by long term isolation. *Eur. J. Pharmacol.* **59**, 293-296.

**Geller, I. and Blum, K.** (1970). The effects of 5-HT on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. *Eur. J. Pharmacol.* **9**, 319-324.

**Geller, I. and Hartmann, R.J.** (1982). Effects of buspirone on operant behaviour of laboratory rats and cynomologus monkeys. *J. Clin. Psychiat.* **43**, 25-32.

**Geller, I., Kula, T. and Seifter, J.** (1962). The effects of chlordiazepoxide and chlorpromazine on a punished discrimination. *Psychopharmacologia (Berl.)* **3**, 374-385.

**Geller, I. and Seifter, J.** (1960). The effects of meprobamate, barbituates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia (Berl.)* **1**, 482-492.

**Geller, I. and Seifter, J.** (1962). The effects of mono-urethans, di-urethans and barbiturates on a punishment discrimination. *J. Pharmacol. Exp. Ther.* **136**, 284-288.

**Gentile, C.G., Jarrell, T.W., Teich, A., McCabe, P.M. and Schneiderman, N.** (1986). The role of amygdaloid central nucleus in the retention of differential pavlovian conditioning of bradycardia in rabbits. *Behav. Brain Res.* **20**, 263-273.

**Gentsch, C., Lichtsteiner, M. and Feer, H.** (1981a). Individual housing of rats causes divergent changes in spontaneous and reactive activity. *Experimentia* **37**, 61-62.

**Gentsch, C., Lichtsteiner, M. and Feer, H.** (1981b). Locomotor activity, defecation score and corticosterone levels during an open field exposure: a comparison among individually and group-housed rats, and genetically selected rat lines. *Physiol. Behav.* **27**, 183-186.

**Gentsch, C., Lichtsteiner, M. and Feer, H.** (1982). $^{3}$H-imipramine binding sites in the rat brain tissue from different groups of rats: a preliminary report. *IRCS Med. Sci.* **10**, 701-702.

**Gentsch, C., Lichtsteiner, M., Frischknecht, H.R., Feer, H. and Siegfried, B.** (1988). Isolation-induced locomotor hyper-activity and hypoalgesia in rats are prevented by handling and reversed by resocialisation. *Physiol. Behav.* **43**, 13-16.

**Gerson, S.C. and Baldessarini, R.J.** (1980). Motor effects of serotonin in the central nervous system. *Life Sci.* **27**, 1435-1451.

**Glaser, T. and Traber, J.** (1987). Binding of the putative anxiolytic TVXQ 7821 to hippocampal 5-hydroxytryptamine (5-$HT_1$) recognition sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **329**, 211-215.

**Glaser, T., Rath, M., Traber, J., Zilles, K. and Schleicher, A.** (1985). Autoradiographic identification and topographical analyses of high affinity serotonin receptor subtypes as a target for the novel putative anxiolytic TVXQ 7821. *Brain Res.* **358**, 129-136.

**Gleeson, S. and Barrett, J.E.** (1990). 5-$HT_{1A}$ agonist effects on punished responding of squirrel monkeys. *Pharmacol. Biochem. Behav.* **37**, 335-337.

**Glennon, R.A.** (1987). Central serotonin receptors as targets for drug research. *J. Neurochem.* **30**, 1-12.

**Gloor, P., Olivier, A. and Quesney, L.F.** (1981). The role of the amygdala in the expression of psychic phenomena in temporal lobe seizures. In: *'The amygdaloid complex'* (Ed. Ben-Ari, Y.), Elsevier, New York, pp. 489-497.

**Glowinski, J., Cheramy, A. and Glorgulieff, M.F.** (1979). *In vivo* and *in vitro* release of dopamine. In: *'The Neurobiology of Dopamine'* (Eds. Horn, A.S., Korf, J. and Westerink, B.H.C.), Academic Press, London, pp. 199-216.

**Goa, K. L. and Ward, A.** (1986). Buspirone - a preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. *Drugs* **32**, 114-129.

**Gobbi, M., Cavanus, G., Miari, A. and Mennini, T.** (1991). Effect of acute and chronic administration of buspirone on serotonin and benzodiazepine receptor subtypes in the rat brain: an autoradiographic study. *Neuropharmacology* **30**, 313-321.

**Gold, M.S., Redmond, D.E. and Kleber, H.D.** (1979). Noradrenergic hyperactivity in opiate withdrawal supported by clonidine reversal of opiate withdrawal. *Am. J. Psychiat.* **136**, 100-102.

**Goldberg, H.L. and Finnerty, R.J.** (1979). The comparative efficacy of buspirone and diazepam in the treatment of anxiety. *Am. J. Psychiat.* **136**, 1184-1187.

**Goldberg, M. E., Salama, A. I., Patel, J. B. and Malick, J. B.** (1983). Novel non-benzodiazepine anxiolytics. *Neuropharmacology* **22**, 1499-1504.

**Gonon, F. and Buda, M.** (1985). Regulation of dopamine release by impulse flow and by autoreceptors as studied by *in vivo* voltammetry in the rat striatum. *Neurosci.* **14**, 765-774.

**Gonon, F.G., Buda, M.J., Cespuglio, R., Jouvet, M. and Pujol, J.F.** (1980). *In vivo* electrochemical detection of catechols in the neostriatum of anaesthetised rats: dopamine or DOPAC? *Nature* **286**, 902-904.

**Gonon, F.G., Navarre, F. and Buda, M.J.** (1984). In vivo monitoring of dopamine release in the rat brain with differential normal pulse voltammetry. *Anal. Chem.* **56**, 573-575.

**Goodwin, G.M. and Green, A.R.** (1985). A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-$HT_1$ and 5-$HT_2$ receptors. *Br. J. Pharmacol.* **84**, 743-753.

**Goodwin, G.M., De Souza, R.J. and Green, A.R.** (1985). The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). *Neuropharmacology* **24**, 1187-1194.

**Gothert, M. and Huth, H.** (1980). Alpha-adrenoceptor-mediated modulation of 5-hydroxy-tryptamine release from rat brain cortex slices. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **313**, 21-26.

**Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski J. and Hamon, M.** (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: $^3$H-PAT. *Nature* **305**, 140-142.

**Graeff, F.G. and Schoenfeld, R.I.** (1970). Tryptaminergic mechanisms in punished and non-punished behaviour. *J. Pharmacol. Exp. Ther.* **173**, 277-283.

**Grahame-Smithe, D.G.** (1971). 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. *J. Neurochem.* **18**, 1053-1066.

**Grahame-Smithe, D.G.** (1974). How important is the synthesis of brain 5-hydroxytryptamine in the physiological control of its central function? *Adv. Biochem. Psychopharmacol.* **10**, 83-91.

**Green, A.R. and Heal, D.J.** (1985). The effects of drugs on serotonin-mediated behavioural models. In *'Neuropharmacology of serotonin'* (Ed. Green, A.R.), Oxford University Press, Oxford, pp. 326-365.

**Green, A.R. and Grahame-Smith, D.G.** (1975). 5-HT and other indoles in the central nervous system. In: *'Handbook of Psychopharmacology'* (Eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H.), Plenum Press, New York, **Vol. 3**, pp. 169-245.

**Green, A.R., Johnson, P. and Nimgaonkar, V.L.** (1983a). Increased 5-$HT_2$ receptor number in brain as a probable explanation for the enhanced 5-hydroxy-tryptamine-mediated behaviour following repeated electroconvulsive shock administration to rats. *Br. J. Pharmacol.* **80**, 173-177.

**Green, A.R., O'Shaughnessy, K., Hammond, M., Schachter, M. and Grahame-Smith, D.G.** (1983b). Inhibition of 5-hydroxytryptamine-mediated behaviour by the putative 5-$HT_2$ antagonist pirenpirone. *Neuropharmacology* **22**, 573-578.

**Guisado, E., Fernandez-Tome, P., Garzon, J. and Del Rio, J.** (1980). Increased dopamine receptor binding in the striatum of rats after long term isolation. *Eur. J. Pharmacol.* **65**, 463-464.

**Guy, A.P. and Gardner, C.R.** (1985). Pharmacological characterisation of a modified social interaction model of anxiety in the rat. *Neuropsychobiology* **13**, 194-200.

**Hagan, R.M., Butler, A., Hill, J.M., Jordan, C.C., Ireland, S.J. and Tyers, M.B.** (1987). Effect of the 5-$HT_3$ receptor antagonist, GR38032F, on responses to injection of a neurokinin into the ventral tegmental area of the rat brain. *Eur. J. Pharmacol.* **138**, 303-305.

**Hamlin, K.E. and Fischer, F.E.** (1951). The synthesis of 5-hydroxytryptamine. *J. Am. Chem. Soc.* **73**, 5007-5008.

**Handley, S.L. and Brown, J.** (1982). Effects on the 5-hydroxytryptamine-induced head-twitch of drugs with selective actions on alpha$_1$ and alpha$_2$-adrenoceptors. *Neuropharmacology* **21**, 507-510.

**Handley, S.L. and Mithani, S.** (1984). Effects of α-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **327**, 1-5.

**Handley, S.L. and Singh, L.** (1986). Neurotransmitters and shaking behaviour - more than a 'gut-bath' for the brain? *T.I.P.S.* **7**, 324-328.

**Handley, S.L. and Thomas, K.V.** (1979). Potentiation of startle response by d- and l-amphetamine: the possible involvement of pre- and postsynaptic alpha-adrenoceptors and other transmitter systems in the modulation of a tactile startle response. *Psychopharmacology* **64**, 105-111.

**Heal, D.J., Philpot, J., O'Shaughnessy, K.M. and Davies, C.L.** (1986). The influence of central noradrenergic function on 5-$HT_2$-mediated head-twitch response in mice: possible implications for the actions of antidepressant drugs. *Psychopharmacology* **89**, 414-420.

**Heaton, J.C.P., Njung'e, K. and Handley, S.L.** (1988). Behavioural profile of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective 5-$HT_2$ agonist. *Br. J. Pharmacol.* **94**, 388P.

**Henke, P.G.** (1980a). The centromedial amygdala and gastric pathology in rats. *Physiol. Behav.* **25**, 107-112.

**Henke, P.G.** (1980b). The amygdala and restraint ulcers in rats. *J. Comp. Physiol. Psychol.* **94**, 313-323.

**Hernandez, L., Paez, X. and Hamlin, C.** (1983). Neurotransmitter extraction by local intracerebral microdialysis in anaethetized rats. *Pharmacol. Biochem. Behav.* **18**, 159-162.

**Herrick-Davis, K. and Titeler, M.** (1988). Detection and characterization of the serotonin 5-$HT_{1D}$ receptor in rat and human brain. *J. Neurochem.* **50**, 1624-1631.

**Heuring, R.E. and Peroutka, S.J.** (1987). Characterization of a novel $^3$H-5-hydroxtryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* **7**, 894-903.

**Heuring, R.E., Schlegel, J.R. and Peroutka, S.J.** (1986). Species variation in RU 24969 interactions with non-5-$HT_{1A}$ binding sites. *Eur. J. Pharmacol.* **122**, 279-282.

**Higgins, G.A., Jones, B.J. and Oakley, N.R.** (1987). Compounds selective for the 5-$HT_{1A}$ receptors have anxiolytic effects when injected into the dorsal raphe nucleus of the rat. *Br. J. Pharmacol.* **92**, 658P.

**Higgins, G.A., Jordan, C.C. and Skingle, M.** (1991). Evidence that the unilateral activation of 5-$HT_{1D}$ receptors in the substantia nigra of the guinea-pig elicits contralateral rotation. *Br. J. Pharmacol.* **102**, 305-310.

**Hille, B.** (1968). Pharmacological modifications of the sodium channels of frog nerve. *J. Gen. Physiol.* **51**, 199-219.

**Hillegaart, V., Ahlenius, S. and Larsson, K.** (1989). Effects of local application of 5-HT into the median and dorsal raphe nuclei on male rat sexual and motor behaviour. *Behav. Brain Res.* **33**, 279-286.

**Hitchcock, J.M. and Davis, M.** (1986a). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. *Behav. Neurosci.* **100**, 11-22.

**Hitchcock, J.M. and Davis, M.** (1986b). Amygdala lesions block fear-enhanced startle using either visual or auditory conditioned stimuli. *Soc. Neurosci. Abstracts* **12**, 752.

**Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikstrom, H., Arvidsson, L.-E.,Hacksell, U. and Nilsson, J.L.G.** (1982). 8-Hydroxy-2-(di-n-propylamino) tetralin, 8-OHDPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J. Neural Transm.* **55**, 169-188.

**Hjorth, S. and Magnusson, T.** (1988). The 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn-Schmiedeberg's Arch Pharmacol.* **338**, 463-471.

**Hjorth, S. and Sharp, T.** (1990). Mixed agonist/antagonist properties of NAN-190 at 5-$HT_{1A}$ receptors: behavioural and *in vivo* brain microdialysis studies. *Life Sci.* **46**, 955-963.

**Hock, F.J. and Scheich, H.** (1986). Functional activity in the brain of socially deprivated rats produced by an active avoidance test after Razobazam (Hoe 175) treatment: a 2-deoxyglucose study. *Behav. Neural Biol.* **46**, 398-409.

**Hoehn-Saric, R., Merchant, A.F., Keyser, M.L. and Smith, V.K.** (1981). Effects of clonidine on anxiety disorders. *Arch. Gen. Psychiat.* **38**, 1278-1282.

**Hoffman, B.J. and Mezey, E.** (1989). Distribution of serotonin$_{1C}$ receptor mRNA in adult rat brain. *FEBS Letts.* **247**, 453-462.

**Hopkins, D.A. and Holstege, G.** (1978). Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp. Brain Res.* **32**, 529-547.

**Howard, J.L. and Pollard, G.T.** (1977). A model of anxiety and a screening procedure for anxiolytics. In: *'Animal Models in Psychiatry and Neurology'* (Eds. Hanin, I. and Usdin, E.), Pergamon Press, New York.

**Hoyer, D., Engel, G. and Kalkman, H.O.** (1985). Molecular pharmacology of 5-HT$_1$ and 5-HT$_2$ recognition sites in the rat and pig brain membranes: radioligand binding studies with [$^3$H]5-HT, [$^3$H]8-OH-DPAT, (-)[$^{125}$I]iodocyanopindolol, [$^3$H]mesulergine and [$^3$H]ketanserin. *Eur J. Pharmacol.* **118**, 13-23.

**Hoyer, D. and Middlemiss, D.N.** (1989). Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *T.I.P.S.* **10**, 130-132.

**Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M.** (1986a). Serotonin receptors in the human brain. I. Characterisation and autoradiographic localisation of 5-HT$_{1A}$ recognition sites. Apparant absence of 5-HT$_{1B}$ recognition site. *Brain Res.* **376**, 85-96

**Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M.** (1986b). Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT$_{1C}$ and 5-HT$_2$ recognition sites. *Brain Res.* **376**, 97-107.

**Hoyer, D. and Schoeffter, P.** (1988). 5-HT$_{1D}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in calf substantia nigra. *Eur. J. Pharmacol.* **147**, 145-147.

**Hoyer, D., Srivatsa, S., Pazos, A., Engel, G. and Palacios, J.M.** (1986c). [$^{125}$I]LSD labels 5-HT$_{1C}$ recognition sites in pig choroid plexus membranes. Comparison with [$^3$H]mesulergine and [$^3$H]5-HT binding. *Neurosci. Lett.* **69**, 269-274.

**Hughes, R.N. and Pither, J.M.** (1987). Chronic imipramine effects on exploratory behaviour in rats. *Pharmacol. Biochem. Behav.* **27**, 359-362.

**Humble, M., Aberg-Wistedt, A. Wistedt, B. and Bertilsson, L.** (1986). A pilot study of ritanserin (a selective serotonin-2 receptor antagonist) in panic disorder and agoraphobia. Clinical and biochemical effects. *Proc. 15th C.I.N.P. Congr. Puerto Rico*, P165.

**Hutson, P.H., Donohoe, T.P. and Curzon, G.** (1987). Hypothermia induced by the putative 5-$HT_{1A}$ agonists LY165163 and 8-OH-DPAT is not prevented by 5-HT depletion. *Eur. J. Pharmacol.* **143**, 221-228.

**Hutson, P.H., Sarna, G.S., O'Connell, M.T. and Curzon, G.** (1989). Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OH-DPAT into the nucleus raphe dorsalis. *Neurosci. Lett.* **100**, 276-280.

**Imperato, A. and Di Chiara, G.** (1984). Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the *in vivo* release of endogenous dopamine and metabolites. *J. Neurosci.* **4**, 966-977.

**Imperato, A., Puglisi-Allegra, S., Casolini, P. and Angelucci, L.** (1991). Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. *Brain Res.* **538**, 111-117.

**Innes, D.L. and Tansy, M.F.** (1980). Gastric mucosal ulceration associated with electrochemical stimulation of the limbic system. *Brain Res. Bull.* **5**, 33-36.

**Ison, J.R., McAdam, D.W. and Hammond, G.R.** (1973). Latency and amplitude changes in the acoustic startle reflex of the rat produced by variation in auditory prestimulation. *Physiol. Behav.* **10**, 1035-1039.

**Iversen, S.D.** (1984). 5-HT and anxiety. *Neuropharmacology* **23**, 1553-1560.

**Iwahara, S. and Sakama, E.** (1972). Effects of chlordiazepoxide upon habituation of open-field behavior in white rats. *Psychopharmacologia (Berl.)* **27**, 285-292.

**Iwata, J., LeDoux, J.E., Meeley, M.P., Arneric, S. and Reis, D.J.** (1986a). Intrinsic neurons in the amygdala field projected to by the medial

geniculate body mediate emotional response conditioned to acoustic stimuli. *Brain Res.* **383**, 195-214.

**Iwata, J., LeDoux, J.E. and Reis, D.J.** (1986b). Different efferent projections of the geniculoamygdala pathway mediate autonomic and behavioral concomitants of conditioned fear: possible completion of the conditioned response circuits. *Soc. Neurosci. Abstracts* **12**, 977.

**Jacobs, B.L.** (1976). An animal behaviour model for studying central serotonergic synapses. *Life Sci.* **19**, 777-786.

**Jacobs, B.L. and Gelperin, A.** (Eds.) (1981). *'Serotonin transmission and Behaviour'*. MIT Press, Boston.

**Jacobs, B.L., Heym, J. and Steinfels, G.F.** (1984). Physiology and behavioral analysis of raphe unit activity. In: *'Handbook of Psychopharmacology'* (Eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H.), Plenum Press, New York, **Vol. 18**, 343-395.

**Jacobson, I., Sandberg, M. and Hamberger, A.** (1985). Mass transfer in brain dialysis devices - a new method for the estimation of extracellular amino acid concentration. *J. Neurosci. Meth.* **15**, 263-268.

**Jones, B.J., Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J., Oakley, N.R. and Tyers, M.B.** (1988). The potential anxiolytic activity of GR38032F, a 5-$HT_3$ receptor antagonist. *Br. J. Pharmacol.* **93**, 985-993.

**Jones, B.J., Oakley, N.R. and Tyers, M.B.** (1987). The anxiolytic activity of GR38032F, a 5-$HT_3$ receptor antagonist, in the rat and cynomolgus monkey. *Br. J. Pharmacol.* **90**, 88P.

**Jones, G.H., Hernandez, T.D., Robbins, T.W. and Marsden, C.A.** (1988). Enhanced striatal response to d-amphetamine as revealed by intracerebral dialysis following social isolation in rats. *Br. J. Pharmacol.* **94**, 249P.

**Jones, G.H., Marsden, C.A. and Robbins, T.W.** (1990). Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. *Psychopharmacology* **102**, 364-372.

**Jones, G.H., Robbins, T.W. and Marsden, C.A.** (1989). Isolation-rearing retards the acquisition of schedule-induced polydipsia in rats. *Physiol. Behav.* **45**, 71-77.

**Julius, D., Huang, K.N., Livelli, T.J., Axel, R. and Jessell, T.M.** (1990). The 5-HT2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors. *Proc. Natl. Acad. Sci. USA* **87**, 928-932.

**Julius, D., MacDermott, A.B. Axel, R. and Jessell, T.M.** (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science* **241**, 558-564.

**Juraska, J.M., Henderson, C. and Muller, J.** (1984). Differential rearing experience, gender and radial maze performance. *Dev. Psychobiol.* **17**, 209-215.

**Kaessermann, H.P. and Dixon, A.K.** (1986). Further validation of the SAP test, a simple behavioural conflict test in mice. *Eur. Behav. Pharmacol. Soc. Antwerp and Beerle*, Belgium. **S19**, No.54.

**Kahn, R.S., Van Praag, H.M., Wetzler, S., Asnis, G.M. and Barr, G.** (1988). Serotonin and anxiety revisited. *Biol. Psychiat.* **23**, 189-208.

**Kahn, R.S. and Westenberg, H.G.M.** (1985). L-5-Hydroxytryptophan in the treatment of anxiety disorders. *J. Affect. Disord.* **8**, 197-200.

**Kalen, P., Rosegren, E., Lindvall, O. and Bjorklund, A.** (1989). 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. *Eur. J. Neurosci.* **1**, 181-186.

**Kalen, P., Strecker, R.E., Rosengren, E. and Bjorklund, A.** (1988). Endogenous release of neuronal serotonin and 5-hydroxyindoleacetic acid in the caudate-putamen of the rat as revealed by intracerebral microdialysis coupled to high-performance liquid chromatography with fluorimetric detection. *J. Neurochem.* **51**, 1422-1435.

**Kaumann, A.J., Brown, A.M. and Raval, P.** (1991). Putative 5-$HT_4$-like receptors in piglet left atrium. *Br. J. Pharmacol.* **102**, 98P.

**Kehne, J.H., Cassella, J.V. and Davis, M.** (1988). Anxiolytic effects of buspirone and gepirone in the fear-potentiated startle paradigm. *Psychopharmacology* **94**, 8-13.

**Kehoe, P. and Blass, E.M.** (1986). Opioid mediating of separation stress in 10-day-old rats: reversal of stress with maternal stimuli. *Dev. Psychobiol.* **19**, 385-398.

**Kellicut, M.H. and Schwartzbaum, J.S.** (1963). Formation of a conditioned emotional response (CER) following lesions of the amygdaloid complex in rats. *Psychol. Rev.* **12**, 351-358.

**Kendall, D.A. and Nahorski, S.R.** (1985). 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J. Pharmacol. Exp. Ther.* **233**, 473-479.

**Kennett, G.A., Whitton, P., Shah, K. and Curzon, G.** (1989). Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-$HT_{1C}$ receptor antagonists. *Eur. J. Pharmacol.* **164**, 445-454.

**Ketelaars, C.E.J. and Bruinvels, J.** (1989). The anti-conflict effect of cyproheptadine is not mediated by its 5-hydroxytryptamine antagonistic property. *Life Sci.* **44**, 1743-1749.

**Kidd, E.J.** (1990). Functional adaptation of 5-HT receptors. *Ph.D. Thesis*, Nottingham University, U.K.

**Kilpatrick, G.J., Jones, B.J. and Tyers, M.B.** (1987). Identification and distribution of 5-$HT_3$ recptors in rat brain using radioligand binding. *Nature* **330**, 746-748.

**Kilts, C.D., Commissaris, R.L., Cordon, J.J. and Roth, R.H.** (1982). Lack of central 5-hydroxtryptamine influence on the anticonflict activity of diazepam. *Psychopharmacology* **78**, 156-164.

**Kimelberg, H.K.** (1986). Occurrence and functional significance of serotonin and catecholamine uptake by astrocytes. *Biochem. Pharmacol.* **35**, 2273-2281.

**Korte, S.M. and Bohus, B.** (1990). The effect of ipsapirone on behavioural and cardiac responses in the shock-probe/defensive burying test in male rats. *Eur. J. Pharmacol.* **181**, 307-310.

**Kostowski, W. and Czlondowski, A.** (1973). The activity of some neuroleptic drugs and amphetamine in normal and isolated rats. *Pharmacology* **10**, 82-87.

**Kostowski, W., Plaznik, A. and Stefanski, R.** (1989). Intrahippocampal buspirone in animal models of anxiety. *Eur. J. Pharmacol.* **168**, 393-396.

**Kraemer, G.W., Ebert, M.H., Lake, C.R. and McKinney, W.T.** (1984). Hypersensitivity to d-amphetamine several years after early social deprivation in rhesus monkeys. *Psychopharmacology* **82**, 266-271.

**Krettek, J.E. and Price, J.L.** (1978). Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J. Comp. Neurol.* **178**, 225-254.

**Kudoh, Y.** (1986). Clinical phase II open trial of buspirone in Osaka area, Japan. *Proc. 15th C.I.N.P. Congr. Puerto Rico*, P156.

**Kuhn, D.M., Wolf, W.A. and Youdim, M.B.H.** (1986). Serotonin neurochemistry revisited: a new look at some old axioms. *Neurochem. Int.* **8**, 141-154.

**Kurtz, K.H. and Siegel, A.** (1966). Conditioned fear and magnitude of startle response: A replication and extension. *J. Comp. Physiol. Psychol.* **62**, 8-14.

**Lader, M. and Olajide, D.** (1987). A comparison of buspirone and placebo in relieving benzodiazepine withdrawal symptoms. *J. Clin. Psychopharmacol.* **7**, 11-15.

**Lader, M.H. and Wing, L.** (Eds.) (1966). In: *'Physiological measures, sedative drugs and morbid anxiety'*, Oxford University Press, Oxford.

**Lal, H., Bennett, D. and Ellmesallamy, F.** (1982). Anxiogenic potential of ß-carboline compounds as bioassayed by generalisation to interoceptive stimuli produced by pentylenetetrazole. *Soc. Neurosci. Abstracts* **8**, 571.

**Lal, H. and Shearman, G.T.** (1980). Interoceptive discriminative stimuli in the development of CNS drugs and a case of an animal model of anxiety. *Ann. Rep. Med. Chem.* **15**, 51-58.

**Lal, H. and Shearman, G.T.** (1982). Attenuation of chemically-induced anxiogenic stimuli as a novel method for evaluating anxiolytic drugs: a comparison of clobazam with other benzodiazepines. *Drug Dev. Res.* **Suppl 1**, 127-134.

**Laurent, J-P., Margold, M., Hunkel, V. and Haefely, W.** (1983). Reduction by two benzodiazepines and pentobarbitone of the multiunit activity in substantia nigra, hippocampus, nucleus locus coeruleus and dorsal raphe nucleus of "encephale isole" rats. *Neuropharmacology* **22**, 501-512.

**Leathley, M., Taylor, M.A.I. and Goudie, A.J.** (1990). Effects of the 5-$HT_3$ antagonist GR38032F on benzodiazepine withdrawal in rats. *Br. J. Pharmacol.* **99**, 243P.

**Leaton, R.N. and Borszcz, G.S.** (1985). Potentiated startle: Its relation to freezing and shock intensity in rats. *J. Exp. Psychol.: Anim. Behav. Processes* **11**, 421-428.

**Lehman, H.E. and Ban, T.A.** (1971). Effects of psychoactive drugs on conflict avoidance in human subjects. *Acta Nerv. Sup.* **13**, 82-85.

**Leonhardt, S., Herrick-Davis, K. and Titeler, M.** (1989). Detection of a novel serotonin receptor subtype (5-$HT_{1E}$) in human brain: interaction with a GTP-binding protein. *J. Neurochem.* **53**, 465-471.

**Leysen, J.E., Gommeren, W., Van Gompel, P., Wynants, J., Janssen, P.F.M. and Laduron, P.M.** (1985). Receptor binding properties in vitro and in vivo of ritanserin. A very potent and long acting serotonin-$S_2$ antagonist. *Mol. Pharmacol.* **27**, 600-611.

**Leysen, J.E., Niemegeers, C.J.E., Van Neuten, J.M. and Laduron, P.M.** (1982). [$^3$H]Ketanserin (R41 468), a selective $^3$H-ligand for serotonin$_2$ receptor binding sites. *Mol. Pharmacol.* **21**, 301-314.

**Leysen, J.E., Van Gompel, P., Gommeren, W., Woestenborghs, R. and Janssen, P.A.J.** (1986). Down regulation of serotonin-$S_2$ receptor sites in rat brain by chronic treatment with the serotonin-$S_2$ antagonists: ritanserin and setoperone. *Psychopharmacology* **88**, 434-444.

**Lidbrink, P., Corrodi, H., Fuxe, K. and Olson, L.** (1973). The effects of benzodiazepines, meprobamate and barbituates on central monoamine neurons. In: *'The Benzodiazepines'* (Eds. Garrattini, S., Mussini, E. and Randall, L.O.), Raven Press, New York, pp. 203-224.

**Lister, R.G. and File, S.E.** (1983). Changes in regional concentrations in the rat brain of 5-hydroxtryptamine and 5-hydroxyindoleacetic acid during development of tolerance to the sedative action of chlordiazepoxide. *J. Pharm. Pharmacol.* **35**, 601-603.

**Lubbert, H., Hoffman, B.J., Snutch, T.P., Van Dyke, T., Levine, A.J., Hartig, P.R., Lester, H.A. and Davidson, N.** (1987). cDNA cloning of a serotonin 5-$HT_{1C}$ receptor by electrophysiological assays of mRNA-injected *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **84**, 4332-4336.

**Maayani, S., Wilkinson, C.W. and Stollak, J.S.** (1984). 5-Hydroxytryptamine receptor in rabbit aorta: characterization by butyrophenone analogs. *J. Pharmacol. Exp. Ther.* **229**, 346-350.

**Mabry, P.D. and Campbell, B.A.** (1977). Developmental Psychopharmacology. In: *'Handbook of Psychopharmacology'* (Eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H.), Plenum Press, New York, **Vol. 7**, pp. 393-444.

**MacIntosh, F.C. and Oborin, P.E.** (1953). Release of acetylcholine from intact cerebral cortex. *Abstracts of the XIX Int. Physiol. Congress* 580-581.

**Mansbach, R.S. and Barrett, J.E.** (1987). Discriminative stimulus properties of buspirone in the pigeon. *J. Pharmacol. Exp. Ther.* **240**, 364-369.

**Markstein, R., Hoyer, D. and Engel, G.** (1986). 5-HT1A-receptors mediate stimulation of adenylate cyclase in rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**, 335-341.

**Marsden, C.A.** (1985). *In vivo* monitoring of pharmacological and physiological changes in endogenous serotonin release and metabolism. In: *'Neuropharmacology of Serotonin'*, (Ed. Green, A.R.,), Oxford University Press, Oxford, pp. 218-252.

**Marsden, C.A.** (1989). 5-Hydoxytryptamine receptor subtypes and new anxiolytics: an appraisal. In: *'Psychopharmacology of Anxiety'* (Ed. Tyrer, P.), Oxford University Press, Oxford, pp. 3-27.

**Marsden, C.A. and Martin, K.F.** (1986). Involvement of 5-$HT_{1A}$ and $\alpha_2$-receptors in the decreased 5-hydroxytryptamine release and metabolism in rat suprachiasmatic nucleus after intravenous 8-hydroxy-2-(di-n-propylamino) tetralin. *Br. J. Pharmacol.* **89**, 277-286.

**Marsden, C.A., Martin, K.F., Routledge, C., Brazell, M.P. and Maidment, N.T.** (1986). Application of intracerebral dialysis and *in vivo* voltammetry to

pharmacological and physiological studies of amine neurotransmitters. *Ann. N. Y. Acad. Sci.* **473**, 106-125.

**Marsden, C.A., Sleight, A.J., Fone, K.C.F., Johnson, J.V., Crespi, F., Martin, K.F., Garratt, J.C. and Bennett, G.W.** (1989). Functional identification of 5-HT receptor subtypes. *Comp. Biochem. Physiol.* **93A**, 107-114.

**Martin, K.F., Hannon, S.D., Phillips, I. and Heal, D.J.** (1991). Opposing roles for 5-$HT_1$ and 5-$HT_3$ receptors in controlling 5-HT release in rat hippocampus *in vivo*. *Br. J. Pharmacol.* (in press).

**Martin, K.F. and Mason, R.** (1987). Isapirone is a partial agonist at 5-hydroxtryptamine$_{1A}$ receptors in the rat hippocampus: electrophysiological evidence. *Eur. J. Pharmacol.* **121**, 135-140.

**Mason, W.A., Davenport, R. and Menzel, E.** (1968). Early experience and the social development of rhesus monkeys and chimpanzees. In: *'Early Experience and Behavior'* (Eds. Newton, G. and Levine, S.), Thomas, Illinois.

**Mathews, W.D. and Smith, C.D.** (1980). Pharmacological profile of a model for central serotonin receptor activation. *Life Sci.* **26**, 1397-1403.

**Mawe, G.M., Branchek, T.A. and Gershon, M.D.** (1986). Peripheral neural serotonin receptors: identification and characterization with specific antagonists and agonists. *Proc. Natl. Acad. Sci. USA* **83**, 9799-9803.

**McAllister, W.R. and McAllister, D.E.** (1971). Behavioral measurement of conditioned fear. In: *'Aversive conditioning and learning'* (Ed. Brush, F.R.), Academic Press, New York, pp. 105-179.

**McKearney, J.W. and Barrett, J.E.** (1975). Punished behavior: increases in responding after d-amphetamine. *Psychopharmacologia (Berl.)* **41**, 23-26.

**McKenna, D.J., Nazarali, A.J., Hoffman, A.J., Nichols, D.E., Mathis, C.A. and Saavedra, J.M.** (1989). Common receptors for hallucinogens in the brain: a comparative autoradiographic study using [$^{125}$I]LSD and [$^{125}$I]DOI, a new psychotomimetic radioligand. *Brain Res.* **476**, 45-56.

**McKenna, D.J. and Peroutka, S.J.** (1989). Differentiation of 5-hydroxytryptamine receptor subtypes using $^{125}$I-R-(-) 2,5 dimethoxy-4-iodo-phenylisopropylamine ($^{125}$I-R-(-)-DOI) and $^{3}$H ketanserin. *J. Neurosci.* **9**, 3482-3490.

**McKinney, W.T.** (1984). Animal models of depression: an overview. *Psychiat. Dev.* **2**, 77-96.

**Meert, T.F. and Colpaert, F.C.** (1986). The shock probe conflict procedure. A new assay responsive to benzodiazepines, barbituates and related compounds. *Psychopharmacology* **88**, 445-450.

**Mefford, I.N.** (1981). Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: measurement of catecholamines, serotonin and metabolites in rat brain. *J. Neurosci. Meth.* **3**, 207-224.

**Mennini, T. and Gobbi, M.** (1990). Effect of acute and chronic buspirone treatments on serotonin receptor subtypes in the rat: an autoradiographic study. *The Second IUPHAR Satellite Meeting on Serotonin, Basel, Switzerland.* July 11-13th, P51.

**Merlo Pich, E. and Samanin, R.** (1986). Disinhibitory effects of buspirone and low doses of sulpiride and haloperidol in two experimental anxiety models in rats: possible role of dopamine. *Psychopharmacology* **89**, 125-130.

**Methot, C. and Deutsch, R.** (1984). The effects of diazepam on a conditioned emotional response in the rat. *Pharmacol. Biochem. Behav.* **20**, 459-499.

**Miczek, K.A.** (1973). Effects of scopolamine, amphetamine and chlordiazepoxide on punishment. *Psychopharmacologia (Berl.)* **28**, 373-389.

**Middlemiss, D.N.** (1985). The putative 5-$HT_1$ receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from the rat frontal cortex slices by stimulation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.* **37**, 434-437.

**Middlemiss, D.N. and Fozard, J.R.** (1983). 8-Hydroxy-2-(di-n-propylamino) tetralin discriminates between subtypes of the 5-$HT_1$ recognition site. *Eur. J. Pharmacol.* **90**, 151-153.

**Middlemiss, D.N., Suman-Chauhan, N., Smith, S.M., Picton, C., Shaw, D. and Bevan, Y.** (1990). Subpopulations of 5-$HT_{1D}$ recognition sites in guinea-pig, rabbit, dog and human cortex. *The second IUPHAR satellite meeting on serotonin, Basel, Switzerland, July 11-13,* P30.

**Molineaux, S.M., Jessell, T.M., Axel, R. and Julius, D.** (1989). 5-$HT_{1C}$ receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. USA* **86**, 6793-6797.

**Montgomery, K.C.** (1955). The relation between fear induced by novel stimulation and exploratory behavior. *J. Comp. Physiol. Psychol.* **48**, 254-260.

**Morgan, M.J.** (1973). Effects of post-weaning environment on learning in the rat. *Anim. Behav.* **21**, 429-442.

**Morgan, M.J. and Einon, D.F.** (1975). Incentive motivation and behavioural inhibition in socially-isolated rats. *Physiol. Behav.* **15**, 405-409.

**Morgan, M.J., Einon, D.F. and Nicholas, D.** (1975). The effects of isolation rearing on behavioural inhibition in the rat. *Q. J. Exp. Psychol.* **27**, 615-634.

**Morgenson, G.J. and Calaresu, F.R.** (1973). Cardiovascular responses to electrical stimulation of the amygdala in the rat. *Exp. Neurol.* **39**, 166-180.

**Morinan, A. and Parker, V.** (1985). Are socially-isolated rats anxious? *Br. J. Pharmacol.* **86**, 460P.

**Mork, A. and Geisler, A.** (1990). 5-Hydroxytryptamine receptor agonists influence calcium-stimulated adenylate cyclase activity in the cerebral cortex and hippocampus of the rat. *Eur. J. Pharmacol.* **175**, 237-244.

**Morley, B.L. and Worsham, E.** (1978). The effects of prolonged handling, scopolamine and physostigmine on the activity of isolated and socially reared rats. *Physiol. Psychol.* **6**, 83-88.

**Moser, P.** (1989a). Buspirone, ipsapirone and 5-$HT_{1A}$-receptor agonists show an anxiogenic-like profile in the elevated plus-maze. In: *'Behavioural pharmacology of 5-HT'* (Eds. Bevan, P., Cools, A.R. and Archer, T.), Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp. 371-375.

**Moser, P. C.** (1989b). An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. *Psychopharmacology* **99**, 48-53.

**Moyer, K.E. and Korn, J.H.** (1965). Behavioral effects of isolation in the rat. *Psychon. Sci.* **3**, 503-504.

**Mundey, M.K., Marsden, C.A. and Fletcher, A.** (1991). Effect of isolation rearing on 5-$HT_{1A}$ autoreceptor function in the dorsal raphe nucleus. *Br. J. Pharmacol.* (in press).

**Murphy, J.M., McBride, W.J., Lumeng, L. and Li, T.-K.** (1982). Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats. *Pharmacol. Biochem. Behav.* **16**, 145-149.

**Mylecharane, E.J.** (1989). The classification of 5-hydroxytryptamine receptors. *Clin. Exp. Pharmacol. Physiol.* **16**, 517-522.

**Mylecharane, E.J. and Martin, G.R.** (1991). The apparent heterogeneity in functional 5-$HT_2$ receptors revealed by antagonists may be species-related. *Br. J. Pharmacol.* (in press).

**Naranjo, J.R. and Fuentes, J.A.** (1985). Association between hypoalgesia and hypertension in rats after short-term isolation. *Neuropharmacology* **24**, 167-172.

**Newberry, N.R., Cheshire, S.H. and Gilbert, M.J.** (1991). Evidence that the 5-$HT_3$ receptor of the rat, mouse and guinea-pig superior cervical ganglion may be different. *Br. J. Pharmacol.* **102**, 615-620.

**Njung'e, K. and Handley, S.L.** (1991). Evaluation of marble-burying behavior as a model of anxiety. *Pharmacol. Biochem. Behav.* **38**, 63-67.

**Osborne, N.N.** (1982). Assay, distribution and functions of serotonin in nervous tissues. In: *'Biology of Serotonergic Transmission'* (Ed. Osborne, N.N.), John Wiley and Sons Ltd., Chichester, pp. 7-28.

**Pannocchia, A., Praloran, N., Arduino, C., Della Dora, N., Bazzan, M., Schinco, P., Buraglio, M., Pileri, A. and Tamponi, G.** (1987). Absence of (-)[3H]desmethoxy-verapamil binding sites on human platelets and lack of evidence for voltage-dependent calcium channels. *Eur. J. Pharmacol.* **142**, 83-91.

**Parent, A., Descarries, L. and Beaudet, A.** (1981). Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [$^3$H]5-hydroxytryptamine. *Neurosci.* **6**, 115-138.

**Parker, V. and Morinan, A.** (1986). The socially-isolated rat as a model for anxiety. *Neuropharmacology* **25**, 663-664.

**Partridge, W.M.** (1979). The role of the blood brain barrier transport of tryptophan and other neutral amino acids in the regulation of substrate limited pathways of brain amino acid metabolism. *J. Neural Transm. Suppl.* **15**, 43-54.

**Paxinos, G. and Watson, C.** (1986). The rat brain in stereotaxic coordinates, 2nd Edition, Academic Press Ltd., London.

**Pazos, A., Cortes, R. and Palacios, J.M.** (1985). Quantitive autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin$_2$ receptors. *Brain Res.* **346**, 231-249.

**Pazos, A., Hoyer, D. and Palacios, J.M.** (1984). The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. *Eur J. Pharmacol.* **106**, 539-546.

**Pazos, A. and Palacios, J. M.** (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. serotonin-1 receptors. *Brain Res.* **346**, 205-230.

**Pedigo, N.W., Yamamura, H.I. and Nelson, D.L.** (1981). Discrimination of multiple [$^3$H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**, 220-226.

**Pei, Q., Zetterstrom, T. and Fillenz, M.** (1989a). Measurement of extracellular 5-HT and 5-HIAA in hippocampus of freely moving rats using microdialysis: long term applications. *Neurochem. Int.* **15**, 503-509.

**Pei, Q., Zetterstrom, T. and Fillenz, M.** (1989b). Both systemic and local administration of benzodiazepine agonists inhibit the in vivo release of 5-HT from ventral hippocampus. *Neuropharmacology* **28**, 1061-1066.

**Pellow, S.** (1986). Anxiolytic and anxiogenic drug effects in a novel test of anxiety: are exploratory models of anxiety in rodents valid? *Meth. Find. Exp. Clin. Pharmacol.* **8**, 557-565.

**Pellow, S., Chopin, P., File, S.E. and Briley, M.** (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Meth.* **14**, 149-167.

**Pellow, S. and File, S.E.** (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol. Biochem. Behav.* **24**, 525-529.

**Pellow, S., Johnston, A.L. and File, S.E.** (1987). Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG 7142 using the elevated plus-maze test in the rat. *J. Pharm. Pharmacol.* **39**, 917-928.

**Peroutka, S.J., Huang, S. and Allen, G.S.** (1986). Canine basilar artery contractions mediated by 5-hydroxytryptamine$_{1A}$ receptors. *J. Pharmacol. Exp. Ther.* **237**, 901-906.

**Peroutka, S.J. and Snyder, S.H.** (1979). Multiple serotonin receptors: differential binding of [$^3$H]-5-hydroxytryptamine, [$^3$H]-lysergic acid diethylamide and [$^3$H]-spiroperidol. *Mol. Pharmacol.* **161**, 687-699.

**Petersen, E.N. and Lassen, J.B.** (1981). A water lick conflict paradigm using drug experienced rats. *Psychopharmacology* **75**, 236-239.

**Petersen, E.N. and Scheel-Kruger, J.** (1982). The GABAergic anticonflict effect of intraamygdaloid benzodiazepines demonstrated by a new water lick conflict paradigm. In: *'Behavioural Models and the Analysis of Drug Action. Proceedings of the 27th OHOLO Conference'* (Eds. Spiegelstein, M.Y. and Levy, A.). Elsevier, Amsterdam, pp. 467-473.

**Petkov, V.V., Constantinova, E. and Grachovska, T.** (1985). Changes in brain opiate receptors in rats with isolation syndrome. *Pharmacol. Res. Commun.* **17**, 575-584.

**Post, S. and Mai, J.K.** (1980). Contribution to the amygdaloid projection field in the rat: A quantitative autoradiographic study. *J. Himforschung* **21**, 199-225.

**Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., Leslie, R.A., Barnes, N.M., Naylor, R.J., Jones, B.J., Nelson, D.R., Palacios, J.M., Slater, P. and Reynolds, D.J.M.** (1990). Concensus meeting agrees distribution of 5-HT$_3$ receptors in mammalian hindbrain. *T.I.P.S.* **11**, 135-137.

**Pratt, J., Jenner, P., Reynolds, E.H. and Marsden, C.D.** (1979). Clonazepam induces decreased serotonin activity in mouse brain. *Neurophamacology* **18**, 791-799.

**Pratt, O.E.** (1979). Kinetics of tryptophan transport across the blood-brain barrier. *J. Neural Trans.* **Suppl. 15**, 29-42.

**Price, J.L. and Amaral, D.G.** (1981). An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J. Neurosci.* **1**, 1242-1259.

**Pritchett, D.B., Bach, A.W.J., Wozny, M., Taleb, O., Dal Toso, R., Shih, J.C. and Seeburg, P.H.** (1988). Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *E.M.B.O.* **7**, 4135-4140.

**Puglisi-Allegra, S. and Oliverio, A.** (1983). Social isolation: effect on pain threshold and stress-induced analgesia. *Pharmacol. Biochem. Behav.* **19**, 679-681.

**Raiteri, M., Maura, G., Bonanno, G. and Pittaluga, A.** (1986). Differential pharmacology and function of two 5-$HT_1$ receptors modulating transmitter release in rat cerebellum. *J. Pharmacol. Exp. Ther.* **237**, 644-648.

**Raiteri, M., Maura, G., Folghera, S., Cavazzani, P., Andrioli, G.C., Schlicker, E., Schalnus, R. and Gothert, M.** (1990). Modulation of 5-hydroxy-tryptamine release by presynaptic inhibitory $\alpha_2$-adrenoceptors in the human cerebral cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **342**, 508-512.

**Rapport, M.M.** (1949). Serum vasoconstrictor (serotonin) V. Presence of creatine in the complex. A proposed structure of the vasoconstrictor principle. *J. Biol. Chem.* **180**, 961-969.

**Ray, O.S.** (1964). Tranquilizer effects on conditioned suppression. *Psychopharmacologia (Berl.)* **5**, 136-146.

**Redfern, W.S. and Williams, A.** (1989). Acute effects of centrally-acting drugs on the behaviour of rats in an elevated X-maze and partially-shaded holeboard. *Br. J. Pharmacol.* **98**, 683P.

**Redmond, D. E.** (1982). Does clonidine alter anxiety in humans ? *T.I.P.S.* **3**, 477-480.

**Redmond, D.E. and Huang, Y.H.** (1979). New evidence for a locus coeruleus norepinephrine connection with anxiety. *Life Sci.* **25**, 2149-2162.

**Richardson, B.P. and Engel, G.** (1986). The pharmacology and function of 5-$HT_3$ receptors. *T.I.N.S.* **7**, 424-428.

**Richardson, B.P., Engel, G., Donatsch, P. and Stadler, P.A.** (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature* **316**, 126-131.

**Robbins, T.W., Jones, G.H. and Sahakian, B.J.** (1989). Transmitters and attentional disorder: a perspective from animal studies. In: *'Attentional Deficit Disorder and the Childhood Hyperactivity Syndrome'* (Eds. Sagvolden, T. and Archer, T.), Erlbaum Publishers, London, pp. 199-222.

**Rosen, J.B. and Davis, M.** (1986). Electrical stimulation of the amygdala enhances acoustically- and electrically-elicited startle. *Soc. Neurosci. Abstracts* **12**, 517.

**Ross, S.B.** (1982). The characteristics of serotonin uptake systems. In *'Biology of Serotonergic Transmission'*, (Ed. Osborne, N.N.), John Wiley and Sons Ltd., Chichester, pp. 159-195.

**Routledge, C. and Marsden, C.A.** (1985). Application of high-performance liquid chromatography with electrochemical detection to the study of neurotransmitters *in vivo*. *Biochem. Soc. Trans.* **13**, 1058-1061.

**Routledge, C. and Marsden, C.A.** (1987). Comparison of the effects of selected drugs on the release of hypothalamic adrenaline and noradrenaline measured in vivo. *Brain Res.* **426**, 103-111.

**Rowan, M.J. and Anwyl, R.** (1986). Serotonergic mechanism for the inhibitory effect of non-benzodiazepine anxiolytics in the rat hippocampal slice. *Br. J. Pharmacol.* **88**, 299P.

**Sahakian, B.J., Burdness, C., Luckhurst, H. and Trayhurn, P.** (1982). Hyperactivity and obesity: the interaction of social isolation and cafeteria feeding. *Physiol. Behav.* **28**, 117-124.

**Sahakian, B.J., Robbins, T.W. and Iversen, S.D.** (1977). The effects of isolation rearing on exploration in the rat. *Anim. Learn. Behav.* **5**, 193-198.

**Sahakian, B.J., Robbins, T.W., Morgan, M.J. and Iversen, S.D.** (1975). The effects of psychomotor stimulants on stereotypy and locomotor activity in socially-deprived and control rats. *Brain Res.* **84**, 195-205.

**Samanin, R.** (1983). Drugs affecting serotonin and feeding. In: *'Biochemical Pharmacology of Obesity'* (Ed. Curtis-Prior, P.B.), Elsevier, Amsterdam.

**Sanders-Bush, E. and Martin, L.L.** (1982). Storage and release of serotonin. In: *'Biology of Serotonergic Transmission'*, (Ed. Osborne, N.N.), John Wiley and Sons Ltd., Chichester, pp. 63-94.

**Sarna, G.S., Hutson, P.H., Tricklebank, M.D. and Curzon, G.** (1983). Determination of brain 5-hydroxytryptamine turnover in freely moving rats using repeated sampling of cerebrospinal fluid. *J. Neurochem.* **40**, 383-388.

**Saxenda, B., Grof, P., Syrotuik, J., Denew, P. Shrotriya, R. and Lancaster, S.** (1986). Buspirone - a novel non-benzodiazepine agent: therapeutic efficacy and withdrawal effects in anxious patients. *Proc. 15th C.I.N.P. Congr. Puerto Rico*, P158.

**Schenk, S., Britt, M.D. and Atalay, J.** (1982). Isolation rearing decreases opiate receptor binding in rat brain. *Pharmacol. Biochem. Behav.* **16**, 841-842.

**Schuurman, T., Spencer, D.G. and Traber, J.** (1986). Behavioural effects of the 5-$HT_{1A}$ receptor ligand ipsapirone (TVXQ 7821): a comparison with 8-OH-DPAT and diazepam. *Psychopharmacology* **89**, p. 554 Abs.162.

**Schwaber, J.S., Kapp, B.S., Higgins, G.A. and Rapp, P.R.** (1982). Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. *J. Neurosci.* **2**, 1424-1438.

**Schwartz, D., Hernandez, L. and Hoebel, B.G.** (1989). Fenfluramine administered systemically or locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. *Brain Res.* **482**, 261-270.

**Scobie, S.R. and Garske, G.** (1970). Chlordiazepoxide and conditioned suppression. *Psychopharmacologia (Berl.)* **16**, 272-280.

**Segal, D.S., Knapp, S., Kuczenski, R. and Mandell, A.J.** (1973). The effects of environmental isolation on behaviour and regional rat brain tyrosine hydroxylase and tryptophan hydroxylase activities. *Behav. Biol.* **8**, 47-53.

**Seitz, P.F.D.** (1959). Infantile experience and adult behaviour in animal subjects II. Age of separation from the mother and adult behaviour in the cat. *Psychom. Med.* **21**, 353-378.

**Sharp, T., Backus, L.I., Hjorth, S., Bramwell, S.R. and Grahame-Smith, D.G.** (1990). Further investigation of the in vivo pharmacological properties of the putative 5-$HT_{1A}$ antagonist, BMY 7378. *Eur. J. Pharmacol.* **176**, 331-340.

**Sharp, T., Bramwell, S., Clark, D. and Grahame-Smith, D.G.** (1989c). In vivo measurement of extracellular 5-hydroxytryptamine in hippocampus of the anaesthetized rat using microdialysis: changes in relation to 5-hydroxytryptaminergic neuronal activity. *J. Neurochem.* **53**, 234-240.

**Sharp, T., Bramwell, S.R. and Grahame-Smith, D.G.** (1989a). 5-$HT_1$ agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. *Br. J. Pharmacol.* **96**, 283-290.

**Sharp, T., Bramwell, S.R., Hjorth, S. and Grahame-Smith, D.G.** (1989b). Pharmacological characterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release in vivo as measured by microdialysis. *Br. J. Pharmacol.* **98**, 989-997.

**Sharp, T., Maidment, N.T., Brazell, M.P., Zetterstrom, T., Ungerstedt, U., Bennett, G.W. and Marsden, C.A.** (1984). Changes in monoamine metabolites measured by simultaneous in vivo differential pulse voltammetry and intracerebral microdialysis. *Neurosci.* **12**, 1213-1221.

**Sharp, T., Zetterstrom, T., Christmanson, L. and Ungerstedt, U.** (1986). p-Chloramphetamine releases both serotonin and dopamine into rat brain dialysates in vivo. *Neurosci. Lett.* **72**, 320-324.

**Shearman, G.T. and Lal, H.** (1979). Discriminative stimulus properties of pentylenetetrazol and bemegride: Some generalization and antagonism tests. *Psychopharmacologia (Berl.)* **64**, 315-319.

**Shearman, G.T. and Lal, H.** (1980). Generalization and antagonism studies with convulsant, GABAergic and anticonvulsant drugs in rats trained to

discriminate pentyleletetrazol from saline. *Neuropharmacology* **19**, 473-479.

**Shenker, A., Maayani, S., Weinstein, H. and Green, J.P.** (1987). Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea-pig hippocampal membranes. *J. Pharmacol. Exp. Ther.* **31**, 357-367.

**Shephard, R.A., Buxton, D.A. and Broadhurst, P.L.** (1982). Drug interactions do not support reduction in serotonin turnover as the mechanism of action of benzodiazepines. *Neuropharmacology* **21**, 1027-1032.

**Shibata, K., Kataoka, Y., Yamashita, K. and Ueki, S.** (1986). An important role of the central amygdaloid nucleus and mammillary body in the mediation of conflict behavior in rats. *Brain Res.* **372**, 159-162.

**Sills, M.A., Wolfe, B.B. and Frazer, A.** (1984). Determination of selective and nonselective compounds for the 5-$HT_{1A}$ and 5-$HT_{1B}$ receptor subtypes in the rat frontal cortex. *J. Pharmacol. Exp. Ther.* **231**, 480-487.

**Simiand, J., Keane, P.E. and Morre, M.** (1984). The staircase test in mice: a simple and efficient procedure for primary screening of anxiolytic agents. *Psychopharmacology* **84**, 48-53.

**Sneddon, J.M.** (1973). Blood platelets as a model for monoamine-containing neurones. *Prog. Neurobiol.* **1**, 151-198.

**Soderpalm, B., Hjorth, S. and Engel, J.A.** (1989). Effects of 5-$HT_{1A}$ receptor agonists and L-5-HTP in Montgomery's conflict test. *Pharmacol. Biochem. Behav.* **32**, 259-265.

**Sotelo, C., Cholley, B., El Mestikawy, S., Gozlan, H. and Hamon, M.** (1991). Direct immunohistochemical evidence of the existence of 5-$HT_{1A}$ autoreceptors on serotoninergic neurons in the midbrain raphe nuclei. *Eur. J. Neurosci.* **2**, 1144-1154.

**Soubrie, P.** (1986). Reconciling the role of central serotonin neurons in human and animal behavior. *Behav. Brain Sci.* **9**, 319-364.

**Spevack, A.A., Campbell, C.T. and Drake, L.** (1975). Effect of amygdalectomy on habituation and CER in rats. *Physiol. Behav.* **15**, 199-207.

**Sprouse, J.S. and Aghajanian, G.K.** (1986). (-) Propanolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-$HT_{1A}$ selective agonists. *Eur. J. Pharmacol.* **128**, 295-298.

**Squires, R.F. and Braestrup, C.** (1977). Benzodiazepine receptors in rat brain. *Nature* **266**, 732-734.

**Stamford, J.A.** (1989). *In vivo* voltammetry - prospects for the next decade. *T.I.N.S.* **12**, 407-412.

**Stein, L. and Berger, B.O.** (1969). Paradoxical fear-increasing effects of tranquilizers: evidence of repression of memory in the rat. *Science* **166**, 253-256.

**Stein, L., Wise, C.D. and Belluzzi, J.D.** (1975). Effects of benzodiazepines on central serotonergic mechanisms. In: *'Mechanisms of action of benzodiazepines'* (Eds. Costa, E. and Greengard, P.), Raven Press, New York, pp. 29-63.

**Stein, L., Wise, C.D. and Belluzzi, J.D.** (1977). Neuropharmacology of reward and punishment. In: *'Handbook of Psychopharmacology'* (Eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H.), Plenum Press, New York, **Vol. 8**, pp. 25-53.

**Stein, L., Wise, C.D. and Berger, B.D.** (1973). Antianxiety action of benzodiazepines: decrease in activity of serotonin neurons in the punishment systems. In: *'The Benzodiazepines'* (Eds. Garattini, S., Mussini, E. and Randall, L.O.), Raven Press, New York, pp. 299-326.

**Steinbusch, H.W.M.** (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat - cell bodies and terminals. *Neurosci.* **6**, 557-618.

**Strange, P.G.** (1990). States and subtypes of the 5-$HT_2$ serotonin receptor: interpretation of the data. *J. Neurochem.* **53**, 1085.

**Syme, L.A.** (1973). Social isolation at weaning: some effects on two measures of activity. *Anim. Learn. Behav.* **1**, 161-163.

**Takeuchi, Y., McLean, J.H. and Hopkins, D.A.** (1982). Reciprocal connections between the amygdala and parabrachial nuclei: ultrastructural demonstration by degeneration and axonal transport of HRP in the cat. *Brain Res.* **239**, 583-588.

**Taylor, D.P., Eison, M.S., Riblet, L.A. and VanderMaelen, C.P.** (1985). Pharmacology and clinical effects of buspirone. *Pharmacol. Biochem. Behav.* **23**, 687-694.

**Taylor, D.P., Riblet, L.A., Stanton, H.C., Eison, A.S., Eison, M.S. and Temple, D.L.** (1982). Dopamine and antianxiety activity. *Pharmacol. Biochem. Behav.* **17 (Suppl. 1)**, 25-32.

**Tenen, S.S.** (1967). Recovery time as a measure of CER strength: effects of benzodiazepines, amobarbital, chlorpromazine, and amphetamine. *Psychopharmacologia (Berl.)* **12**, 1-7.

**Thiebot, M-H.** (1986). Are serotonergic neurons involved in the control of anxiety and in the anxiolytic activity of benzodiazepines? *Pharmacol. Biochem. Behav.* **24**, 1471-1477.

**Thiebot, M-H., Hamon, M. and Soubrie, P.** (1982). Attenuation of induced anxiety in rats by chlordiazepoxide: role of the raphe dorsalis benzodiazepine binding sites and serotonergic neurones. *Neurosci.* **7**, 2287-2294.

**Thiebot, M-H., Hamon, M. and Soubrie, P.** (1984). Serotonergic neurones and anxiety-related behaviour in rats. In: *'Psychopharmacology of the Limbic System'* (Eds. Zarifian, E. and Trimble, M.R.), John Wiley and Sons Ltd., New York, pp. 164-173.

**Thiebot, M-H., Jobert, A. and Soubrie, P.** (1980). Chlordiazepoxide and Gaba into raphe dorsalis release the conditioned behavioural supression induced in rats by a conflict procedure without nociceptive component. *Neuropharmacology* **19**, 633-641.

**Thiebot, M-H. and Soubrie, P.** (1983). Behavioural pharmacology of the benzodiazepines. In: *'The Benzodiazepines: From Molecular Biology to Clinical Practice'* (Ed. Costa, E.), Raven Press, New York, pp. 67-92.

**Thoa, N.B., Tizabi, Y. and Jacobowitz, D.M.** (1977). The effect of isolation on catecholamine concentration and turnover in discrete areas of the rat brain. *Brain Res.* **131**, 259-269.

**Traber, J., Davies, M.A., Dompert, W.U., Glaser, T., Schuurman, T. and Seidel, P-R.** (1984). Brain serotonin receptors as a target for the putative anxiolytic TVXQ 7821. *Brain Res. Bull.* **12**, 741-744.

**Traber, J. and Glaser, T.** (1987). 5-$HT_{1A}$ receptor-related anxiolytics. *T.I.P.S.* **8**, 432-437.

**Tricklebank, M.D.** (1984). Behavioural effects of 8-hydroxy-2-(di-n-propylamino) tetralin, a putative 5-$HT_{1A}$ receptor agonist. *Br. J. Pharmacol.* **81**, 26P.

**Tricklebank, M.D., Forler, C. and Fozard, J.R.** (1985a). The involvement of subtypes of the 5-HT1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino) tetralin in the rat. *Eur. J. Pharmacol.* **106**, 271-282.

**Tricklebank, M.D., Forler, C., Middlemiss, D.N., and Fozard, J.R.** (1985b). Subtypes of the 5-HT receptor mediating the behavioural response to 5-methoxy-N,N-dimethyltryptamine in the rat. *Eur. J. Pharmacol.* **117**, 15-24.

**Trulson, M.E., Preussler, D.W., Howell, G.A. and Fredrickson, C.J.** (1982). Raphe unit activity in freely moving cats: effects of benzodiazepines. *Neuropharmacology* **21**, 1045-1050.

**Twarog, B.M. and Page, I.H.** (1953). Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.* **174**, 157-161.

**Tye, N.C., Everitt, B.J. and Iversen, S.D.** (1977). 5-hydroxytryptamine and punishment. *Nature* **268**, 741-743.

**Tye, N.C., Iversen, S.D. and Green, A.R.** (1979). The effects of benzodiazepines and serotonergic manipulations on punished responding. *Neuropharmacology* **18**, 689-695.

**Tyers, M.B.** (1989). A review of the evidence supporting the anxiolytic potential of 5-$HT_3$ receptor antagonists. In: *'Behavioural Pharmacology of 5-HT"* (Eds. Bevan, P., Cools, A.R. and Archer, T.), Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp. 353-359.

**Uhde, T.W., Boulenger, J-P., Jimerson, D.C. and Post, R.M.** (1984). Caffeine: relationship to human anxiety, plasma and cortisol. *Psychopharmacol. Bull.* **20**, 426-430.

**Ungerstedt, U.** (1984). Measurement of neurotransmitter release by intracranial dialysis. In: *'Measurement of Neurotransmitter Release In Vivo'*, (Ed. Marsden, C.A.), John Wiley and Sons Ltd., Chichester, pp. 81-105.

**Ungerstedt, U., Herrera-Marschitz, M., Jungnelius, U., Stahle, L., Tossman, U. and Zetterstrom, T.** (1982). Dopamine synaptic mechanisms reflected in studies combining behavioural recordings and brain dialysis. *Adv. In The Biosci.* **37**, 219-231.

**Unnerstall, J.R., Kuhar, M.J., Neihoff, D.L. and Palacios, J.M.** (1981). Benzodiazepine receptors are coupled to a subpopulation of gamma-aminobutyric acid (Gaba) receptors: evidence from a quantitative autoradiographic study. *J. Pharmacol. Exp. Ther.* **218**, 797-804.

**Upton, N. and Blackburn, T.P.** (1991). Anxiolytic-like activity of the selective 5-$HT_3$ receptor antagonist, BRL 46470A, in the rat elevated X-maze test. *Br. J. Pharmacol.* **102**, 253P.

**Uvnas, B. and Aborg, C.-H.** (1984). Cation exchange - a common mechanism in the storage and release of biogenic amines stored in granules (vesicles)? *Acta Physiol. Scand.* **120**, 99-107.

**Valzelli, L.** (1977). Social experience as a determinant of normal behaviour and drug effect. In: *'Handbook of Psychophramacology'* (Eds. Iversen, L.L., Iversen, S.D. and Snyder, S.H.), Plenum Press, New York, **Vol. 7**, pp. 369-392.

**VanderMaelen, C. P., Matheson, C. K., Wilderman, R. C. and Patterson, L. A.** (1986). Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration buspirone, a non-benzodiazepine anxiolytic drug. *Eur. J. Pharmacol.* **129**, 123-130.

**VanderMaelen, C.P. and Wilderman, R.C.** (1984). Buspirone a non-benzodiazepine anxiolytic drug, causes inhibition of serotonergic dorsal raphe neurons in the brain slice. *Soc. Neurosci. Abs.* **10**, 259.

**Van Neuten, J.M., Schuurkes, J.A.J., De Ridder, W.J.E., Kuyps, J.J.M.D. and Janssens, W.J.** (1986). Comparative pharmacological profile of ritanserin and ketanserin. *Drug Dev. Res.* **8**, 187-195.

**Varrault, A., Leviel, V. and Bockaert, J.** (1991). 5-$HT_{1A}$-sensitive adenylyl cyclase of rodent hippocampal neurons: effects of antidepressant

treatments and chronic stimulation with agonists. *J. Pharmacol. Exp. Ther.* **257**, 433-438.

**Veening, A.R., Swanson, L.W. and Sawchenko, P.E.** (1984). The organization of projections from the central nucleus of the amygdala to brain stem sites involved in central autonomic regulation: A combined retrograde transport-immunohistochemical study. *Brain Res.* **303**, 337-357.

**Verge, D., Daval,G., Marcinkiewicz, M., Patey, A., El Mestikawy, S., Gozlan, H. and Hamon, M.** (1986). Quantitative autoradiography of multiple 5-$HT_1$ receptor subtypes in the brain of control or 5,7-dihydroxy-tryptamine-treated rats. *J. Neurosci.* **6**, 3474-3482.

**Vogel, J.R., Beer, B. and Clody, D.E.** (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia (Berl.)* **21**, 1-7.

**Waeber, C., Dietl, M.M., Hoyer, D. and Palacios, J.M.** (1989b). 5-$HT_1$ receptors in the vertebrate brain. Regional distribution examined by autoradiography. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340**, 486-494.

**Waeber, C., Schoeffter, P., Palacios, J.M. and Hoyer, D.** (1989a). 5-$HT_{1D}$ receptors in guinea pig and pigeon brain. Radioligand binding and biochemical studies. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **340**, 479-485.

**Wang, R.Y. and Aghajanian, G.K.** (1977). Inhibition of neurones in the amygdala by dorsal raphe stimulation: mediation through a direct serotonergic pathway. *Brain Res.* **120**, 85-102.

**Weinstock, M., Speiser, Z. and Ashkenazi, R.** (1978). Changes in brain catecholamine turnover and receptor sensitivity induced by social deprivation in rats. *Psychopharmacology* **56**, 205-209.

**Weissman, B.A., Barrett, J.E., Brady, L.S., Witkins, J.M., Mendelson, W.B., Paul, S.M. and Skolnick, P.** (1984). Behavioural and neurochemical studies on the anticonflict actions of buspirone. *Drug Dev. Res.* **4**, 83-93.

**Westerink, B.H.C., Damsma, G., Rollema, H., De Vries, J.B. and Horn, A.S.** (1987a). Scope and limitation of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems. *Life Sci.* **41**, 1763-1776.

**Westerink, B.H.C. and De Vries, J.B.** (1988). Characterization of in vivo dopamine release as determined by brain microdialysis after acute and subchronic implantations: methodological aspects. *J. Neurochem.* **51**, 683-687.

**Westerink, B.H.C. and Tuinte, M.H.J.** (1986). Chronic use of intracerebral dialysis for the in vivo measurement of 3,4-dihydroxyphenylethylamine and its metabolite, 3,4-dihydroxyphenylacetic acid. *J. Neurochem.* **46**, 181-185.

**Westerink, B.H.C., Tuntler, J., Damsma, G., Rollema, H. and De Vries, J.B.** (1987b). The use of tetrodotoxin for the characterization of drug-enhanced dopamine release in conscious rats studied by brain dialysis. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336**, 502-507.

**Wilkinson, L.O., Abercrombie, E.D., Rasmussen, K. and Jacobs, B.L.** (1987). Effect of buspirone on single unit activity in locus coeruleus and dorsal raphe nucleus in behaving cats. *Eur. J. Pharmacol.* **136**, 123-127.

**Willner, P.** (1984). The validity of animal models of depression. *Psychopharmacology* **83**, 1-16.

**Wilmot, C.A., VandeWende, C. and Spoerlein, M.T.** (1986). Behavioural and biochemical studies of dopamine receptor sensitivity in differently housed mice. *Psychopharmacology* **89**, 363-369.

**Wise, C.D., Berger, B.D. and Stein, L.** (1972). Benzodiazepines: anxiety-reducing activity by reduction of serotonin turnover in the brain. *Science* **177**, 180-183.

**Wise, R.A. and Dawson, V.** (1974). Diazepam-induced eating and lever pressing for food in sated rats. *J. Comp. Physiol. Psychol.* **86**, 930-941.

**Wong, D.T., Threlkeld, P.G., Lumeng, L. and Li, T.-K.** (1990). Higher density of serotonin-1A receptors in the hippocampus and cerebral cortex of alcohol-preferring P rats. *Life Sci.* **46**, 231-235.

**Wood, W.E. and Greenough, W.T.** (1974). Effect of grouping and crowding on learning in isolation-reared adult rats. *Bull. Psychon. Sci.* **3**, 65-67.

**Xiong, W.-C. and Nelson, D.L.** (1989). Characterization of a [$^3$H]-5-hydroxytryptamine binding site in rabbit caudate nucleus that differs from the 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1C}$ and 5-$HT_{1D}$ subtypes. *Life Sci.* **45**, 1433-1442.

**Yap, C.Y. and Taylor, D.A.** (1983). Involvement of 5-HT2 receptors in the wet-dog shake behaviour induced by 5-hydroxytryptophan in the rat. *Neuropharmacology* **22**, 801-804.

**Yevich, J.P., Temple, D.L., New, J.S., Taylor, D.P. and Riblet, L.A.** (1983). Buspirone analogues. I. Structure-activity in a series of N-aryl and heteroarylpiperazine derivatives. *J. Med. Chem.* **26**, 194-203.

**Yocci, F.D., Wright, R.N., Magraf, R.R. and Eison, A.S.** (1990). 8-OH-DPAT and buspirone analogs inhibit the ketanserin-sensitive quipazine-induced head-shake response in rats. *Pharmacol. Biochem. Behav.* **35**, 251-254.

**Young, S.N. and Sourkes, T.L.** (1977). Tryptophan in the central nervous system: regulation and significance. In: *'Advances in Neurochemistry'*, (Eds. Agranoff, B.W. and Aprison, M.H.), Plenium Press, New York, **2**, pp. 133-191.