ASPECTS OF MONOAMINE INVOLVEMENT IN SOME AVERSIVELY-MOTIVATED BEHAVIOURS.

SIDDIKA MITHANI

Thesis submitted for the Degree of

Doctor of Philosophy

The University of Aston In Birmingham

August 1984.

To

AMIJAN AND PAPPA

without whom this work could never have been done.

University of Aston in Birmingham

Aspects of Monoamine Involvement in Some Aversively-Motivated Behaviours.

Siddika Mithani.

Submitted for the Degree of Doctor of Philosophy

1984

Summary

A study has been made of drugs acting at α-adrenoceptors and 5-HT receptors in some aversively-motivated behaviours (AMB).

An elevated X-maze was used as a model of anxiety and was validated by investigating the effects of known anxiolytic and putative anxiogenic agents. In this model the α_2-adrenoceptor agonists had an anxiolytic-like effect, whereas the antagonists appeared to be anxiogenic. The α_1-adrenoceptor agonists as well as the 5-HT receptor agonist quipazine also had anxiogenic-like activity in this model and the α_1-adrenoceptor antagonist prazosin and the 5-HT receptor antagonist ketanserin had anxiolytic-like effects. These results were confirmed by examining the effects of the agents on the Geller-Seifter conflict test - a widely accepted test for anxiety. These latter results not only provided evidence for the involvement of noradrenergic and serotonergic systems in these AMB, but also validated the X-maze as a reliable and rapid test for examining such behaviour.

Evidence from behavioural interaction studies have suggested that the effect of α-adrenoceptor ligands in these models is not modulated by 5-HT receptors. Conversely, α-adrenoceptors do not appear to modulate the behavioural effects of the 5-HT receptor ligands. Biochemical determinations of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-indole acetic acid (5-HIAA) in various brain regions after drug administration have shown that all these agents modify serotonergic activity. The importance of these changes have been discussed with special reference to studies that have investigated biochemical and behavioural interactions between anxiolytics and serotonergic systems - a field which appears to be under much controversy.

Bilateral lesions of the locus coeruleus (L.C.) did not affect exploratory behaviour in the X-maze. The effect of α-adrenoceptors and 5-HT receptor ligands as well as diazepam and adrenocorticotrophic hormone (ACTH) on conflict behaviour in L.C. lesioned animals was no different from sham-operated controls suggesting that the L.C. is not involved in these AMBs.

KEY WORDS

α-adrenoceptors, 5-HT receptors, aversively-motivated behaviour, anxiety.

ACKNOWLEDGEMENTS

I wish to to thank Professor M.F.G.Stevens for allowing me to undertake this work in the laboratories of the Pharmacy Department of Aston University. I am especially grateful to Dr. S.L.Handley for supervising this thesis and for her help and encouragement during the preparation of this thesis. I am also grateful to the Pharmaceutical Society of Great Britain for financial support.

My thanks are due to my colleague Mr Lakhbir Singh for performing the locus coeruleus lesions and for the assistance in the biochemical assays of noradrenaline and dopamine. I also acknowledge the able assistance of the technical staff of the Pharmacy Department especially Mr Alan Richardson, Mr David Briggs for his assistance in writing the computer programs, Mr Paul Beham and all the staff in the Animal Unit.

My thanks are also due to the staff of the library at the University of Aston for the facilities and services provided during the course of this work, and also to Mrs Hazel Brown who typed this thesis.

Finally, I wish to thank my parents and family members for their patience and understanding while this work was in progress. I am also indebted to my fiance, Giby, for his tolerance and forbearance throughout the preparation of this thesis.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

1. Distribution of noradrenergic neurones in the brain.

Detailed studies by Ungerstedt (1971) revealed three separate noradrenergic systems: descending systems to the spinal cord, ascending pathways from the lower brain stem and pathways originating in the locus coeruleus (L.C.). The descending pathways which originate in the A1 and A2 regions of the medulla enter the spinal cord and terminate in the dorsal and ventral horns. Other neurones from these cell groups along with neurones from A5 and A7 regions ascend in the reticular formation, turn ventrally along the medial lemniscus and continue in the medial forebrain bundle (MFB). This system gives rise to nerve terminals in the lower brain stem, midbrain and diencephalon and innervates the whole hypothalamus.

The ascending catecholaminergic axons originating in pontine and medullary cell groups are confined to two major fibre systems. The first is the central tegmental tract - fibres of which ascend in the medulla oblongata, pons, mesencephalon and diencephalon. The second is the dorsal periventricular system which is a prominent periventricular-periaqueductal system running within the dorsal longitudinal fasciculus in its extention through medulla oblongata, pons, mesencephalon and diencephalon (see Lindvall and Bjorkland, 1974).

Three pathways originate in the L.C. or A6 cell group. A descending pathway innervates the lower brain stem nuclei, a lateral pathway innervates the cerebellum and an ascending pathway forms the dorsal noradrenergic bundle (db). This pathway runs dorsally to that previously described and forms a completely separated bundle of neurones in the midbrain. It then descends to join the other ascending noradrenergic neurones in the MFB at the level of the hypothalamus. This pathway innervates the hippocampus and cortex, possibly via the amygdala and hypothalamus, thus is able to influence many regions.

More sensitive fluorescence methods have confirmed the location of pathways described and have enabled more detailed studies of the pathways originating in the L.C. (Lindvall and Bjorkland, 1974). Branches of noradrenergic neurones have thus been found to leave the MFB to innervate the thalamus, amygdala, some hypothalamic nuclei, the septum, striatum and olfactory lobe (Moore, 1980).

2. Adrenergic receptors.

Early theories that the effects of sympathetic nerve stimulation were mediated by noradrenaline (NA) (Sympathin E) and adrenaline (Sympathin I) were changed by Ahlquist in 1948. He suggested that there were two distinct types of adrenotropic receptors as determined by their relative responsiveness to a series of racemic amines most closely related structurally to adrenaline. Receptors associated with most excitatory functions and one important inhibitory function (that of intestinal relaxation) were designated α, whereas receptors associated with most of the inhibitory functions and one important excitatory one were designated β.

Further work showed that β-receptors were not all identical, but could be divided into subtypes (Lands et al, 1967), which has led to the development of drugs which stimulate only bronchial (β_2) or block only cardiac (β_1) type receptors. Recently, it has been suggested (Langer, 1974) that α-receptors should also be subdivided into α_1 and α_2 types, since many experiments have indicated the presence of an α receptor on the presynaptic nerve terminal, which differs in sensitivity to drugs from those found on peripheral effector organs. These receptors have, in many cases, inhibitory pharmacological actions, but do not resemble β-receptors in their sensitivity to drugs. Their pharmacological effects appear to be mediated indirectly by a reduction in the amount of transmitter released on stimulation, and their probable presynaptic location has led to the concept of presynaptic receptor-mediated regulation of transmitter release (see Langer, 1974). However, the validity of this presynaptic receptor feedback regulation mechanism has recently been questioned, since evidence was provided that presynaptic antagonists prolonged the potassium efflux from nerve varicosities during the action potential (Kalsner and Quillan, 1984). It has therefore been suggested that these agents prolong depolarisation and the associated period of transmitter release, rather than disrupting an ongoing system sensing and responding to fluctuations in the extracellular transmitter levels (see Kalsner and Quillan, 1984).

The subdivision of α-adrenoceptors into α_1 (those found on peripheral effector organs) and α_2 (those on the presynaptic nerve

terminal) types, have been based entirely on the potency order of the agonists and antagonists in various peripheral vascular systems. In vascular smooth muscle, Drew and Whiting (1979) demonstrated the presence of two postsynaptic α-adrenoceptors - one that was prazosin sensitive, and the other, prazosin insensitive. In rat preparations, it has been shown that at peripheral sites α_1- and α_2-adrenoceptors do exist pre- as well as postsynaptically (Kobinger and Pichler, 1980a). Further, a good correlation between central and both peripheral effects, pre- as well as postsynaptic, with the α_2-adrenoceptor agonists and antagonists have further confirmed the existence of postsynaptic α_2-adrenoceptors (Kobinger and Pichler, 1980b).

The existence of presynaptic facilitatory β adrenoceptors has also been suggested (Langer et al, 1975). These receptors are thought to be sensitive to low concentrations of NA; low frequencies of stimulation release only small quantities of NA which would result in the activation of these receptors - leading to an increase in NA release; where synaptic levels of NA reach a sufficiently high concentration, presynaptic α-adrenoceptors would be activated thus decreasing release. The mechanism of presynaptic β-adrenoceptor stimulation appears to be mediated through an increase in cyclic adenosine monophosphate levels in the noradrenergic nerve endings (Langer, 1977). However, Stjarne and Brundin (1975) demonstrated that presynaptic α-mediated negative feedback inhibition operates at stimulation frequencies similar to those at which β-adrenoceptors should be effective.

Binding studies have shown that receptors which resemble pharmacologically α_1- and α_2-adrenoceptors found peripherally are also present in brain tissue (U'Prichard et al, 1978; Tanaka and Starke, 1980) as are β-adrenoceptors. Taube et al (1977) demonstrated that yohimbine decreased NA release from cortical slices and suggested that drugs which have been shown to possess some selectivity for presynaptic receptors are also present in modifying central NA release. Furthermore, it was shown that systemic administration of prazosin will also block α_1-adrenoceptors in the brain (Menkes et al, 1981) indicating the involvement of central adrenoceptors during systemic administration of drugs that are able to cross the blood brain barrier.

3. Pharmacology of drugs acting at α-adrenoceptors

The selectivity of drugs acting at α-adrenoceptors only, will be reviewed here since this study has examined the effects of α-adrenoceptor ligands; agents acting at β-adrenoceptors have not been examined.

A range of drugs acting at α-adrenoceptors have been studied to compare their effects on α_1- and α_2-adrenoceptors. Among the α_1-adrenoceptor agonists in the rat cardiovascular system, methoxamine, naphazoline, phenylephrine and oxymetazoline have been shown to be potent on α_1-adrenoceptors (Drew, 1976). In the pithed rat, St 587 induced vasoconstriction - an effect that would be antagonised by prazosin but not yohimbine, indicating that this agent was selective for α_1-adrenoceptors (De Jonge et al, 1981), and Kobinger and Pichler (1982) showed that St 587 was more selective than methoxamine at α_1-adrenoceptors. The selectivity of phenylephrine and methoxamine on α_1-adrenoceptors has been confirmed in the rabbit pulmonary artery, although oxymetazoline was demonstrated to be more potent on α_2-adrenoceptors (Starke et al, 1975).

With regard to the α_2-adrenoceptor agonists, clonidine has been shown to be a potent α_2-adrenoceptor agonist in the rabbit pulmonary artery (Starke et al, 1975) although in the rat cardiovascular system, it is equipotent at both types of receptors (Drew, 1976). The partial agonist effect of clonidine at α_1-adrenoceptors has also been shown in cerebral cortical neurones (Bradshaw et al, 1982) suggesting that clonidine may not be a full agonist at central α_2-adrenoceptors. In the rat anococcygeus preparation, azepexole was shown to act preferentially at α_2-adrenoceptors (Coates and Weetman, 1982) and guanabenz has also been shown to act as an agonist at central α_2-adrenoceptors (Jarrott et al, 1979).

The selectivity of α-adrenoceptor antagonists is also well reported. Phenoxybenzamine has been found to selectively block α_1-adrenoceptors in the rat cardiovascular system (Drew, 1976) and prazosin has a similar effect (Doxey et al, 1977). Both these agents have been found to have little effect at α_2-adrenoceptors in the rat cardiovascular system and vas deferens, whereas phentolamine displays

no selectivity at all for either receptor (Drew, 1976; Doxey et al, 1977). Yohimbine and piperoxane have been shown to preferentially block α_2-adrenoceptors, although both these agents show small α_1-adrenoceptor blocking activity (Starke et al, 1975; Drew, 1976). In vivo and in vitro ligand binding studies have demonstrated that RS 21361 is a selective blocker at α_2-adrenoceptors (Michel et al, 1981) and Doxey et al (1983) showed that in the pithed rat, the rank order for α_2/α_1 selectivity was: idazoxan > RS 21361 > yohimbine > piperoxane > phentolamine > WB 4101 > prazosin. However, high doses of idazoxan was shown to inhibit the effect of phenylephrine suggesting that it may also block α_1-adrenoceptors at high doses (ibid).

The above cited studies demonstrate the wide range of selectivity which has been found to exist among both agonists and antagonists for the two receptors. To a certain extent, this makes it possible to determine pharmacologically the type of receptors involved in many physiological and behavioural effects by investigating the action of these drugs on functions.

4. Possible roles of NA in the CNS.

The diffuse projection of noradrenergic neurones in the CNS suggests that NA is likely to be associated with a variety of behavioural and physiological functions. NA has been variously implicated in blood pressure regulation (Haeusler, 1974), motor activity (Anden et al, 1970), body temperature regulation (Feldberg and Myers, 1964) and pain sensation (Gardella et al, 1970).

The involvement of noradrenergic systems in the control of feeding has been reported. There are at present, two theories involving feeding behaviour, reviewed by Hoebel (1977). One involves an α-receptor mediated feeding response, possibly located in the hypothalamus, combined with a β-receptor-mediated satiety response. However, a second theory suggests that NA produces satiety by α-adrenoceptors, thus reducing feeding, and that β receptor activation also reduces feeding, but by inducing taste aversion.

NA is also thought to be involved in drinking mechanisms. Both β-adrenoceptor stimulants and α-adrenoceptor blockers appear to induce drinking, which may be due to increased renin release from the kidney (Setler, 1977) and is probably a peripheral response. However,

intracerebroventricular (icv) NA was shown to inhibit drinking (ibid), although the importance of this effect is uncertain.

4.1 Behavioural effects of NA.

Self stimulation experiments combined with pharmacological manipulations have implicated NA in reward systems. Drugs which released NA were shown to increase self stimulation, whereas α-methylparatyrosine (α-mpt) decreased it (Stein et al, 1977). Behavioural arousal was shown to occur when catecholaminergic activity was increased by means of l-dopa, amphetamine or inhibition of the breakdown of the amines, whereas blockade of central α-adrenoceptors by phenoxybenzamine and dibenamine induced sedation (Jouvet, 1977). Furthermore, clonidine caused sedation (Delbarre and Schmitt, 1971) suggesting that the involvement of NA in the sleep/waking cycle seems to be one of an increased availability of NA resulting in increased waking, while a decrease leads to sedation.

NA has also been shown to play a major role in depression, together with 5-HT systems. Behavioural work on NA depleted rats showed hunger drive deficits, lethargy in novel environment, decreased rearing, and overconfidence, whereas 5-HT depleted animals were more agitated, frightened in an open-field environment and showed an increase in food consumption (Ellison, 1977). The catecholamine theory of depression postulates that some, if not all, depressions may be associated with a relative deficiency of NA and/or 5-HT in functionally important sites in the brain (see Schildkraut and Kety, 1967). However, there is an alternative monoamine hypothesis which holds that the primary defect lies in the hyperactivity of serotonergic and/or noradrenergic postsynaptic receptors, and the signs of reduced monoamine metabolism are interpreted as secondary to the primary receptor defect (see Sulser et al, 1978).

4.2 NA and learning.

Many studies have been made on the effect of NA on learning and memory. In general, drugs facilitating catecholaminergic activity may facilitate acquisition and retention, and also consolidation of recently acquired information (Hunter et al, 1977; Dunn, 1980). Drugs which reduce catecholaminergic activity appear to reduce performance of well-learned responses, an effect which may involve both NA and DA

(Hunter et al, 1977). Mason and Iversen (1975) showed that dorsal bundle (db) lesioned rats had an impaired ability to extinguish a response after it was no longer rewarded. Acquisition learning was unaffected, but extinction of this response when learned, was slower, suggesting that the db pathway is involved in response inhibition rather than in memory (Mason and Iversen, 1977). However, other studies have shown that NA may be involved in memory formation since inhibition of dopamine-β-hydroxylase appears to reduce learning, an effect which may be reversed by the icv administration of NA (Hunter et al, 1977).

Lesions of the L.C. have been shown to impair learned responses such as that of running for food in an L-shaped runway (Anzelark et al, 1973). Ogren et al (1980) showed that a marked degeneration of the L.C. (with DSP-4) impaired the acquisition of both, one way and two way avoidance, the effects of which were blocked by the NA uptake blocker, desipramine, therefore suggesting the involvement of L.C. in learning. However, Mason and Fibiger (1979a) demonstrated that db lesioned animals (with 6-OHDA) acquired the two-way avoidance response quicker than controls and were more resistant to extinction, suggesting perhaps that all L.C. pathways must be destroyed before learning is reduced. Inhibition of conditioned avoidance by clonidine has been shown to be due to an effect on central α-adrenoceptors (Hawkins and Monti, 1979), thus it is possible that this drug may reduce noradrenergic transmission in pathways other than db to bring about this effect.

Experiments done on the acquisition of conditioned reinforcement by Mason and Fibiger (1978) demonstrated that 6-OHDA lesioned animals were no different from controls but were more distractable, suggesting that noradrenergic systems were involved in attention. Crow et al (1978) however, observed otherwise. They showed that the susceptibility to auditory stimuli and the rate of habituation and dishabituation to these stimuli in L.C. lesioned animals were no different from controls. Other work has shown that dorsal bundle lesioned rats (using 6-OHDA) were unable to ignore redundant stimuli information thus implying the involvement of noradrenergic innervation in the selective processing of information (Lorden et al, 1980).

4.3 NA and stress.

Stress has been shown to increase cortical NA turnover, an effect which arises from the L.C. (Korf et al, 1973) and may be reduced by benzodiazepines (Corrodi et al, 1971). The increase in brain NA catabolism induced by stress was shown to be blocked by monoamine oxidase inhibitors but not by catechol-o-methyl transferase inhibitors (Bliss et al, 1968). Following acute cold swim stress, hypothalamic NA concentrations were decreased, but returned to baseline within 14 hrs. (Roth et al, 1982), and immobilisation stress was shown to decrease NA and increase MHPG (an indication of NA turnover) in the hypothalamus, amygdala, thalamus, hippocampus, pons/medulla, and cerebral cortex (Tanaka et al, 1982). Cassens et al (1980) demonstrated that 24 hrs after footshock stress, increases in emotional behaviour and MHPG could be elicited by previously neutral environmental stimuli that had been paired with the stress.

The involvement of β-adrenoceptors in stress has also been considered. Nomura et al (1981) have suggested that stress, by increasing intrasynaptic NA levels resulting from an accelerated turnover rate causes β adrenergic receptor subsensitivity. A reduction in the density of β-adrenoceptors was observed in the hypothalamus, cerebral cortex and brain stem after chronic restraint stress (Stone and Platt, 1982) suggesting that a reduction in the number of β-adrenoceptors is one of the biochemical factors underlying the adaptation to stress.

Stress has also been associated with changes in other neurotransmitters. Anisman (1978) showed that stress increases the turnover of NA, DA and 5-HT and under conditions of severe stress, production of NA cannot keep up with the increased utilisation. It is thought that stress also produces a rise in acetylcholine levels (ibid). However, Maynert and Levi (1964) failed to observe any changes in whole brain 5-HT or acetylcholine after physical stress was applied (by exposure to cold or electric shock delivery) in rats or kittens, although decreases in brain NA were recorded.

5. Distribution of serotonergic neurones in the brain.

5-hydroxytryptamine (5-HT) containing neurones are known to be restricted to nine clusters of cells lying in or near the midline or raphe regions of the pons and upper brain stem (see Breese, 1975). The more caudal groups, originally formed B1- B3 (Dahlstrom and Fuxe, 1964) project largely to the medulla and pons. The more rostral 5-HT cell groups (raphe dorsalis, medianus and centralis superior, or B7 - B9) are thought to provide the extensive 5-HT innervation of the telencephalon and diencephalon. The raphe medianus (or B8 group) appears to furnish a very large component of the 5-HT innervation of the limbic system while B7 (dorsal raphe), which contain the greatest number of 5-HT cell bodies project to the neostriatum, cerebral and cerebellar corticas and thalamus. Ventrolateral to B8 scattered laterally within the reticular formation, is B9 nucleus - a group of 5-HT containing cells that have been identified by fluorescence microscopy but do not correspond to any classical neuroanatomical definitions of a nucleus (see Breese, 1975).

Lesion work carried out by Geyer (1976) showed that a selective decrease in 5-HT levels in the striatal region occurred when the dorsal raphe was lesioned. Selective reduction of 5-HT was also recorded in the hippocampal and septal areas during B8 lesioning. It was therefore suggested that there were at least two distinct 5-HT systems. The mesostriatal pathway originates in B7 and projects to the striatum, thalamus and some cortical (primarily dopaminergic) regions. The mesolimbic pathway originates in B8 and innervates such limbic structures as the septal nuclei, hippocampus, cingulate and entorhinal cortices and mamillary bodies (primarily noradrenergic regions).

5.1 Anatomical relationship between noradrenergic and serotonergic systems.

Anterograde labelling and degeneration studies have demonstrated that the raphe dorsalis and raphe medianus project to the L.C. in rats (Conrad et al, 1974), and the raphe pontis projects strongly to the ventrolateral L.C. (Sakai et al, 1977). Noradrenergic neurones of the L.C. were demonstrated to be innervated by processes of 5-HT neurones which provided ultrastructural evidence to support the contention that the activity of noradrenergic neurones is probably

directly modulated by 5-HT (Pickel et al, 1978). Noradrenergic projections to the raphe medianus from the A1 and A2 cell bodies have been implicated in the modulation of serotonergic neuronal function (Massari et al, 1979) indicating the involvement of one in the modulation of the other.

It has also been suggested that in the L.C., 5-HT afferents might have a wide spread influence independent of their synaptic connections, since 5-HT released from nerve terminals could diffuse in tissue and exert an action even on relatively distant targets (Leger and Descarries, 1978). Electron-microscopic autoradiographic techniques have shown that NA terminals directly innervate 5-HT cells in the raphe dorsalis (Baraban and Aghajanian, 1981) and work done by Morgane and Jacobs (1979) also supported the general view of heavy innervation of L.C. from the extra raphe and the raphe nuclei.

6. 5-HT receptors.

Direct binding assays of CNS receptors using ^{3}H-5-HT have indicated the presence of two types of binding sites in a variety of species (Whittaker and Seaman, 1978). Peroutka and Snyder (1979) have suggested that there are two types of 5-HT receptors in the frontal cortex of the rat which they have termed 5-HT$_1$ (preferentially labelled by ^{3}H-5-HT) and 5-HT$_2$ (preferentially labelled by ^{3}H-spiroperidol). The investigation of the effect of various 5-HT receptor agonists to inhibit K+-evoked release of ^{3}H-5-HT demonstrated that the 5-HT autoreceptor and the 5-HT$_1$ binding site have similar pharmacological characteristics and it is therefore possible that these receptors may be identical (Martin and Sanders-Bush, 1982).

Autoradiographic techniques revealed three subpopulations of 5-HT$_1$ sites that were characterised by their anatomical localisation and drug sensitivity; these have been referred to as 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ respectively (Cortes et al, 1984). 8-hydroxy-2-di-n-propylaminotetralin (8-OHDPAT) has been shown to be selective for 5-HT$_{1A}$ receptors whereas the 5-HT$_{1B}$ sites were sensitive to nanomolar concentrations of RU 24969 (Cortes et al, 1984). The 5-HT$_{1C}$ sites were labelled with nanomolar affinities by ^{3}H-5-HT, ^{3}H-LSD and ^{3}H-mesulergine (ibid).

5-HT receptors have also been referred to as S_1 and S_2 receptors characterised on the basis of relative agonist/antagonist potencies (Ennis and Cox, 1982). The S_1 receptor is located on the dopaminergic neuroterminals in the striatum and is postsynaptic to the 5-HT nerve ending. The other receptor, designated S_2 is located on the 5-HT containing neurones in the raphe nuclei (ibid).

A comparison of the S_1 and S_2 receptor types with the binding sites defined by Peroutka and Snyder (1979) on 5-HT_1 and 5-HT_2 does not reveal any similarities irrespective of whether agonists or antagonists are considered (see Ennis and Cox, 1982). Furthermore Middlemiss et al (1980) have shown that very high concentrations of 5-HT agonists are required to displace ^{3}H-spiroperidol from the 5-HT_2 receptor subtype, suggesting that the claim that ^{3}Hspiroperdol labels a 5-HT_2 receptor in frontal cortex of the rat may be erroneous.

To date the classification of 5-HT receptors is unresolved. In some studies they are referred to as 5-HT_1 and 5-HT_2 receptors (Colpaert et al, 1981; Green et al, 1983), whereas others prefer the S_1 and S_2 classification (Ennis and Cox, 1982).

7. Pharmacology of drugs acting at 5-HT receptors.

Studies investigating the selectivity of drugs acting at 5-HT receptors appear to be conflicting perhaps due to the fact that to date the classification of 5-HT receptors is unresolved (see section 6). Furthermore, drugs activity at 5-HT receptors appear to be non-selective for these receptors and have also been shown to influence other neuronal systems. For instance, quipazine was shown to stimulate both pre- and postsynaptic 5-HT receptors in the rat CNS (Blier and de Montigny, 1983), and its post/presynaptic ratio was similar to that of lysergic acid diathylamide (LSD) (ibid). Grabowska et al (1974b) demonstrated that quipazine inhibited catalepsy caused by spiroperidol (a DA blocker), suggesting that it may also interact with DA receptors.

The affinities of 5-HT agonists and antagonists for 5-HT autoreceptors in cortical slices has been investigated, and results showed that methiothepin and metergoline had moderate affinities for the 5-HT autoreceptor, whereas quipazine had weak affinity and ketanserin, cinanserin, cyproheptadine had no antagonistic properties at this receptor (Engel et al, 1982). However, Colpaert and Janssen

(1983) demonstrated that cyproheptadine had partial agonistic effect on the 5-HTP-induced headtwitch; only pirenperone exerted behavioural effects that suggest it to be a pure antagonist. Radioligand binding studies have shown that ketanserin primarily displayed high binding affinity for 5-HT_2 receptors and was inactive at 5-HT_1 receptor sites (Leysen et al, 1981). However, recent studies have shown that the selective 5-HT_2 receptor antagonists pirenperone and ketanserin also have an effect on other neurotransmitter systems. Ketanserin has been shown to block α_1-adrenoceptors (Fozard, 1982) and pirenperone in high doses was demonstrated to possess antidopaminergic activity (Pawlowski et al, 1983).

8. Possible roles of 5-HT in the CNS.

The serotonergic systems are important in the central control of physiological and behavioural mechanisms. 5-HT involvement in the production of sleep (Jouvet, 1973), regulation of body temperature (Feldberg and Myers, 1964) and pain sensitivity (Evans, 1961) have been implicated.

The involvement of 5-HT in feeding behaviour has been extensively studied. 5-HT receptor blockers have been shown to increase food intake, and quipazine produced a marked reduction in intake in rats, which was counteracted by pretreatment with metergoline (see Blundell, 1979). Central and peripheral manipulations of 5-HT, including specific lesioning and depletion of brain 5-HT systems have strongly indicated the role of 5-HT in food consumption, food preferences and body weight (for review, see Blundell, 1979).

A large number of studies have suggested that both serotonergic and catecholaminergic neurones play a role in controlling sexual behaviour; brain 5-HT appears to inhibit, and dopamine enhances, the copulatory behaviour in males whereas hormone-activated copulatory behaviour in the female rat seems to be ihibited by both brain 5-HT and dopamine (for review, see Gessa and Tagliamonte, 1979).

8.1 Behavioural effects of 5-HT.

Pharmacological treatments which result in either increased synaptic 5-HT or stimulation of postsynaptic 5-HT receptors produce a behavioural syndrome in the rat (Grahame-Smith, 1971) consisting of hyperactivity, hyperreactivity, resting tremor, rigidity or hypertonus,

reciprocal forepaw treading, straube tail, hindlimb abduction and lateral head weaving. This syndrome is of special importance in that with the possible exception of the hyperactivity component, it is a pure behavioural index of central 5-HT activity (Green and Grahame-Smith, 1974).

The involvement of serotonergic systems in depression has been suggested on the basis of the effects of antidepressants on 5-HT metabolism (see Abrams, 1978). Also tryptophan was reported to be just as effective as imipramine in depression (Coppen et al, 1972) and produced a reduction in hyperactivity of manic behaviour (Prahge et al, 1974). Ellison (1977) observed that rats depleted of both NA and 5-HT had large behavioural disruptions, were underresponsive to all negative reinforcers, very responsive to electric shocks and most helpless in an openfield situation. However, a review of present literature on 5-HT and depression (Abrams, 1978) has indicated that studies supporting the 5-HT involvement in depression do not bear close scrutiny, and that further work requires to be done in this area.

The monoamine hypothesis of affective disorders suggests that decreases in monoamines are responsible for depressive syndromes (see Schildkraut and Kety, 1967), although Aprison et al (1978) have suggested that people pre-disposed to depression have a reduced release of 5-HT therefore 5-HT receptors are naturally hypersensitive. Prior to exhibition of behavioural symptoms low 5-HT levels are recorded; however, depression seen clinically is a result of an increase in 5-HT release and the effect of this on hypersensitive receptors therefore imply that depression involves an enhancement of serotonergic activity (Aprison et al, 1978).

8.2 5-HT and learning.

It was originally suggested that para-chlorophenylalanine (p CPA)-induced facilitation of avoidance behaviour resulted from an increase in reactivity to novel stimuli (Tenen, 1967). Essman (1978) has reviewed evidence suggesting a relationship between the elevation of brain 5-HT and impairment of learning and memory; for example, when 5-HT was injected icv into mice trained on a passive avoidance response, there was a significant impairment of conditioned response retention, as compared with icv injected saline controls. Furthermore,

it was shown that 5-HT interfered with conditioned avoidance response acquisition, as a function of the intensity of the conditioning footshock without any alteration in the footshock threshold (Essman, 1978), suggesting that 5-HT increments can reduce the learning ability of mice.

8.3 5-HT and stress.

Restraint stress was shown to increase 5-HT turnover (Bliss et al, 1968) and cortical 5-HT turnover was also increased shortly after immobilisation stress, although in the brain stem 5-HT turnover remained unchanged and only increases in 5-HIAA were observed (Morgan et al, 1975). Joseph and Kennett (1981) reported an increase in 5-HIAA after stress indicating an increase in functional activity of 5-HT which in turn, is at least partly dependent upon an increase in brain tryptophan.

Tonic immobility in chickens was found to be decreased if 5-HT was injected peripherally (Wallnau and Gallup, 1977) but central 5-HT administration increased it (ibid) therefore proposing a serotonergic midbrain raphe model wherein there is an inverse relationship between raphe activity and the duration of tonic immobility. However, in rabbits, intracisternal injections of 5-HT decreased the duration of tonic immobility, and data suggest that raphe firing has a positive relationship with tonic immobility (Hatton et al, 1978).

9. The psychology of anxiety.

In their textbook of Abnormal Psychology, Davison and Neale (1974) write: "There is perhaps no other single topic in abnormal psychology that is as important and controversial as anxiety. This emotional state is considered as a symptom of almost all psycholopathologies and in particular of the neurotic disorders. Furthermore, anxiety plays an important role in the study of psychology oF normal people as well, for very few of us go through a week of our lives without experiencing in at least some measure what we would all agree is the emotion of anxiety."

In various psychological theories of anxiety in the clinical and experimental literature, recurring themes can be identified. Anxiety is associated with inability to express impulses or desires and

thus with states of conflict. The inhibition on behaviour occurs as a consequence of experience and in that experience, classical conditioning between the inhibition signal and the drive, plays a major role. There is however, much inconsistency in conceptualising anxiety. In the theoretical and research literature, the terms anxiety, fear, nervousness and tension seem to be employed interchangeably. Those of psychoanalytic persuasion prefer to reserve the term anxiety for fear that is experienced in the absence of external danger, whereas most learning theorists apply the term anxiety to fear learned in the presence of specific harmless stimuli (see Davison and Neale, 1974).

Although the terms fear and anxiety have been used interchangeably, Sullivan (1964) has attempted to differentiate anxiety from fear. He considers that as felt experience, marked fear and uncomplicated anxiety are identical, that is, there is nothing in one's awareness of the discomfort which distinguishes the one from the other. Fear, as a significant factor in any situation is often unequivocal. Anxiety on the other hand, in anything like the accustomed circumstances of one's life, is seldom clearly represented as such in awareness. He also explains that fear is related to some definable situation and is roughly the same for all people, but the significant pattern of situations which arouse anxiety is generally obscure; it can be almost infinitely varied among people and it shows much less obvious effects of habituation.

"Arousal" is another concept used to describe anxiety. It is a term usually used to describe the continuum of behaviour from sleep to increasing vigilance and alertness to emotional excitement and panic. Arousal is often equated with anxiety, and neurophysiological measures of arousal are used to describe the affect "anxiety" (see Fink, 1979). The degree of arousal may be defined as a symptom of anxiety by CNS activity, (EEG and sedation threshold), autonomic measures (blood pressure, heart rate) and skin conductance (see Fink, 1979). In humans at least, it appears that "arousal" as defined by its neurophysiological functions cannot be distinguished from anxiety although they may be distinguished on the basis of "emotion".

10. Animal models of anxiety.

10.1 Non-consummatory behaviour.

a. Exploratory behaviour.

The disinhibitory properties of anxiolytics can be demonstrated in a simple test involving exploratory activity in a novel environment. Marriott and Spencer (1965) examined exploratory behaviour in naive animals in a Y maze, whereas Christmas and Maxwell (1970) used a circular enclosure divided into sections (open field). The hole board apparatus has also been used to examine exploratory behaviour with anxiolytics (Nolan and Parkes, 1973). This consists of a floor with uniformly spaced holes into which animals can poke their heads as they explore the novel environment. Marriott and Smith (1972) used a square enclosure, the base of which was made of 4 metal plates separated by 3 mm gaps.

Another technique developed by Boissier et al (1976) (see Sepinwall and Cook, 1978) is the staircase test which consists of a wooden enclosure with a 5-step staircase. The number of rearings are interpreted as reflecting the animal's emotionality or anxiety, whereas the number of steps climbed is interpreted as an index of exploration.

Exploratory behaviour has been shown to be markedly enhanced in all the above cited tests, with anxiolytics.

b. Punished locomotory behaviour.

In this model, there are 4 electrifiable plates in the floor of the test chamber and a footshock is delivered when animals cross from one plate to another so that locomotor activity is reduced. Diazepam, meprobamate, and phenobarbitone all increased behaviour suppressed by punishment (Boissier et al, 1968).

c. Social interaction test of anxiety.

This model has been developed by File and Hyde (1978) in which the time spent by pairs of male rats in active social interaction is measured under various conditions. Social interaction is highest when the rats are tested in a box with which they are familiar and in a low level of illumination and decreases if the box is unfamiliar or if the light level is increased. Behaviours observed in the active interactions score including grooming, following, sniffing, mounting, nipping, licking, boxing, wrestling, jumping on and crawling under or

over. It has been shown that the decrease in interaction that occurs in control animals is not due to an increase in exploration of an unfamiliar environnent, nor to changes in olfactory cues from the partner. Drugs with an anxio lytic action increase the interaction score and produce a constant level of social interaction across all test conditions (File et al, 1976).

d. Potentiated startle paradigm.

This paradigm involves the association of a cue (light) with an inescapable shock and subsequent pairing of this cue with an acoustic stimulus (tone), leading to the potentiation of the startle produced by the tone. It has been found to be sensitive to the anxiolytic action of benzodiazepines (Davis, 1979a) and morphine (Davis, 1979b) all of which decrease the potentiated startle amplitude.

10.2 Conflict tests.

a. Geller-Seifter.

In this test (Geller and Seifter, 1960) rats are trained to lever press for a food reward on two different alternating schedules. On one, it initially receives food infrequently on a variable interval schedule and on the other one (which is normally signalled by the cue light or tone) food is usually delivered on every lever response. When response is established the high reinforcement schedule is modified so that each reinforcement is accompanied by a footshock. Benzodiazepines and other anxiolytics increase responding in the punished segment at doses that usually depress responding in the unpunished segment.

One of the problems with this test is that the two components of the schedule may not be equally sensitive to the drug effects even if rates of responding on both are matched. However, an anxiolytic profile with chronic as well as acute administration of benzodiazepines is observed and yet there is a rapid tolerance to their sedative effects. This distinction between acute and chronic effects of benzodiazepines is also found clinically (Warner, 1965) - a possible reason why this test is widely used to examine the anxiolytic profiles of drugs (see Cook and Davidson, 1973).

b. Conditioned Emotional Response (CER).

This test developed by Estes and Skinner (1941) has also been used to screen anxiolytics. Rats are trained to press a lever for a food reward on a fixed interval schedule (conditioned reinforcement). When this behaviour is stabilised, a tone is presented regularly every few minutes and the termination of the tone is associated with a shock which is unavoidable and inescapable. The result of this procedure is eventually, a decrease in rate of operant responding during the preshock tone, a disruption of behaviour which Estes and Skinner (1941) attributed to a state of anxiety. However, in this test, the effect of anxiolytics have been very inconsistent (Millenson and Leslie, 1974).

c. Punished drinking.

In this test, thirsty naive rats are periodically administered shocks for licking water (Vogel et al, 1971) - conflict being established between thirst (24 hr water deprivation) and drinking contingent footshock. Benzodiazepines appear to increase the number of shocks taken in this test (ibid).

10.3 Validation of anxiety models.

a. Tests based on consummatory behaviour.

These tests (which include the Geller-Seifter, CER and punished drinking) have been criticised on the basis that drugs may have a direct affect on food and water consumption which may affect their anxiogenic/anxiolytic activity. The validity of the punished drinking test (Vogel et al, 1971) depends on whether drugs being investigated have any direct effects on drinking. For example, Wise and Dawson (1974) found that benzodiazepines had no effect on water consumption, but Maickel and Maloney (1973) found an increase, and Soubrie et al (1976) reported that water consumption increased in an unfamiliar, as well as a familiar environment after treatment with benzodiazepines. Furthermore, oxazepam and lorazepam have been shown to significantly increase the latency to start drinking (Stein and Berger, 1969), an effect difficult to reconcile with an anxiety reducing action of these agents. Hence the effects of drugs on drinking may result in inconsistent data and therefore reduces the reliability of this test for quantifying antianxiety action. However, in the Geller-Seifter

conflict test, anxiolytics have been shown to have a selective effect on punished behaviour (Stein et al, 1973) suggesting that feeding behaviour may override the effects on anxiety in this test, since unpunished behaviour was either decreased (because of the sedative effect of the drug) or remained unchanged (ibid).

b. Tests based on painful stimuli.

In tests involving painful stimuli (such as the Geller-Seifter, CER, potentiated startle, punished locomotor activity and punished drinking), the fact that drugs may change the animal's sensitivity to painful stimuli, therefore decreasing punishment-induced suppression, has also been considered. Morphine, at analgesic doses does not reinstate punished reponding in the Geller-Seifter test (Geller et al, 1963), hence the blockade of painful sensation does not appear to be sufficient to produce a release in punished responding.

c. Tests based on operant conditioning.

In operant conditioning experiments, it is possible that an increase in punished responding may be due to a general stimulatory effect and conversely, a decrease in such behaviour may reflect a depressant effect. This seems not to be the case, since amphetamines do not release low rates of punished responding, and chlorpromazine which has potent CNS depressant effects does not reinstate punished responding (see Iversen, 1980).

In the CER test, the presentation of a stimulus preceding an unavoidable shock resulting in the suppression of ongoing food-maintained behaviour (Estes and Skinner, 1941) is based on the assumption that conditioned anxiety causes the disruption of lever pressing. However, it has been pointed out that the behaviour conditioned to the stimulus was "freezing" and is thus incompatible with active lever pressing, and further work on the schedule parameters of CER have shown that the schedule of reinforcement and the degree of discriminative control modifies considerably the effect of a particular shock on behaviour (see Iversen, 1980). Furthermore, although anxiolytics have occasionally been reported to increase responding in a CER situation (Lauener, 1963) such drugs have frequently been ineffective in this model (see Millensen and Leslie, 1974).

d. Rate dependency.

The effect of drugs on operant conflict behaviour may also be influenced by rate dependency, in that although conflict behavioural schedules control for general changes in response rates (by comparing the effect of drug day with the previous saline control day), they do not control for the possibility that an increased response rate in the punished component of a multiple schedule results from a drug effect on low response rate per se, whether produced by punishment or by some other influence. There is in fact considerable evidence that some drug-induced changes in response rate may depend in just this way on pre-existing response rates, a phenomenon known as "rate-dependency". (For review, see Robbins, 1981). For example, Harris et al (1978) demonstrated that clonidine, fenfluramine and quipazine increased low response rates, while decreasing high rate responding under a fixed-ratio (FR30) schedule of behaviour, which did not incorporate punishment. Furthermore, Dews (1964) reviewed evidence illustrating examples where the action of drugs were also found to be dependent upon the type of schedule used, even with similar levels of food deprivation.

10.4 Choice of Model.

The present study examined the effect of α-adrenoceptor and 5-HT receptor ligands on two models of aversively-motivated behaviour (AMB). One involving operant conflict, and the other based on exploratory activity in a novel environment. In the latter behavioural model, it was desired that the effect of drugs in AMB should be evaluated in animals, and then the brains be used for biochemical determinations of brain 5-HT and 5-HIAA, so that any congruence between anxiety-motivated behaviour and biochemical effects would become apparent. The potentiated startle paradigm could not be used owing to the fact that in this model, animals have to be previously trained to associate a tone or light signal with a painful stimuli (see Davis et al, 1979). With regard to the holeboard test, Nolan and Parkes (1973) reported that only small doses of benzodiazepines increased exploratory activity; larger doses appeared to have no effect. Furthermore, flurazepam failed to show a significant increase in activity in the dose range 0.1 - 12.5 mg/kg (ibid). From this, it appeared that not

all benzodiazepines increase exploratory activity in this behavioural situation, and it would be possible that a drug with anxiolyitic activity would pass undetected, hence this test could not be used.

In the social interaction test of anxiety, animals are required to be housed singly for 5 days before the start of the experiment (see File and Vellucci, 1978). Social isolation has been reported to cause disturbances in brain monoamine levels (see Valzelli, 1978) and because it was desired to examine regional brain concentrations of 5-HT and 5-HIAA in animals subsequent to their evaluation in an anxiety-motivated test, this behavioural model could not be used.

The measurement of exploratory activity in a Y maze, as described by Marriott and Spencer (1965) was also considered for the present study. However, it was felt that this situation did not incorporate a high "fear-inducing" drive; moreover, the situation would be more sensitive to the sedative effects of drugs, since it is essentially measuring exploration, which would be affected by sedation. It was therefore regarded necessary to develop a model based on exploratory activity which incorporated a more potent "fear-inducing" situation; where animals did not have to be isolated, and behaviour would not be influenced by the effect of drugs on feeding, drinking or painful stimuli.

Montgomery (1955) described an elevated Y maze with alternating open and enclosed arms which was used to investigate exploratory behaviour in naive rats. In this Y maze, rats showed a clear and significant preference for the enclosed arm. Montgomery (1955) proposed that such a situation produces two conflicting drives; the drive to explore, and the fear of exploration; the open arms appear to increase the fear drive. The present study investigated the effects of drugs on an elevated X-maze, based on the Y maze described by Montgomery (1955) consisting of two open (opposite) and two enclosed arms. The reason for using an X-maze as opposed to that of Montgomery (1955) was so that the "exploratory" drive and the "fear" drive could be adequately compared. This model has the advantage that "sedation" measure is on the same parameter as "fear" measure. It was found that in X-maze, "fear" could be measured by the ratio of open to total arm entries which would automatically compensate for sedation caused by a

drug. In contrast, in the hole-board test locomotor activity (as a measure of sedation) has to be measured independently (although in the same experiment) in order to assess the extent of sedation (see Chapter 2).

As mentioned earlier, the second test of AMB in this study was based on operant conflict, and initial experiments examine the effects of drugs on the Geller-Seifter test (Geller and Seifter, 1960). This multiple schedule procedure has the advantage that animals trained with stable performance baselines can serve as their own controls and are able to participate in many successive experiments over the course of many months. Also, the test is able to detect sensitive drug effects - that is, potential antianxiety activity can be quantified by the effect on punished behaviour, whereas the unpunished component makes it possible to evaluate non-specific drug effects such as depressant activity (see Cook and Davidson, 1973).

The effects of antianxiety agents in the Geller-Seifter test appear to be specific since a clear separation among drug classes has been observed. Low and medium doses of neuroleptics were shown to have no effect on punished or unpunished behaviour, although high dose levels depressed both types of responding (see Cook and Davidson, 1973). Antidepressants had a similar profile to the neuroleptics and amphetamine tended to increase the punishment-induced response suppression (Cook and Davidson, 1973).

In the present study, following initial experiments using the Geller-Seifter test, other conflict behavioural schedules have also been used, and rate-dependency effects have been considered.

11. The psychobiology of anxiety.

The involvement of various central neurotransmitter systems in anxiety have been based on the effects of drugs acting on those systems on anxiety and in various animal models of anxiety, as well as the effects of known anxiolytics on the functional activity of these neurotransmitters. Owing to the fact that the present study examines the involvement of noradrenergic and serotonergic systems in anxiety, these will be discussed in detail. The involvement of GABAergic and dopaminergic systems have also been implicated, although supporting

evidence is still lacking (see Iversen, 1984).

11.1 Involvement of NA.

a. Effects of drugs modifying noradrenergic function.

The behavioural effects of alteration in the noradrenergic function have suggested the involvement of NA in anxiety. Piperoxane, the α_2-adrenoceptor antagonist, was shown to be anxiogenic (Goldenberg et al, 1947) and yohimbine also caused fear and panic attacks in man (Holmberg and Gershon, 1961). Conversely, the α_2-adrenoceptor agonist clonidine, caused a decrease in anxiety attacks and psychic symptoms (Hoehn-Saric et al, 1981) and the increase in subjective anxiety by yohimbine was antagonised by diazepam and clonidine (Charney et al, 1983). Uhde et al (1983) reported a marked increased in mania and psychosis ratings after clonidine withdrawal, and findings suggested that clonidine had noteworthy antianxiety effects.

In the potentiated startle paradigm, Davis et al (1979) showed that drugs decreasing noradrenergic activity (such as the α_2-adrenoceptor agonist clonidine, and the β-adrenoceptor blocker propranolol) decreased the potentiated startle amplitude in rats and those that increase NA release (such as yohimbine and piperoxane, the α_2-adrenoceptor antagonists) increased it, suggesting that noradrenergic hyperactivity may be a factor in the production of some anxiety states. Phenoxybenzamine also reduced potentiated startle in rats (Davis et al, 1980) and the increase in potentiated startle caused by phenylephrine was antagonised by the selective α_1-adrenoceptor antagonist WB 4101 (Davis and Astrachan, 1981). Similarly, prazosin caused a marked increase in punished drinking (Gardner and Piper, 1982) and clonidine increased punished responding in the Geller-Seifter conflict test (Kruse et al, 1981) suggesting the involvement of both α_1- and α_2- adrenoceptors in anxiety.

β-Adrenoceptor involvement in anxiety has also been studied, and the antianxiety effects of β-adrenoceptor blockers alone are well reported (for review, see Jefferson, 1974). Furthermore, the combination of diazepam and propranolol was found to be more effective in the management of chronic anxiety in humans than diazepam alone (Hallstrom et al, 1981) and propranolol has also been found to potentiate the anticonflict activity of chlordiazepoxide (Sepinwall et

al, 1973). Moreover, Farhoumand et al (1979) suggested that oxprenolol exerted a similar central action to that of lorazepam since both decreased alertness and concentration, and skin conductance after stress.

There is however some conflicting evidence suggesting the involvement of noradrenergic systems in anxiety. For instance, Kruse et al (1981) failed to detect an anticonflict activity in the Geller-Seifter conflict test with phenoxybenzamine, and yohimbine was also without effect (Sepinwall and Cook, 1981). Using selective α- and β- adrenoceptor agents, Stein et al (1973) showed that both antagonists failed to release punishment- suppressed behaviour in the rat conflict test. It was also observed that NA antagonised the depressant effect of oxazepam on unpunished behaviour and therefore suggested that the depressant (rather than the anxio lytic) action of benzodiazepines was mediated by a decrease in NA turnover (ibid).

b. Effects of lesions.

The mechanisms which precipitate fear and anxiety have been studied by examining the effect of stimulating or lesioning specific noradrenergic cell groups. Results from such studies have been conflicting and in particular, the role of L.C. appears to be very controversial. Fear-like behaviour was elicited in the stump-tailed monkey by electrical stimulation of the L.C. or by drugs increasing L.C. activity, while electrolytic lesions of the L.C. caused a deficit in such behaviour (Redmond and Huang, 1979), which led to the hypothesis that the L.C. is a "fear" or "alarm" centre. Benzodiazepines were shown to decrease spontaneous single unit activity in the L.C. at relatively low doses (Grant et al, 1980), and Adams and Geyer (1981) reported that animals with L.C. lesions (using 6-OHDA) exhibited reduced startle responses.

Experiments on aversively and novelty motivated behaviour in the rat have yielded conflicting results. Mason and Fibiger (1979a) showed that lesioning of the dorsal bundle (db) - a major L.C. output pathway (see Introduction, section 1.), by 6-OHDA failed to disrupt the acquisition and performance of a wide variety of aversively-motivated behaviours (AMB). Responses to novelty were disrupted but in such a way as to suggest "if anything, an increased rather than a decreased

fear reaction". Results from another experiment showed that the acquisition of fear-motivated tasks, such as two-way active avoidance, or conditioned emotional response (CER), db lesioned rats (using 6-OHDA) were no different from sham-operated controls, but were more resistant to extinction in the CER task (Mason and Fibiger, 1979b). Crow et al (1978) showed that 6-OHDA lesions later al to the L.C. (so that "mechanical" damage to the L.C. [due to the insertion of the needle into the L.C.] in both groups of animals is kept to a minimum), had no effect on social interaction in the social interaction test of anxiety.

The concept of NA involvement in anxiety is still very unclear. Redmond (1979) has shown that the pharmacological induction of anxiety in humans by the α_2-adrenoceptor antagonists yohimbine and piperoxane, suggest the involvement of noraderenergic systems in anxiety. Mason et al (1978) have observed otherwise. They showed that forebrain NA depletion increased neophobia, since db lesioned animals (with 6-OHDA) showed a decreased consumption of novel solutions than did control rats, which suggests that lesions increase, rather than decrease fear. Chlordiazepoxide, however, which would be expected to increase consumption of novel food and drink, only produced an increase in total food consumption, not in novel foods (Cooper and Crummy, 1978) hence this test may not be sufficiently sensitive to detect changes in the emotional state of the animal.

Results obtained from electrical stimulation and electrolytic lesioning experiments described by Redmond (1979) have been criticised by Mason and Fibiger (1979b) on the grounds that the drugs and procedures used by Redmond (1979) are not sufficiently specific to noradrenergic neurones. It is possible that the effects on other systems which occur as a consequence of electrolytic lesioning or electrical stimulation of the L.C. may be the cause of behavioural changes. Indeed, stimulation of the L.C. does not exclusively affect noradrenergic systems as intracranial self-stimulation with electrodes in the L.C. is not abolished by the destruction of dorsal noradrenergic fibres (Clavier et al, 1976). Furthermore, the species used by Redmond (1979) has in fact been shown to possess serotonergic neurones in the L.C. (Sladek and Walker, 1977) hence, the effects observed with

stimulation and electrolytic lesioning may have been due to a 5-HT neuronal effect.

The evaluation of experimental findings from behavioural studies involving the lesioning of specific noradrenergic pathways has been complicated by the fact that different experimental models of AMB have been used to detect "fear" deficits in animals. Also, where specific lesioning (with 6-OHDA) has been performed, the effects of db lesions have been examined to a large extent. Although db lesioning degenerates a major L.C. output pathway, failure to detect any changes in AMB with db lesions only indicates that forebrain NA may not be involved; the central tegmental tract, which originates from the L.C. is still intact. Cerebellar innervations originating from the L.C. are also intact in db lesions which may suggest that these pathways are involved in AMB. Furthermore, noradrenergic neurones of the L.C. are also innervated by 5-HT neurones from the raphe nuclei (see Section 5.1); with db lesions, these are still intact and it is possible therefore, that the lack of effect on AMB with db lesions is due to a modulatory effect of the 5-HT neurones which innervate the L.C. For this reason, the present study has undertaken to examine the effects of 6-OHDA lesions of the L.C. itself on AMB.

11.2 Involvement of 5-HT.

a. Effect of drugs modifying serotonergic function.

The initial evidence of the involvement of 5-HT in anxiety was provided by Robichaud and Sledge (1961) that pCPA had anticonflict activity in the Geller-Seifter conflict test. Additive anticonflict effects between methysergide and chlordiazepoxide were also observed and cinanserin released punished responding in the Geller-Seifter test (Cook and Sepinwall, 1975). Antipunishment activity also increased after icv administration 5,6-dihydroxytryptamine (Stein et al, 1975). There are however, some discrepancies, especially with regard to the doses of 5-HT agents as well as the type of schedule used. For instance, Graeff (1974) using a multiple fixed-interval - fixed ratio schedule of food reinforcement with punishment in the latter component found that 3-10 mg/kg methysergide increased all responses to about half the peak effect exerted by chlordizepoxide. Stein et al (1975)

showed that in the Geller-Seifter test, 10 mg/kg methysergide increased both punished and unpunished responding, and Sepinwall and Cook (1978), using a schedule similar to that of Graeff (1974) found that 1.25 and 5.0 mg/kg methysergide increased punished and unpunished rates of responding to much less than those obtained after optimal doses of chlordiazepoxide; methysergide 10 mg/kg had no significant effect on punished rates but unpunished behaviour was decreased (ibid).

Winter (1972) observed that 1 - 10 mg/kg methysergide increased punished rates of responding whereas cinanserin as well as α-methyltryptamine had no effect on punished behaviour. Furthermore, Shephard et al (1982) demonstrated that 5-methoxy dimethyltryptamine (5-MeODMT) did not affect punished behaviour alone, or that of chlordiazepoxide, and metergoline failed to alter the anticonflict effects of chlordiazepoxide applied to the dorsal raphe (Thiebot et al, 1982) suggesting that 5-HT antagonism was neither necessary nor sufficient to account for the anticonflict activity of these agents. Furthermore, in a conditioned suppression of drinking test, only one dose of methysergide potentiated the anticonflict activity of diazepam (Kilts et al, 1982) and metergoline did not release punished responding alone and also did not alter the increase in punished responding induced by diazepam (Commisaris and Rech, 1982). These results are difficult to reconcile with the proposal that anxiolytic effects of benzodiazepines are related to serotonergic activity as suggested by Stein et al (1973).

b. Effects of lesions.

5,7-Dihydroxytryptamine (5,7-DHT) lesions of the 5-HT pathways innervating the lateral septum (Clarke and File, 1981) showed a decrease in the incidence of dominant behaviours as measured by the social interaction test of anxiety. Tye et al (1975) showed that animals with 5,7-DHT lesions of the 5-HT forebrain pathways accepted more shocks than sham-operated controls, and although chlordiazepoxide did not reinstate responding to the same degree as control animals, lesioned rats were responding in conflict at a higher rate than controls. They suggest that under chlordiazepoxide, lesioned rats reach their highest possible rate of responding sooner, thus a response releasing effect of normal magnitude was not possible, and attempt to

speculate that 5-HT pathways have a central role in mediating both the effects of shock on responding and the reversal of these effects with benzodiazepines. However, not all evidence is as convincing. It has been recently reported that 5,7-DHT lesions of the raphe dorsalis did not affect behavioural inhibition in control rats nor did it modify the ability of diazepam to release punished responding (Thiebot et al, 1984).

The discrepancies in the above cited studies might be explained by the fact that different tests as well as conflict schedules were used to investigate behaviour. For instance, Tye et al (1975) used a three component multiple variable-interval (VI-25 sec) schedule which consisted of a VI-25 sec period for 10 mins, followed by a "time-out" period, and then a VI-25 sec shock period. On the other hand, Thiebot et al (1984) examined the effects of lesions on a fixed ratio (FR7) of shock presentation, and Clarke and File (1981) investigated the effects of lesions in the social interaction test of anxiety.

11.3 Electrophysiological and biochemical correlates of anxiety.

Electrophysiological studies have shown that diazepam and chlordiazepoxide decreased single unit activity in the L.C. at relatively low doses (Grant et al, 1980), and Corrodi et al (1971) showed that benzodiazepines decreased the impulse activity in the NA neurones of the L.C. which innervate all cortices of the brain suggesting that anxiolytics can act by attenuating L.C. activity.

The effect of benzodiazepines on NA turnover have yielded conflicting results. Stein et al (1973) have shown that whereas the anxiolytic effect of oxazepam is maintained on chronic administration as is the decrease in 5-HT turnover, tolerance develops to both the sedative effects of oxazepam as well as the decrease in NA turnover suggesting that NA turnover may be related to the reduction in unpunished behaviour. However Cook and Sepinwall (1975) found that decreases in NA turnover do not correspond completely to the changes in unpunished behaviour although tolerance develops to this effect. The involvement of 5-HT systems in anxiety has also been suggested on the basis of the effects of benzodiazepines on brain 5-HT turnover. There have been many previous reports that benzodiazepines decrease central 5-HT turnover (see for instance Haefely et al, 1981), and it has been

suggested that their anxiolytic effect may be due to a reduction in the activity of "sertonergic" punishment system (Wise et al, 1972). File and Vellucci (1978) have further suggested that since ACTH causes a large elevation in brain 5-HIAA concentrations, one of the critical events in the induction of anxiety may be the stimulation of 5-HT pathways by ACTH and that the anxiolytics could act by counteracting this effect. Clonidine, which can have anxiolytic effects (see Redmond, 1982) also reduces 5-HIAA (Reinhard and Roth, 1982) and Banki (1977) measuring 5-HIAA levels in the cerebrospinal fluid of depressives found that anxiety and insomnia showed a significant correlation with 5-HIAA levels.

There is however disagreement as to whether benzodiazepines reduce raphe unit activity (Gallagher, 1978). Moreover, the reduction in turnover following these agents has also been attributed to feedback inhibition following enhanced 5-HT receptor stimulation (Nakamura and Fukushima, 1977). In addition, yohimbine which can cause anxiety and panic attacks in man (Holmberg and Gershon, 1961) produced biochemical signs of a reduction rather than an increase in serotonergic activity (Papeschi et al, 1971).

11.4 Behavioural Inhibition System and anxiety.

A model of the neuropsychology of anxiety has ben proposed, and the concept of a behavioural inhibition system (BIS) on the basis of the analysis of behavioural effects of antianxiety drugs in animals (see Gray, 1981). According to Gray (1981), this system responds to a novel stimuli or to those associated with punishment or non-reward by inhibiting on-going behaviour and increasing arousal and attention to the environment. It is the activity of the BIS that constitutes anxiety and that is reduced by antianxiety drugs.

The effects of anxiolytics in the brain also suggest hypotheses concerning the neural substrate of anxiety. Because of the similarities between the behavioural effects of certain lesions and those of anxiolytics (for review, see Gray, 1981) it is proposed that these drugs reduce anxiety by impairing the functioning of a widespread neuronal system including the septo-hippocampal system, the Papez circuit, prefrontal cortex, and ascending monoaminergic and cholinergic pathways which innervate these forebrain structures. Analysis of the

function of this system based on anatomical, behavioural and physiological data, suggests that it acts as a comparator: it compares predicted to actual sensory events and activates the outputs of the BIS where there is a mismatch or when the predicted event is aversive (see Gray, 1981). Gray also suggests that the most likely sites of specific antianxiety effects of anxiolytics are the ascending noradrenergic and serotonergic pathways, ascending dopaminergic pathway to the prefrontal and cingulate cortices (perhaps secondary to the projection from the L.C. to nucleus A10) and much less certainly the ascending cholinergic pathway to the septo-hippocampal system (Gray, 1981).

11.5 Behavioural interactions between NA and 5-HT.

Noradrenergic and serotonergic systems have been associated with many behavioural effects and it therefore possible that these two systems interact in mediating the effects of drugs on AMB. Carlsson et al (1969) suggested that for antidepressants, the level of psychomotor activity and drive were associated with an increase in noradrenergic activity, whilst mood elevating properties involved an increase in serotonergic activity. Berger et al (1971) however, reported that the reward system was mainly noradrenergic while the punishment system appeared to be at least partially serotonergic.

Lesioning of the B7 and B8 cell groups were shown to increase startle response in rats (possibly by enhancing sensitisation) which could be correlated with noradrenergic activation in the hippocampus (Geyer, 1976) and Kostowski (1980) reported that behavioural depression by clonidine was blocked after lesions involved either in the dorsal or median raphe indicating that 5-HT had an inhibitory effect on the release of NA since 5-HT destruction caused hyperactivity of NA neurones.

The elevation of the tailflick response produced by the microinjection of phentolamine into the raphe magnus was antagonised by the intrathecal administration of methysergide (Hammond et al, 1980), and the effect of clonidine in decreasing tonic seizures and mortality caused by pentylenetetrazole was completely prevented by metergoline and methysergide (Lazarova and Samanin, 1983). Furthermore, Handley and Brown (1982) reported that α-adrenoceptor ligands modulated the

head twitch induced by 5-HT, suggesting that a tonic noradrenergic input may be necessary for the occurrence of the head twitch induced by 5-HT. However, other studies have failed to detect such interactions. For instance, in the Geller-Seifter test, methysergide failed to alter the anticonflict activity of clonidine (Kruse et al, 1981) and cyproheptadine had no effect on the increase in startle amplitude produced by phenylephrine (Davis and Astrachan, 1981) although both cyproheptadine and methysergide have been shown to release punished responding in conflict behaviour (see Sepinwall and Cook, 1978).

Failure to detect behavioural interactions between NA and 5-HT in some instances is probably due to the fact that different aspects of behavi our have been considered. This study therefore examines behavioural interactions between NA and 5-HT in AMB.

12. Aims of the project.

1. To study the possible involvement of α-adrenoceptors and 5-HT receptors in some aversively motivated behaviours (AMB) by establishing the effects of agonists and antagonists varying in selectivity for α_1- and α_2- adrenoceptors, and drugs acting at 5-HT receptors, in behavioural tests incorporating an approach- avoidance conflict which may be regarded as sensitive to "fear" and it modulation.

2. To determine whether the L.C. is involved in AMB as exemplified by the above models, and whether the effects of the above agents are mediated via the L.C. system.

3. To examine whether behavioural effects of α-adrenoceptor ligands are modulated by 5-HT systems, and conversely, whether α-adrenoceptors modulate behavioural effects of 5-HT receptor ligands.

4. To determine whether behavioural effects of the above agents can be correlated to changes in regional 5-HT and 5-HIAA concentrations.

1. Animals and Animal Husbandry

Experiments reported in the thesis were performed on male Lister hooded rats, obtained from Bantin and Kingman Limited, except for lesion studies where hooded rats were obtained from Glaxo (Ware). Rats were kept in the Animal Housing Unit at an ambient temperature of 21-23°C and under a 12 hour light/dark cycle (8.00-20.00 hours), and were maintained on a conventional 41B cube diet supplied by Pilsbury Limited with tap water ad libitum except for the rats used for operant conditioning (Chapters 3 - 6) where water was available at all times, but food was limited in order to maintain their body weights.

2. General Experimental Conditions

Animals were held in groups of 6 in a quiet room (except for rats used for operant conditioning, which were caged singly), for at least one week prior to the experiment. All experiments were carried out at temperatures between 18°and 22°C and as far as possible between 10.00 and 18.00 hours, although more rigid conditions were imposed for certain experiments which are described in the appropriate sections.

3. Injection Technique

All drugs were injected intraperitoneally (i.p.). Injections were made by inserting the needle into the abdominal wall towards the diaphragm. Care was taken not to penetrate too deeply thereby damaging the internal organs. Where more than one injection was made by this route in the same animal, care was taken not to use the same injection site. The injection volume was 1ml/kg. Saline was injected in control animals unless stated otherwise. Drug solutions used for the operant conditioning rats were prepared aseptically, in order to minimize microbial contamination in these solutions, and wherever possible sterile drugs and saline was used.

4. Exploratory behaviour assessment.

4.1 Maze-Exploration Model of Conflict Behaviour

The apparatus used was similar to that described by Montgomery (1955). It consisted of an X-shaped maze (made of wood) elevated 70cm from the floor and comprising two (opposite) enclosed and two open arms (with no sides or ends). The arms were 45cm long and 10 cm high. The enclosed arms had sides and ends 10cm high. The central square formed by the armswas open, and the floor of the maze was lined with wire

mesh.

Animals weighing 150 - 200g were held in groups of six in a quiet room for at least one week prior to experiment. On the day before the experiment the cages were transferred to the experimental room and entry barred to all but the experimenter until completion of the experiment. Experiments were performed between 10.00 and 14.00 hours. Animals were assigned randomly to test and vehicle control groups but remained in their home cages. Six rats received each treatment and a vehicle control group was included in every experiment. Drugs were injected ip thirty minutes before placement into the maze, except for ACTH which was injected fifteen minutes beforehand since File and Vellucci (1978) obtained significant results in the social interaction test only between three and thirty minutes.

Immediately before testing, rats were observed undisturbed in the home cage and the degree of gross sedation estimated as 0 (absent), 1 (minimal), 2 (moderate) or 3 (severe). Each rat was placed gently in the centre square of the maze facing the same enclosed arm. The number of open and enclosed arm entries were recorded for a ten minute period by the observer sitting quietly at a distance of 1.5 metres from the centre of the maze and equidistant between the nearest open and enclosed arms. The criterion for an arm entry was a full body-length entry, excluding the tail. On the removal of the rat the maze floor was thoroughly cleaned. Each rat was exposed to the maze once only.

During pilot experiments it was found to be most important that this procedure was adhered to strictly, particularly with regard to maintaining quiet conditions throughout the time the animals were in the experimental room. Any disturbance tended to reduce exploration.

Expression of Results.

The number of open arm entries was determined as a proportion of the total number of entries (i.e. open/total) for each individual animal. Thus the group mean proportion 'P' was expressed as (Open/total) /n. Statistical comparisons were carried out using the Mann-Whitney 'U'-test (two-tailed), and the figures display the changes in open/total ratio calculated relative to the vehicle control baseline for that experiment as:

(P test / P control). 100.

4.2 Plate-crossing activity

The method of Marriott and Smith (1972) was adapted for use with rats to measure the effect of drugs on exploratory activity. The apparatus used was a box 62 cm square with sides 45 cm high. The floor consisted of four wooden plates 30 cm square which were separated from each other by 1 cm gaps.

Animals weighing 150 - 200g were held in groups of six in a quiet room for at least one week prior to experiment; they had been previously exposed to the 'maze' (see Section 4.1) and preliminary experiments showed that plate crossing activity was not affected by previous exposure to the maze (Table 1.2). Animals were placed individually in the box at exactly 50 minutes after injection, always in the same position facing the same wall.

The times at which animals crossed from one plate to another was recorded for a period of 180 seconds. In addition, the presence of autonomic signs and unusual behaviour was also recorded. The effect of drugs on four parameters were studied - the latency to the first and fifth crossings and the total number of crossings in both 90 and 180 seconds. Statistical evaluation was done by means of the Mann-Whitney 'U' test.

5. Operant Conditioning

5.1 Apparatus

This consisted of two Skinner boxes - dimensions 25 x 22 x 20 cm (Operant Conditioning Unit Model 105 - Campden Instruments Limited), with a shock scrambler. Each box contained two levers 6 cm above an electrifiable grid floor, two lights (4 cm above each lever respectively), and an automatic dipper feeder between the two levers for the delivery of a liquid food reward (0.2 cc of sweetened condensed milk diluted 1 part in 2 parts tap water). In all the operant conditioning experiments, only the left lever was active - pressing the right lever did not yield a reward.

Experiments were controlled by an Acorn System 3 computer programmed in OnliBASIC which comprised a visual display unit and a Hitachi Monitor (Hitachi Denshi Limited), a casette interface and a printer interface. The computer was connected to a cassette recorder

(Pioneer) for storing programs and an Epson FX-10 printer (Q-Com Computer Systems Limited), so that a print-out could be obtained for every run.

5.2 Experimental Conditions

Male rats weighing 300 - 400g at the start of the experiment were used. The animals were caged singly in polypropylene cages (40 x 25 cm) and were gradually reduced to 80% of their original body weight, which was maintained at this level by limited feedings. Animals were weighed at least three times a week and the weight was not allowed to vary more than ± 5g.

6. Experimental Schedules of conflict behaviour.

6.1 Geller-Seifter conflict schedule (Geller and Seifter, 1960)

Rats were first trained to associate a left lever press with a sweetened condensed milk reward. Initial magazine training was based on a continuous reinforcement schedule (CRF) where every lever response produced a liquid-food reward. When this response was stabilised, the animals were first trained on a 10 second Variable Interval Schedule (VI-10sec) in which a reward was obtainable on average once every ten seconds. The VI-10 seconds was then progressively extended to a VI-2 minutes, in which a reward was obtainable on average once every two minutes. When lever pressing rates had stabilised, a light stimulus (obtained by the two lights in the box) was introduced every ten minutes (for three minutes) as a signal that every lever response would be food reinforced. When all these contingencies were well established, conflict was introduced during the three minute period. This was done by delivering a shock through the grid floor at every lever response made during the presence of the light stimulus. Thus the rats were simultaneously rewarded with sweetened milk and punished with shock during the three minute light stimulus period (CRFs).

The rate of responding during the punished period (CRFs) could be controlled by appropriately adjusting the intensity and duration of shock. Low shock intensities (0.1 to 0.5 mA) and a short duration of shock (10 to 20 centiseconds) permitted only slight suppression of responding during the CRFs period and a high shock intensity (0.75 mA) with a longer duration of shock (20 to 30 centiseconds) suppressed responding.

Two groups of rats were used. For group 1 (n = 5) footshock intensity and duration was titrated to produce greater than 75% suppression, so that an increase in rate of responding by drugs having an "anxiolytic-like" effect could be easily detected. Conversely, for examining drugs having an "anxiogenic-like" effect footshock intensity and duration in Group 2 (n = 6) was titrated to produce less than 25% suppression.

All drugs were administered ip, immediately before placement in the box. Saline or vehicle was given for comparison on days +1 and -1, and comparisons were made with the immediately preceding day by means of the Wilcoxon matched pairs-signed ranks test. Animals were reinforced daily thus making it possible to investigate the time taken for the responses to return to baseline. Drugs were given at intervals not less than 7 days unless stated otherwise.

6.2 Continuous reinforcement schedule

This behavioural model was similar to that described by de Carvalho et al (1983). Rats were trained in a Skinner box to press the left lever for a condensed milk reward. When stable pressing rates were achieved, the animals were submitted to a 20-minute daily session divided as follows: during the first and third five minute periods, each lever press resulted in a condensed milk reward (non-conflict); during the central five minute period (in between the two non-conflict periods), which was signalled by the left cue light in the box, each lever press was similarly rewarded but also concommitantly punished with an electric footshock. Here, footshock intensity and duration was titrated to reduce the lever-pressing rate to between 33 and 66% of the first five-minute period. The last five minute period was signalled by the right cue light in the box - in this period (the time out period) lever responses were not food reinforced.

Drugs were injected (ip) 30 minutes before the beginning of each session, and saline/vehicle was given for comparison on days +1 and -1. Animals were submitted to the session for at least three days postdrug injection, so that any "carry-over" effects could be established. Comparisons of lever pressing rates on drug day were made with the immediately preceding saline control day by means of the Wilcoxon matched pairs-signed routes test. Drugs were given at intervals not

less than 7 days, unless stated otherwise.

6.3 Variable/fixed reinforcement schedule

This schedule consisted of a 20 min session, divided as follows: during the first five minutes, animals were submitted to a Variable Interval (VI-30 sec) period, where a reward was obtained on average once every 30 seconds (variable interval non-conflict). The next five minutes, (signalled by the left cue light in the box) introduced the continuous reinforcement punished period, where each lever press was similarly rewarded but also concomitantly punished with an electric footshock. This was followed by a continuous reinforcement period, where every lever press resulted in a condensed milk reward. The footshock intensity and duration was titrated to reduce the lever-pressing rate of the second period to between 33 and 66% of the third five minute period. The last five minute period was signalled by the right cue light in the box - in this period (Time Out) lever responses were not reinforced.

Drugs were injected (ip) 30 minutes before the beginning of each session, and the saline/vehicle was given for comparison on days +1 and -1. Comparisons of lever-pressing rates on drug day were made with the immediately preceding saline control day by means of the Wilcoxon matched pair-signed ranks s test. Drugs were injected at intervals not less than 7 days unless stated otherwise.

6.4 Variable interval reinforcement schedule

In this schedule animals were trained to press levers on a 30-second variable interval (VI-30 seconds). Animals were submitted to a 20 minute session divided as follows: during the first and third five minute periods, a condensed milk reward was obtainable on a VI-30 second schedule - i.e., on average, once every 30 seconds. The central five minute period also consisted of a VI-30 second schedule, but every reward obtained was concommitantly punished with an electric footshock. This period was signalled by the left cue light in the box, and footshock intensity and duration was titrated to produce a consistent level of suppression. The last five minute period was signalled by the right cue light in the box - in this period (Time Out), lever responses were not reinforced.

Drugs were injected (ip) 30 minutes before the beginning of each

session, and saline or vehicle was given for comparison on days +1 and -1. Animals were reinforced every day, and comparisons of lever pressing rates on drug day were made with the immediately preceding saline control day by means of the Wilcoxon matched pairs-signed ranks tests. Drugs were given at intervals not less than 7 days, unless stated otherwise.

6.5 Variableinterval reinforcement schedule with footshock at every lever response during punished period.

This schedule was similar to the Variable Interval reinforcement schedule described in Section 6.4. Animals were submitted to a 20 minute session divided as follows: during the first and third five minute periods, a condensed milk reward was obtainable on a VI-30 second schedule - i.e., on average, once every 30 seconds. During the central five minutes, animals were still on a VI-30 second schedule, but every lever response was punished with an electric footshock. This period was signalled by the left cue light in the box, and footshock intensity and duration was titrated to produce a consistent level of suppression. The last five minute period was signalled by the right cue light in the box - in this period (Time Out) lever responses were not reinforced.

Drugs were injected (ip) 30 minutes before the beginning of each session, and saline or vehicle was given for comparison on days +1 and -1. Drugs were given at intervals not less than 7 days, unless stated otherwise. Animals were reinforced every day, and comparisons of lever pressing rates on drug day were made with the immediately preceding saline control day by means of the Wilcoxan matched pairs-signed ranks test.

7. Stereotaxic lesioning of the Locus Coerulus (L.C.)

These were performed by Mr Lakhbir Singh. Rats (inbred at Glaxo) weighing 270 ± 10g were pre-treated with 1.0 mg/kg atropine sulphate and 15 mins later anaesthetised with pentobarbitone (60 mg/kg). Animals were then positioned in a stereotaxic instrument such that the upper incisor bar was 5.0 mm above the interaural line, and

the skull exposed. A hole was drilled in the skull and a 30 gauge cannula lowered unilaterally to the following coordinates:-

-8.20 mm from the bregma.
0.80 mm lateral from midline.
5.50 mm below dura.

The treated animals received 5mcg of 6-OHDA base dissolved in saline ascorbate (0.2 mg/kg ascorbic acid in saline) which was infused in 1.5mcl over 3 mins. Control animals received 1.5mcl of saline ascorbate over a 3 min period. The cannula was left in place for further 1 min to allow diffusion of the drug or saline-ascorbate and then withdrawn. The skin was then sutured.

Eight controls and ten treated animals were used in the study. Maze experiments were performed 21 days after surgery, and drugs were injected subsequently for operant behaviour experiments. Conditioning commenced 2 days after surgery, and animals were maintained on a food restricted diet (for conditioning purposes) 7 days after surgery.

In order to confirm a lesion in each of the treated animals, after the completion of behavioural experiments, animals were decapitated, then brains removed and dissected into various regions (as described in section 8), biochemical assays were then carried out for noradrenaline and dopamine (see Section 9.1).

8. Brain dissection

Dissections were performed by the method of Glowinski and Iversen (1966). Rats were killed by decapitation, their brains carefully removed and placed on an ice-cooled glass slide. Seven regions were separated of which six were used for assay, the cerebellum being discarded.

The rhombencephalon was first separated from the rest of the brain. This region was further divided by a transverse section into the cerebellum and the pons/medulla region. A transverse section was then made at the level of the optic chiasma, which separated the cerebrum into two parts B (parietal) and C (frontal).

The hypothalamus was then dissected out of part B by taking the anterior commisure as a horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit.

The striatum was then dissected out with the external walls of the lateral ventricles as the internal limit and the corpus collosum as the external limit. The frontal parts of the striatum were also dissected out from part C. The midbrain was gently separated out from the remaining part of the brain and the hippocampus was carefully removed. The remainder of parts B and C were then combined to form the cortex. Typical values obtained for the weights of various brain regions assayed are as shown in Table 1.0.

9. Biochemical Studies.

9.1 Biochemical assay for monoamines.

Noradrenaline and dopamine concentrations in various brain regions were determined spectrofluorometrically by the method of Cox and Perhach (1973) which was slightly modified. This method was used in sham operated and L.C. lesioned animals in order to determine the accuracy of the lesions performed.

Brain samples were homogenised in 3 ml of cold, acidified n-butanol. The homogenates were shaken mechanically for 5 mins and then centrifuged for 5 mins at 3000 r.p.m. 2.5 ml of the supernatant was withdrawn and transferred to a centrifuge tube containing 2.5 ml distilled water and 5.0 mg n-heptane. The tubes were then shaken for 5 mins and centrifuged at 3000 r.p.m. for 5 mins.

The organic layer was aspirated off and 2.5 ml of the aqueous phase was transferred to a screw-capped test tube containing 200 mg of alumina; 1.0 ml of 2.0M sodium acetate containing 0.1% EDTA was added, and the tubes were gently shaken for 19 mins and then centrifuged at 3000 r.p.m. for 5 mins. The aqueous phase was aspirated from the alumina.

The alumina was then washed by shaking with 2.0 ml distilled water for 5 mins and centrifuged at 3000 r.p.m. for 5 mins. The aqueous phase was discarded and 2.0 ml of 0.1N acetic acid were added to the alumina. The tubes were then shaken for 10 mins and centrifuged at 3000 r.p.m. for 5 mins; 1.0 ml of the aqueous phase was transferred to a small test tube and 0.2 ml of 0.01% EDTA was added to this, after which the pH was adjusted to 6.5 using 2N sodium hydroxide. Following this 0.1 ml of 0,1N iodine was added to the tube in order to oxidise the catecholamines. After exactly 2 mins, 0.2 ml of alkaline sulphite

was added to the tube in order to stop the oxidation of the catecholamines. The pH of this solution was then adjusted to about 5.4 by the addition of 0.2 ml 5N acetic acid. The mixture was then heated in a boiling water bath for exactly 2 mins after which the tubes were cooled in ice for 5 mins. Fluorescence was then read at 385 nm excitation and 485 nm emission in an Aminco-Bowman spectrophotofluorimeter for noradrenaline. Following this, the solutions were heated in a boiling water bath for 3 mins and fluorescence was read at 320 nm excitation and 370 nm emission for dopamine.

0.3ml standard solutions containing 75, 150 and 300 mg of catecholamines were extracted by the same procedure.

9.2 Biochemical estimation of 5-HT and 5-HIAA

a. Extraction

The method of Curzon and Green (1970) with modification by Gould (1979) was followed. Each brain region was homogenised (Citenco Limited) in 3 ml ice-cold acidified n-butanol. The homogenate was then shaken for 10 minutes on an automatic shaker (Griffin Ltd) allowed to stand in the freezer for 5 minutes (in order to maintain the low temperature) and centrifuged at 2,500 rpm (1000g) for 5 minutes in a bench centrifuge (MSE LTD).

2.5 ml of the supernatant from the acidified n-butanol homogenate was added to 5 ml n-heptane and 0.4 ml 0.1M Hydrochloric acid (containing 2% w/v cysteine hydrochloride in order to prevent oxidation). This mixture was shaken and centrifuged as above. 4 ml of the supernatant was removed and added to 0.8 ml Phosphate buffer at pH7.0. The remainder of the supernatant organic layer together with any tissue disc which may have formed in the interface was then aspirated off and the aqueous layer was assayed for 5-HT (see later).

The organic layer together with the Phosphate buffer was shaken and centrifuged as above, the supernatant was aspirated off and the aqueous layer was assayed for 5-HIAA.

0.3 ml of standard solutions were extracted by the same procedure.

b. Assay

0.1 ml of the aqueous phase (containing either 5-HT or 5-HIAA)

was added to 0.65 ml of o-phthalaldehyde solution (OPT) and 0.05 ml 1% w/v cysteine hydrochloride solution. The tube contents were shaken and heated in a water bath at 80 C for 20 minutes. The solutions were then removed, allowed to cool and the fluorescence was read on a Aminco-Bowman Spectrophotofluorimeter at excitation and emission wavelengths 360/470 nm respectively. The excitation and emission slits were 3.0 mm. The reaction products for 5-HT and 5-HIAA were identical and at these wavelength parameters produced maximum fluorescence (Fig 1.0). The relationship between indole concentration and fluorescence was found to be linear (typical correlation coefficient values - 5-HT r = 0.992; 5-HIAA r = 0.990).

Standard solutions

A stock solution containing:

5-hydroxytryptamine (free base)	50mcg/ml
5-hydroxyindole-acetic acid	50mcg/ml

- all in 0.1% w/v cysteine hydrochloride solution was used. This was stored in aliquots of 1.5 ml at -30 C. An aliquot was thawed once only as 5-HT is unstable to freezing and thawing (Gould, 1979).

The stock solution was diluted 1 in 50, 1 in 100, and 1 in 200 for use in this assay. Recovery standards of 0.1mcg/ml 5-HT or 5-HIAA were also used.

Extraction Recoveries

Recovery standards were prepared by adding 5-HT and 5-HIAA directly to the OPT/cysteine mixture. The theoretical recovery of the indoles was calculated assuming partition coefficients between aqueous and organic layers to be 100%, and the actual recoveries were expressed as a percentage of this value. The recovery values found were:

5-HT	78.4%	n=4
5-HIAA	65.6%	n=4

c. Comments

(a) The addition of cysteine hydrochloride solution at stages indicated was important in order to prevent oxidation of the indoles.

(b) The pH of the Phosphate buffer was critical; a pH of 7.0 was required for 5-HIAA extraction. Increase in the pH to 7.2 and 7.4 showed decreases in extraction of 5-HIAA.

Calculations of results

The levels of 5-HT and 5-HIAA in various brain regions were expressed as ng/g of tissue. Data was assessed for significance by the student's t test.

9.3 General Cleaning of Glassware

Special attention was paid to the cleaning of glassware to ensure accurate results in the spectroflourimetric assay. General glassware was soaked in Decon 90 solution (5% v/v solution prepared from Decon 90 concentrate) for 24 hours. Several rinses were made with hot and cold tap water and finally double distilled water. Glassware was then dried in a hot air oven (Laboratory and Electrical Enginering Co.Ltd.). The glass cuvettes (used to measure the amount of fluorescence) were soaked in concentrated nitric acid for 48 hours, then rinsed out several times with hot and cold tap water and finally double distilled water, and then left to dry.

10. Drugs and vehicles used.

Weights of all drugs referred to in the text are expressed as the salt.

ACTH	Sigma
Amylobarbitone Sodium	Eli-Lilly
Atropine Sulphate	Sigma
Azepexole	Boehringer Ingelheim
Clonidine HCL	Hoehringer Ingelheim
Diazepam	Roche
D-Glucose	BDH
Glycerol	BDH
Guanabenz	Wyeth
Gum Acacia	Sigma
Idazoxan (RX 781094)	Reckitt and Colman
Ketanserin	Janssen Pharmaceuticals
l-Phenylephrine HCl	Sigma
Pentobarbitone Sodium	May & Baker
Picrotoxin	Sigma
Piperoxane HCl	May & Baker
Pirenperone	Janssen Pharmaceuticals
Prazosin HCl	Pfizer

Quipazine Maleate	Miles Laboratories
RS 21361	Syntex Laboratories
St 587	Boehringer Ingelheim
Thymoxamine HCl	Warner
Yohimbine HCl	Sigma

All drugs were dissolved or suspended in normal saline, apart from the following:

diazepam -obtained in injectable form and diluted with distilled water. In experiments where diazepam was injected chronically, it was suspended in 2.5% gum acacia.

ketanserin -moistened with distilled water, dissolved in 0.1M HCl, then returned to pH7 with 0.1M NaOH and made to volume with water.

pirenperone -moistened with distilled water, dissolved in 0.1M HCl, then returned to pH7 with 0.1M NaOH and made to volume with water.

prazosin -dissolved in 5% glycerol / 5% glucose solution.

quipazine -moistened with distilled water, dissolved in 0.1M HCl, then returned to pH6 with 0.1M NaOH, and made to volume with water.

11. Reagents used in the biochemical assays.

Acetic acid (glacial)	BDH
Alumina (standard)	Sigma
n-Butanol (analar)	BDH
Cysteine HCl (anahydrous)	Sigma
Decon-90	BDH
Ethanol (absolute)	BDG
n-Heptane (analar)	BDH
Hydrochoric acid (HCl)(analar)	BDH
5-hydroxytryptamine sulphate creatinine complex	Sigma
5-hydroxyindole-acetic acid	Sigma
Iodine	Sigma
Nitric acid (analar)	BDH
o-phthalaldehyde	Sigma

Potassium di-dydrogen ortho-phosphate	Griffin and George Ltd.
Sodium acetate	Sigma
disodium-ethylene diamne tetra-acetate(EDTA)	Sigma
di-sodium hydrogen ortho-phosphate dodecahydrate (analar)	BDH
Sodium sulphite	Sigma

(a) Acidified Butanol

n-Butanol containing 0.85 ml concentrated hydrocloric acid per litre.

(b) Cysteine hydrochloride 1% in distilled water.

This solution was kept frozen or used freshly prepared.

(c) 5-hydroxytryptamine creatinine sulphate complex in distilled water 1 mg/ml (base equivalent), and 5-hydroxyindole acetic acid 1 mg/ml.

These solutions were diluted with distilled water to give a concentrated standard containing

5-hydroxytryptamine	50mcg/ml
5-hydroxyindole acetic acid	50mcg/ml

This solution was kept in the freezer in aliquots of 1 ml, and thawed once only, since 5-HT was unstable to freezing and thawing.

(d) o-phthalaldehyde reagent.

o-phthalaldehyde crystals dissolved in concentrated hydrochloric acid 4mcg/ml. (4 mg in 100 ml).

(e) Phosphate buffer

Disodium hydrogen orthophosphate ($Na_2HPO_4.12H_20$) 2.3 g/100ml and potassium dihydrogen orthophosphate (KH_2PO_4) 0.908 g/100 ml. 200 mg cysteine hydrochloride was then added to the buffer, and pH returned to pH7 by the addition of disodium hydrogen orthophosphate solution.

(f) 0.01% EDTA - this was prepared by dissolving 37.2g of disodium ethylenediamine tetra acetate (EDTA) in 1M sodium acetate and made up to a volume of 1 litre.

The pH was then adjusted to about 6.7 - 7.0 by the addition of sodium hydroxide.

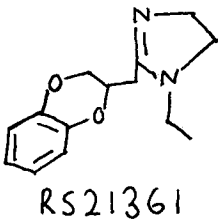
(g) 0.1N iodine - was prepared by dissolving 1.27g iodine in 100ml of absolute ethanol.

(h) Alkaline Sulphite - 1.0 ml of sodium sulphate (Na_2SO_3) solution (2.5 g of anhydrous salt dissolved in 10 ml of water) is diluted with 9.0 ml of 5N sodium hydroxide just before use.

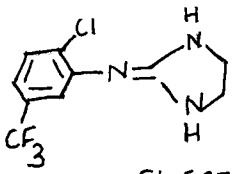
(i) Borate buffer - 31.4 g of boric acid was dissolved in 1 litre of distilled water, and 55 ml of 10N sodium hydroxide was added to this. The solution was then saturated with n-butanol and sodium chloride and adjusted to pH 10-11 with sodium hydroxide, if necessary.

(j) Alumina - approximately 200g of chromatographic-grade alumina was refluxed in 1 litre of 1N HCl for 30 mins, then washed with 20 changes of distilled water until the pH of the washings had risen to between 4 and 5. Finally, it was left to dry overnight at room temperature and then heated to 200°C for 2 hrs.

12. Chemical structures of RS 21361 and St 587.



RS21361



St 587

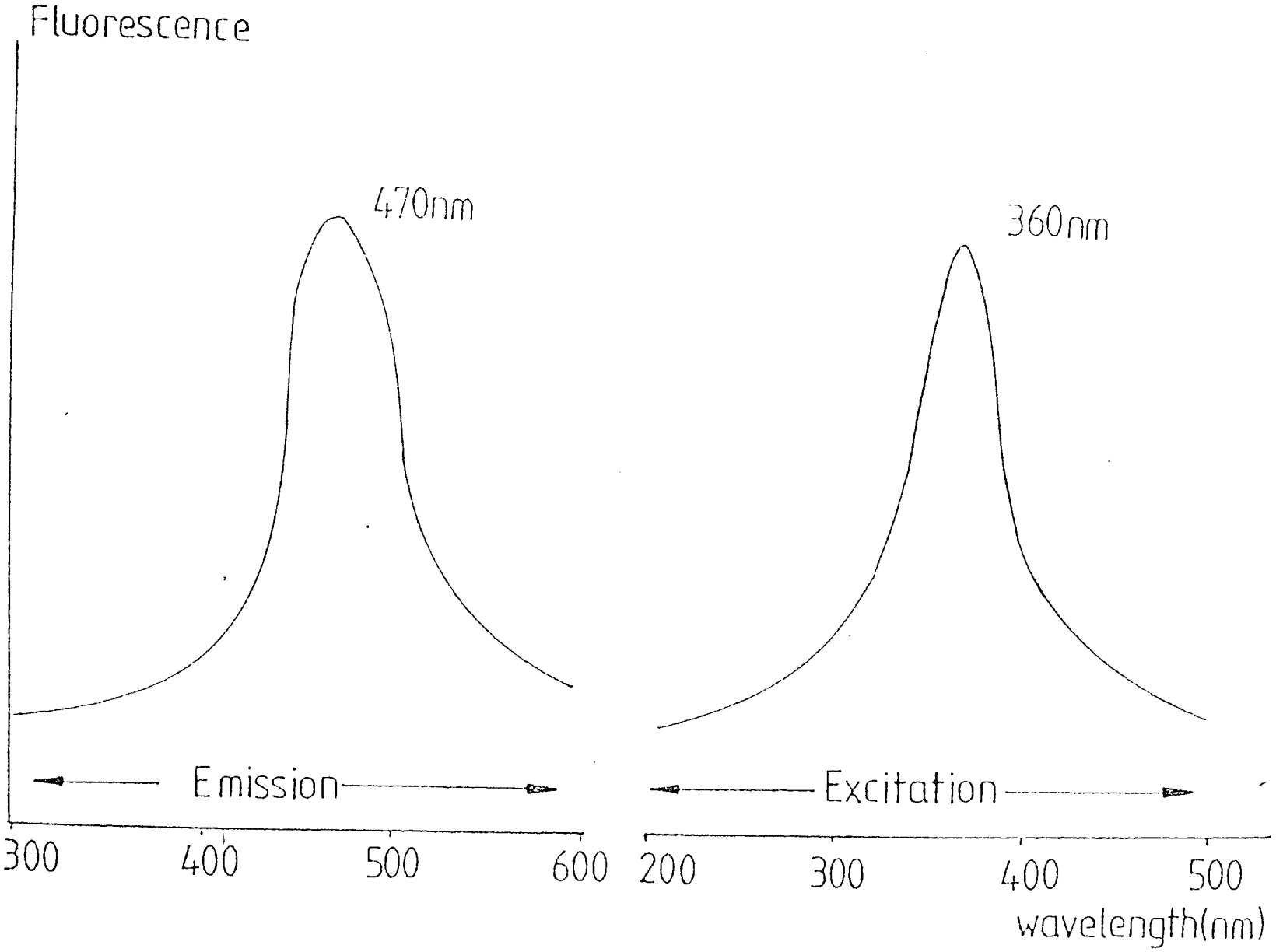


Fig 1. Excitation and emission spectra of the product of the 5-HT assay.

Brain Region	Weight (mg ± s.d.)
Pons-medulla	196 ± 16
Hypothalamus	77 ± 17
Striatum	111 ± 16
Midbrain	123 ± 12
Hippocampus	103 ± 12
Cortex	718 ± 31

Table 1.0 Mean weights for various brain regions dissected from twenty animals.
Results are expressed as mean ± standard deviation.

CHAPTER 1.

THE EFFECT OF DIAZEPAM AND α-ADRENOCEPTOR LIGANDS ON PLATE CROSSING ACTIVITY.

CHAPTER 1

Introduction

The effect of anxiolytics on exploratory activity in various test situations is well documented. Amylobarbitone was shown to increase exploratory behaviour in a Y maze (Rushton and Steinberg, 1966) and Marriott and Spencer (1965) also showed that chlordiazepoxide and meprobamate significantly increased the ambulation activity in rats placed in Y-shaped runway.

In the open-field test, amylobarbitone, meprobamate and oxazepam increased ambulation (Christmas and Maxwell, 1970); similar results were obtained in the holeboard test (Nolan and Parkes, 1973) and the plate crossing activity test (Marriott and Smith, 1972) where the benzodiazepines also increased exploratory activity. The effect of anxiolytics on exploratory behaviour in various novel situations have shown that these agents are able to restore inhibited responses to normal.

A suitable method for quantifying and investigating drugs modulating fear and anxiety would be to examine the effects of these agents in animals exposed to a novel environment. In the maze exploration study (Chapter 2) an approach-avoidance conflict situation was incorporated (as described by Montgomery, 1955) where the effect of drugs on exploratory activity was thought to be influenced to a large extent, by the effect of the drug on the "fear of exploration" and to a lesser extent, the drive to explore.

The purpose of this study was to examine the effects of α-adrenoceptor ligands and drugs acting at 5-HT receptors on fear-motivated behaviour, and to ascertain whether these effects could be related to their effects on 5-HT metabolism in various brain regions (Chapter 8/9). In these experiments, a method similar to that of Marriott and Smith (1972) was used, since it was shown that in this model, benzodiazepines increased exploration in animals that were naive to the novel environment, whereas previous exposure to this novel situation showed an increase in plate crossing activity of a transient nature, which was followed by a decrease in activity (Marriott and

Smith, 1972).

This suggests that the action of benzodiazepines was on a component only present in animals faced with a novel situation, possibly the "fear" drive (as described by Montgomery, 1955), and that the effect of benzodiazepines on exploratory behaviour was not due to a direct action on ambulatory activity. Hence, it would appear that drugs modulating fear and anxiety would affect plate crossing activity, and therefore this would be a valid and reliable test for examining the effect of drugs on fear-motivated behaviour.

Results

1. The effect of drugs on plate crossing activity.

The parameters used to compare drug-treated animals to controls were similar to those used by Brown (1980). The time taken for the rat to make the first crossing was used, since this would be expected to be most sensitive to any change in the emotional state of the animal. The time taken to make five crossings was also measured since this was found to be fairly consistent in control animals. With rats, it was found that the majority of exploration took place up to 180 sec., hence the number of crossings in 90 and 180 secs were also recorded.

1.1 The effect of previous exposure to the maze on plate crossing activity.

Previous exposure to the maze (10 mins before introducing the animal to the plate crossing situation) did not affect plate crossing activity (compared to controls that had not been exposed to the maze). Conversely, maze exploration was not different in animals that were previously exposed to the plate crossing activity test, compared to naive controls (Table 1.1). In subsequent experiments therefore, maze exploration was investigated 30 mins after drug/vehicle injection and plate crossing activity was examined 10 mins thereafter.

1.2 The effect of diazepam on plate crossing activity.

Doses of 2.0 and 5.0 mg/kg caused an increase in the total number of crossings (only the higher dose increasing it significantly). The latency to the fifth crossing tended to decrease with increasing dose of diazepam (Table 1.2). Diazepam 0.5 mg/kg did not affect any of the parameters measured. Chronic administration of diazepam (5.0 mg/kg daily for 6 days) had no effect on the latency to the first or the fifth crossing, although the total number of crossings were slightly increased (Table 1.2).

1.3 The effect of α_2-adrenoceptor agonists.

Both azepexole (2.0 mg/kg) and clonidine (0.01 and 0.025 mg/kg) had inconsistent effects on all the parameters measured, although azepexole 2.0 mg/kg did show tendency to decrease the latency to the first and fifth crossing, while increasing the total number of crossings (Table 1.3).

1.4 The effect of α_2-adrenoceptor antagonists.

A significant increase in the latency to the first and fifth crossings was obtained with RS 21361 (5 mg/kg) (Table 1.3). The total number of crossings decreased significantly at this dose (Table 1.3). However, yohimbine (1.25 and 2.5 mg/kg) did not have any significant effects.

1.5 The effect of α_1-adrenoceptor agonists.

Only the highest dose of phenylephrine (2.5 mg/kg) significantly reduced the total number of crossings, although St 587 (0.5 and 1.0 mg/kg) also tended to decrease this parameter (Table 1.4). Phenylephrine (0.25 - 2.5 mg/kg) tended to increase the latency to the first crossing as did St 587 (0.5 and 1.0 mg/kg) although only the highest dose of phenylephrine showed a significant increase in this parameter (Table 1.3). The latency to the fifth crossing remained unchanged with both these agents (Table 1.4).

1.6 The effect of α_1-adrenoceptor antagonists.

Prazosin (0.025 - 1.0 mg/kg) and thymoxamine (0.5 mg/kg) had no significant effect on plate crossing activity (Table 1.4), except the highest dose of prazosin which significantly increased the latency to the first crossing.

Discussion

The results with control animals show that plate crossing activity was greatest during the first 90 seconds, and preliminary experiments also revealed that pre-exposing the animals to the maze 10 mins beforehand did not alter plate crossing activity.

The effect of diazepam on plate crossing activity showed that only one dose produced an effect that was expected, that is, an increase in the total number of crossings. Furthermore, this effect was weak and at that dose, the total number of crossings in the control animals was relatively low (compared to controls for other doses); this could account for the weak, but significant effect observed with this dose of diazepam.

Generally, results are in agreement with Boissier et al (1968) who failed to find significant increases in plate crossing in mice when shocks were not given after each crossing. In mice that were shocked at each crossing, the benzodiazepines increased plate crossing activity (ibid). However, results are not in agreement with those of Marriott and Smith (1972) who demonstrated that diazepam (given orally) increased plate crossing activity in mice, in which the dose-response relationship was biphasic - lower doses increased activity, whereas the higher doses decreased it. The decrease at higher doses was due to the incapacitating effects which ranged from general motor depression to ataxia (ibid).

The α_2-adrenoceptor agonists clonidine and azepexole had no effect on plate crossing activity. In mice, clonidine was shown to produce an increase in plate crossing activity of a similar magnitude to that produced by amylobarbitone and diazepam (Brown, 1980). However, Dandiya and Patni (1973) reported that intra- ventricular administration of clonidine decreased the ambulation and rearing in rats in a open field situation. Similarly, Herman et al (1976) found that clonidine (0.1 mg/kg but not 0.05 mg/kg) decreased ambulation, rearing and defecation in an open field and reduced the peeping of rats in a hole test. Intracerebro- ventricular adminstration of 6-OHDA potentiated the depressant action of clonidine (Herman et al, 1976). Furthermore, Strombom (1975) reported that clonidine (0.025 - 0.8 mg/kg) depressed the exploratory behaviour of mice in a Y shaped runway

maze at doses that do not affect motor activity.

It is possible that the depressant effect of clonidine on exploratory activity as observed by Herman et al (1970), Strombom (1975) and Dandiya and Patni (1973) is due to the relatively high dose of clonidine used - for example, 0.025 mg/kg was shown to cause moderate sedation (Chapter 2) and the exploratory activity may have been influenced by the mild sedative effect of this agent. The lack of effect of clonidine on plate crossing activity in rats may be due to a species difference since Brown (1980) reported an increase in activity with mice. Also the possibility that this model adapted for investigating effects on rats may not be sensitive enough to detect small anxiolytic-like effects cannot be excluded, since the anxiolytic-like effects of the α_2-adrenoceptor agonists are much smaller than those of diazepam (Chapter 2; Chapter 3).

The selective α_2-adrenoceptor antagonist RS 21361 (Michel et al, 1981) significantly decreased the number of plate crossings and increased the latency to the first crossing. Although this effect was as predicted since the anxiogenic-like effect of RS 21361 has been demonstrated in both maze exploration (Chapter 2) and Geller Seifter conflict (Chapter 3) tests, the total number of crossings in the control group for this test appeared to be relatively higher than control values for other drugs. Moreover, yohimbine did not significantly alter either of the parameters even at the doses where its anxiogenic-like effect had been demonstrated (Chapter 2). Results from a study in mice indicated that yohimbine produced a dose-dependent decrease in plate crossing activity - the effect of which was reduced by diazepam (Brown, 1980).

Of the α_1-adrenoceptor agonists, phenylephrine and St 587, only the highest dose of phenylephrine caused a decrease in plate crossing activity. This is consistent with the finding that methoxamine decreased plate crossing activity in mice (Brown, 1980) although it is also possible that the decrease is due to the sedative effect of phenylephrine, as estimated in the homecage (Chapter 2). The α_1-adrenoceptor antagonists prazosin and thymoxamine had no significant effects on plate crossing activity, although in the light of the effects of phenylephrine, and those reported elsewhere (Chapter 2;

Chapter 3) it would be expected to increase plate crossing activity.

The effects of α-adrenoceptor ligands on fear-motivated behaviour are discussed in Chapter 2. The anxiolytic- or anxiogenic-like effects of these drugs could not be confirmed or established in this model, since even with diazepam, a very weak effect was obtained, therefore, it was not possible to evaluate the effect of these agents on fear-motivated behaviour.

The use of plate crossing activity for investigating behaviour in a novel situation or as a model for passive avoidance has been limited to mice (Boissier et al, 1968; Slotnick and Jarvick, 1966; Marriott and Smith, 1972; Brown 1980). The change of species to rats may have contributed to the difficulties found in obtaining consistent, and therefore significant, results with the administration of drugs. Hence the effect of these drugs on plate crossing activity in mice may be more useful in confirming and establishing the role of α-adrenoceptors in fear-motivated behaviour.

It is also possible that the adaptation of this model for use in rats was unsatisfactory. Further experiments are required to examine the effect of plates of different sizes on exploratory behaviour, and also varying the distance between the plates, since it is also possible that the dimensions of the apparatus used here were not sensitive enough to be able to detect small changes in activity caused by the drugs. Furthermore, there is one important consideration. Although preliminary experiments indicated that pre-exposing animals to the maze 10 mins beforehand did not alter plate crossing activity, the possibility that it could have altered the drug effects, since animals were exposed to a novel situation previously, cannot be excluded.

Owing to the inconsistent effects obtained with the α-adrenoceptor ligands in this study, at the doses where activity in other behavioural models were observed (Chapter 2, Chapter 3) and in particular, the fact that diazepam showed no real effect (or perhaps very weak effect) in this model, the plate crossing activity test was abandoned, and subsequently only the maze exploration study was used to evaluate the effect of drugs on fear-motivated behaviour.

FIRST EXPOSURE	PLATE CROSSING ACTIVITY				MAZE-EXPLORATION		
	Latency to first crossing (sec)	Latency to first crossing (sec)	Total number of crossings in 90 secs.	Total number of crossings in 180 secs.	Number of open entries in 10 mins.	Total number of entries in 10 mins.	Open/Total (ratio)
MAZE-EXPLORATION	3.5 ± 0.9	34.0 ± 15.9	6.8 ± 0.7	10.0 ± 2.4	2.7 ± 0.8	13.2 ± 4.8	0.18 ± 0.04
PLATE CROSSING	3.3 ± 0.6	37.2 ± 16.5	7.5 ± 0.8	12.6 ± 1.3	2.5 ± 0.4	11.8 ± 3.3	0.22 ± .05

TABLE 1.1 Effects of previous exposure of rats to maze-exploration on plate crossing activity.

Significance of differences from pre- and post-exposure

* $2p < 0.05$ ** $2p < 0.01$ (Mann Whitney 'U' test).

Values are expressed as mean ± standard error.

Animals were exposed to the maze for 10 mins, and to the plate crossing activity box for 180 secs. Time interval between the exposures was 10 mins.

Drug/dose (mg/kg)		Latency to first crossing (secs)	Latency to fifth crossing (sec)	Number of crossings in 90 seconds	Number of crossings in 180 seconds
Diazepam	0.5	9.3 ± 3.1 (6.0 ± 1.7)	75 ± 11 (67 ± 11)	6.0 ± 0.5 (5.8 ± 0.6)	8.0 ± 1.2 (8.2 ± 1.5)
	2.0	5.8 ± 1.8 (6.0 ± 1.7)	50 ± 12 (67 ± 11)	9.2 ± 1.9 (5.8 ± 0.6)	11.5 ± 2.7 (8.2 ± 1.5)
	5.0	19.0 ± 7.5 (15.8 ± 3.9)	104 ± 30 (133 ± 34)	6.0 ±1.1 (4.0 ± 1.0)	10.0 ± 3.0 (5.5 ± 0.5)**
Chronic diazepam (daily for 6 days)	5.0	27.0 ± 10.6 (41.3 ± 4.1)	138 ± 30 (180 ± 0.0)**	4.0 ± 1.4 (2.4 ± 0.3)*	6.0 ± 2.0 (3.6 ± 0.3)

TABLE 1.2 Effect of acute and chronic diazepam on plate crossing activity in a 180 second period, measured 50 minutes after injection.

Values are expressed as mean ± standard error. (Saline control values are in brackets).

Significance of differences from correspondng vehicle control.

* $2p < 0.05$ ** $2p < 0.01$ (Mann-Whitney 'U' test).

Drug/dose (mg/kg)		Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 90 seconds	Number of crossings in 180 seconds
Azepexole	2.0	4.0 ± 1.2 (6.0 ± 3.1)	53.0 ± 7.7(60.3 ± 16.1)	9.0 ± 1.0 (6.1 ± 0.5)	12.0 ± 0.9 (9.4 ± 1.4)
Clonidine	0.01	36.7 ± 18.0 (34.3 ± 17.0)	111 ± 48 (102 ± 35)	3.8 ± 0.8 (3.5 ± 0.4)	4.8 ± 0.9 (5.5 ± 1.2)
	0.025	34.6 ± 25.5 (34.2 ± 17.0)	114 ± 48 (102 ± 35)	3.2 ± 0.3 (3.5 ± 0.4)	6.0 ± 1.0 (5.5 ± 1.2)
RS 21361	5.0	30.8 ± 17.6 (5.5 ± 1.0)**	146 ± 26 (54 ± 7.0)*	4.5 ± 1.5 (7.9 ± 0.9)	5.0 ± 2.2 (10.0 ± 1.3)*
Yohimbine	1.25	23.3 ± 9.4 (24.2 ± 8.9)	137 ± 45 (160 ± 30)	3.0 ± 0.8(3.0 ± 0.6)	5.6 ± 1.1 (4.3 ± 0.6)
	2.5	17.5 ± 6.9 (24.2 ± 8.9)	129 ± 54 (160 ± 30)	4.0 ± 0.3 (3.0 ± 0.6)	5.6 ± 1.1 (4.3 ± 0.6)

TABLE 1.3 Effect of α_1-adrenoceptor agonists and antagonists on plate crossing activity in a 180 second period, measured 50 minutes after injection.

Values are expressed as mean ± standard error; Saline control values are in brackets.

Significance of differences from corresponding vehicle control

* $2p < 0.05$ ** $2p < 0.01$ (Mann Whitney 'U' test)

Drug/dose (mg/kg)		Latency to first crossing (sec)	Latency to fifth crossing (sec)	Number of crossings in 90 seconds	Number of crossings in 180 seconds
Phenylephrine	0.25	29.2 ± 9.5 (24.3 ± 8.9)	139 ± 42 (160 ± 31)	3.0 ± 0.5 (3.0 ± 0.9)	5.6 ± 1.1 (4.3 ± 0.6)
	1.0	12.4 ± 5.0 (116 ± 3.9)	126 ± 24 (83 ± 16)	4.0 ± 0.9 (5.0 ± 0.5)	4.4 ± 0.6 (7.2 ± 1.0)
	2.5	10.3 ± 3.8 (17.0 ± 7.2)*	180 ± 0 (118 ± 28)*	5.8 ± 0.5 (4.2 ± 1.3)	0.8 ± 0.5 (5.6 ± 1.7)**
ST 587	0.1	6.7 ± 1.4 (14.1 ± 10.8)	146 ± 23 (137 ± 28)	3.8 ± 0.3 (4.3 ± 1.0)	6.0 ± 1.0 (5.5 ± 1.1)
	0.5	34.2 ± 16.5 (27.7 ± 35.4)	155 ± 45 (160 ± 15)	3.2 ± 0.6 (3.3 ± 0.8)	4.2 ± 0.8 (5.7 ± 0.6)
	1.0	42.7 ± 54.3 (27.7 ± 35.4)	138 ± 35 (160 ± 45)	3.2 ± 0.6 (3.3 ± 0.8)	5.2 ± 0.3 (5.7 ± 0.6)
Prazosin	0.025	21.8 ± 7.6 (14.1 ± 10.8)	106 ± 23 (137 ± 29)	4.2 ± 0.7 (4.3 ± 1.0)	5.0 ± 0.4 (5.5 ± 1.1)
	0.05	17.6 ± 4.7 (20.0 ± 6.6)	117 ± 22 (82 ± 11)	4.0 ± 0.6 (5.0 ± 0.6)	5.0 ± 0.7 (7.7 ± 0.8)
	0.1	16.2 ± 4.9 (15.8 ± 3.9)	95 ± 26 (133 ± 34)	6.0 ± 1.1 (4.0± 1.0)	6.2 ± 0.9 (5.5 ± 0.5)
	0.25	17.5 ± 6.9 (24.3 ± 8.9)	115 ± 16 (160 ± 31)	4.0 ± 1.0 (3.0 ± 0.9)	5.6 ± 0.7 (4.3 ± 0.6)
	0.5	9.5 ± 1.3 (116 ± 3.9)	88 ± 18 (82 ± 16)	5.0 ± 1.2 (5.0 ± 0.5)	5.6 ± 0.8 (7.2 ± 1.0)
	1.0	45.0 ± 6.6 (17.0 ± 7.2)	167 ± 10 (118 ± 28)	2.8 ± 0.5 (4.2 ± 1.3)	4.2 ± 1.0 (5.6 ± 1.7)
Thymoxamine	0.5	20.0 ± 6.6 (17.6 ± 4.7)	117 ± 22 (82 ± 11)	5.0 ± 0.6 (4.0 ± 0.6)	7.7 ± 0.7 (5.7 ± 0.8)

TABLE 1.4 Effect of α_1-adrenoceptor agonists and antagonists on plate crossing activity in a 180 second period, measured 50 minutes after injection.

Values are expressed as mean ± standard error (Saline control values are in brackets).

Significance of differences from corresponding vehicle control

* $2p < 0.05$; ** $2p < 0.01$ (Mann Whitney 'U' test)

CHAPTER 2

THE EFFECT OF α-ADRENOCEPTOR AND 5-HT RECEPTOR LIGANDS ON MAZE EXPLORATION.

CHAPTER 2

Introduction

The purpose of this study was to consider whether α-adrenoceptor ligands and drugs acting at 5-HT receptors had a modulatory effect on fear-motivated behaviour, and whether this could be related to their effects on 5-HT metabolism in various regions of the rat brain (Chapter 8/9), since it has been suggested that the anxio lytic effect of benzodiazepines for example, may be due to a reduction in the activity of a serotonergic "punishment" system (Wise et al, 1973).

Montgomery (1955) examined the manner of exploration of open and enclosed arms in an elevated 'Y' maze. He attributed the rats' preference for the enclosed arm(s) to differences in the extent to which each elicited fear and exploratory drives. He proposed that novel stimulation evokes "both the fear drive and the exploratory drive, thus generating an approach-avoidance conflict". He considered that both the open and enclosed arms evoke the exploratory drive, but that the former evokes a greater strength of "fear drive" than the latter, thus resulting in a greater relative exploration of the enclosed arm(s). If this explanation is correct, then it would be expected that anxio lytic drugs would increase the relative exploration of open arms while anxiety-inducing agents would decrease it. Since several drugs were to be investigated in a range of doses and a simple and rapid test capable of yielding easily quantifiable data was required, a model based on these experiments was therefore devised, in which rats explored an X-shaped elevated maze with two open and two enclosed arms. This model was initially tested out using known anxiolytics and putative anxiogenic agents.

1. Maze dimensions and components

Preliminary experiments demonstrated that for rats weighing 150-200 g the maze dimensions were accurate enough to give consistent values for control animals. Increasing the length of the individual arms did not affect exploration significantly, and it was found that the width of the arms was sufficient enough to allow the animal to turn around within a particular arm quite comfortably, but was not too wide so as to affect the "fear drive" produced by the open arms.

On the X maze, various combinations of arms was looked at. Results showed that in a situation where two open arms were next to each other, exploration in these arms was greater than if they were opposite to each other, since it appeared to be effectively just an 'L' shaped alley. Three enclosed arms and one open arm reduced exploration in the latter, since animals only entered the three enclosed arms. Conversely, with three open arms and one enclosed, exploration in the open arms increased only by the number of head entries: full body entries were not affected.

It was found that the wire mesh lining on the floor of the maze aided exploration, since animals had a good "grip" on the floor. Also, if the top of the enclosed arms were covered in wire mesh, it caused a distraction, and animals would not explore. Furthermore, if the top of the enclosed arms was also covered (with wood), this reduced exploration further - animals would stay in the enclosed arms for most of the time.

All control animals showed a preference for the enclosed arms. For 60 control vehicle pretreated animals the mean of the ratio of open/total entries was 0.28 $\pm$ 0.07(s.e.m.), i.e., 28% of the entries were into the open arms. The ratio of open to total entries was not dependent on the total number of entries (r=0.22, slope 0.003).

2. The Effect of Anxiolytic and Anxiogenic agents.

a. Amylobarbitone

Amylobarbitone (15 and 30 mg/kg) caused an increase in the proportion of open arm entries (only 30 mg/kg was statistically significant) and sedation was observed at both doses (Fig.2.1). Amylobarbitone did not have a significant effect on total entries (Table 2.1).

b. Acute (single dose) diazepam.

Diazepam (0.5-5.0 mg/kg) caused a marked increase in the proportion of open arm entries (all except 0.5 mg/kg dose were statistically significant), (Fig.2.1). At these doses, diazepam did not have a consistent effect on total entries - total entries were significantly reduced at 1.0, 5.0 and 10.0 mg/kg (Table 2.1). Sedation was observed at 2.0 and 5.0 mg/kg and was so intense at 10.0 mg/kg that no exploration occured. Hence the dose response relationship with diazepam was 'U' shaped. Lower doses markedly increased open to total entries, whereas higher doses caused smaller increases, which appeared to be due to the incapacitating effect of high doses of diazepam - these ranged from general motor depression, to sedation and ataxia.

c. Chronic diazepam

In these experiments, vehicle or diazepam was injected daily for 10 days and animals were tested 30 mins after the last injection. Two doses (1.0 and 5.0 mg/kg) were looked at - both caused a significant increase in relative open arm exploration (Fig 2.1). There was no sedation after chronic 1.0 mg/kg diazepam, but animals were still sedated on the last day with 5.0 mg/kg although sedation was comparatively less with chronic dosing than with the acute, single dose diazepam 5.0 mg/kg (Fig 2.1). Chronic diazepam did not have a significant effect on total entries - unlike 5.0 mg/kg acute diazepam where total entries were significantly reduced (Table 2.1).

d. ACTH

ACTH was inactive at 0.05 mg/kg but markedly reduced relative open arm exploration at 0.075 mg/kg (Fig 2.1). At 0.075 mg/kg, ACTH significantly reduced both open and total entries, but did not cause sedation.

e. Picrotoxin

Picrotoxin significantly reduced the proportion of open arm entries (Fig 2.1). Both doses significantly decreased open arm entries; at 2.0 mg/kg total entries were not affected while at 4.0 mg/kg these were reduced despite the complete absence of sedation (Table 2.1).

Picrotoxin pretreated animals were hyperactive, both in the home cage and in the maze: startle responses occured to slight sounds

or movements such as that made by another rat. Sometimes this would initiate a "chain reaction" in the home cage, with the startle response of one rat setting off similar responses in the other rats. This did not occur with ACTH.

3. The Effect of α-Adrenoceptor Agonists and Antagonists.

a. α_2-Adrenoceptor Agonists

The effect of these agents are shown in Fig 2.2. The picture obtained depended on the dose. Azepexole 1.0 and 2.0 mg/kg caused a significant increase in open arm exploration, although 4.0 mg/kg had no effect. At 4.0 mg/kg total entries were significantly reduced although open arm exploration was not affected (Table 2.2). With Clonidine, 0.01 mg/kg significantly increased the proportion of open arm entries (Fig 2.2), but with higher doses (0.025-0.075 mg/kg) a reduction in the proportion of open arm entries was observed. All doses of Clonidine except 0.005 mg/kg significantly reduced total entries (Table 2.2) but the effect was not dose-dependent. Home cage sedation increased with the dose of Clonidine.

The effect of guanabenz (0.1-1.0 mg/kg) was similar to that observed with Clonidine. 0.1 and 0.25 mg/kg significantly increased the proportion of open arm entries, while the higher dose significantly reduced it (Fig 2.2). Sedation was observed at 0.25 and 1.0 mg/kg guanabenz - doses at which total entries were significantly reduced (Table 2.2).

In general, lower doses of the α_2-adrenoceptor agonists tended to increase the proportion of open arm entries, whereas at higher doses the effect became less - or in the case of Clonidine and guanabenz it was reversed. Also, total entries showed a general, dose dependent decrease in line with increasing home cage sedation.

b. α_2-Adrenoceptor Antagonists

The effects of these agents are shown in Table 2.2 and Fig 2.2. Yohimbine (1.25-5.0 mg/kg) produced a dose dependent decrease in the proportion of open arm entries. Total entries decreased at higher doses despite the absence of sedation. The animals were hyperactive at all doses. Both doses of RS21361 (5.0 and 10 mg/kg), idazoxan (0.125 and 0.25 mg/kg) and piperoxane (5.0 and 10.0 mg/kg) reduced the proportion of open arms entries, the higher dose in each case being

more effective than the lower (Fig 2.2). Piperoxane and RS21361 did not significantly affect total entries although idazoxan reduced these at both doses (Table 2.2). In no case was sedation noted and hyperreactivity was present in all treatment groups.

c. α_1-Adrenoceptor Agonists

Phenylephrine (0.25-2.5 mg/kg) and St587 (0.1-2.0 mg/kg) produced dose related decreases in relative open arm exploration. All doses of phenylephrine, and St587 1.0 and 2.0 mg/kg significantly decreased the proportion of open arm entries (Fig 2.3). Total exploration decreased at higher doses of phenylephrine (1.0 and 2.5 mg/kg) and St587 (1.0-2.0 mg/kg) although sedation was not marked (Table 2.3). Hyperreactivity resembling that seen with picrotoxin was seen after both drugs at all doses, except the highest dose of St587.

d. α_1-Adrenoceptor Antagonists.

Prazosin (0.025-0.1 mg/kg) increased the proportion of open arm entries. At higher doses however (0.5-1.0 mg/kg) this was reversed into a significant decrease (Fig 2.3) with a concommitant fall in overall exploration. At doses of 0.5 and 1.0 mg/kg prazosin, sedation was severe. Phymoxamine 0.5 mg/kg increased the proportion of open arm entries, although this was not statistically significant (Fig 2.3). When the dose was doubled however, there was a significant decreased in open arm exploration. Total entries were reduced significantly only at 0.5 mg/kg (Table 2.3) - the dose at which there was an increase in the proportion of open arm entries - although only the highest dose (1.0 mg/kg) produced mild sedation. Hyperreactivity was not seen with either agent.

4. The Effect of drugs acting at 5-HT_2-receptors.

a. Quipazine

Quipazine (0.5-2.0 mg/kg) produced significant decreases in the proportion of open arm entries which were dose-related although there was no sedation at any of the doses (Fig 2.4). Quipazine did not have a significant effect on total entries but at higher doses (1.0 and 2.0 mg/kg) there was a slight decrease in total entries (Table 2.4). Hyperreactivity was seen at all doses and at 1.0 and 2.0 mg/kg the

characteristic 5-HT syndrome (head weaving, forepaw treading) was also observed in the home cage 5 mins after quipazine was injected.

b. 5-HT_1-receptor antagonists.

At 0.025 mg/kg, ketanserin had no significant effect on any of the parameters measured. Ketanserin 0.05 mg/kg significantly increased the proportion of open arm entries and at 0.1 mg/kg a slight increase in open arm exploration was observed, although 0.2 mg/kg reversed this effect (Fig 2.4). Ketanserin did not have a significant effect on total entries (Table 2.4) and none of these doses produced sedation.

Pirenperone (0.05-0.2 mg/kg) did not have any significant effect on the proportion of open arm entries, although 0.1 and 0.2 mg/kg slightly increased it (Fig 2.4). Pirenperone did not have any consistent effects on total entries (Table 2.4) and also did not produce any sedation. Hyperreactivity was not seen with either of the 5-HT receptor antagonists.

5. The Effect of Clonidine in the presence of prazosin

In order to investigate whether the reduction in the proportion of open arm entries caused by higher doses of the α_2-adrenoceptor agonists is due to their effect on α_1-adrenoceptors, the effect of clonidine was investigated in the presence of prazosin 0.025 mg/kg injected 15 mins previously. Behaviour in the maze was looked at 30 mins after clonidine injection. Fig 2.5 shows that prazosin 0.025 mg/kg shown previously to be a sub-anxiolytic dose (Fig 2.3) abolished the relative reduction in open arm entries induced by 0.075 mg/kg clonidine (one way analysis of variance - Kruskal Wallis test). Both clonidine alone, and clonidine pretreated with prazosin groups reduced total entries significantly (Table 2.5) and sedation was very intense in both these groups.

6. The Effect of Quipazine in the present of pirenperone

In order to investigate whether the "anxiogenic-like" activity of quipazine (Fig 2.4) is due to its activity at the 5-HT_2-receptor, the effect of quipazine 1.0 mg/kg was investigated in the presence of the 5-HT_2-selective antagonist pirenperone injected 15 mins previously. Fig 2.6 shows that pirenperone 0.1 mg/kg abolished the relative reduction in open arm entries induced by quipazine (one way analysis of

variance - Kruskal-Wallis test). Total entries were not significantly affected in any of the drug treated groups. (Table 2.5).

7. Noradrenergic - serotonergic interactions.

a. α_2-Adrenoceptor - 5-HT_2 interaction.

The effect of pirenperone on the reduction in the proportion of open arm entries induced by yohimbine was investigated to see whether the anxiogenic-like effect of α_2-adrenoceptor antagonists are modulated by 5-HT$_2$ receptors. Fig 2.7(b) shows that pirenperone 0.1 mg/kg had no effect on the reduction in the proportion of open arm entries caused by yohimbine 1.25 mg/kg. Both yohimbine and pirenperone caused a slight decrease in total entries and this was further reduced in the yohimbine pretreated with pirenperone group, although none of these decreases were statistically significant.

b. α_1-Adrenoceptor-5-HT_2 interaction

In order to investigate whether 5-HT -receptors were involved in the "anxiogenic-like" activity of α_1-adrenoceptor agonists the effect of pirenperone 0.1 mg/kg on the reduction in the proprtion of open arm entries induced by phenylephrine (0.25 mg/kg) was investigated. Fig 2.7(a) shows that pirenperone failed to modify the decrease in the proportion of open arm entries induced by phenylephrine. Total entries were not significantly affected, although the phenylephrine, and pirenperone + phenylephrine group both showed a slight decrease in total entries (Table 2.5).

c. 5-HT_2- α_1-Adrenoceptor interaction

The effect of prazosin on the reduction in the proportion of open arm entries induced by quipazine was investigated to see whether the "anxiogenic-like" effect of quipazine was modified by α_1-adrenoceptors. Prazosin 0.025 mg/kg was injected 15 mins prior to quipazine (0.5 mg/kg), and behaviour in the maze was tested 30 mins after quipazine injection. Fig 2.8 shows that prazosin did not modify the effect of quipazine on the proportion of open arm entries. Total entries also remained unchanged (Table 2.5).

Discussion

The results with control animals show that the X maze used here behaved similarly to the Y maze of Montgomery (1955), in that rats showed a clear and significant preference for the enclosed arm.

The effects of drugs on exploratory activity in a novel environment could be exerted through several mechanisms. These include effects on the "fear" and "curiosity" drives (Montgomery, 1955) and also changes secondary to sedation. From this hypothesis, there is therefore reason to suppose that while both open and enclosed arms evoke an "exploratory" or "curiosity" drive, the open arms evoke a much greater strength of "fear" drive. What is not known is whether sedation per se would have any differential effect on exploration of the two types of arms. Sedative drugs reduce motor activity, and both enclosed and open arm entries would be affected by sedation. However, the enclosed arm entries are also likely to be affected by changes in fear drive, the proposal being that there is a relative difference in this drive between the two arms (Montgomery, 1955). It was therefore important to have an independent estimate of sedation which was derived in a non-exploratory situation and also without handling, as this might affect subsequent maze behaviour. A gross estimate of sedation was therefore obtained by observing the undisturbed animals in the home cage immediately prior to testing.

The relative exploration of open to total arms responded as predicted to the anxiolytic and putative anxiogenic agents. Both diazepam and amylobarbitone produced an increase in the proportion of open arm entries (the open to total ratio) even in the presence of severe sedation. Since total exploratory activity was not increased by any dose except 2.0 mg/kg diazepam, it is unlikely that this shift in ratio was due to an enhancement of "exploratory drive" or locomotor activity. Results are consistent with the findings that diazepam increased the number of punished crossings in plate crossing activity experiments (Boissier et al, 1968) and that amylobarbitone decreased the potentiated startle response in rats (Chi, 1965). ACTH has been proposed to possess anxiogenic activity on the basis of its effect on

the social interaction test in the rat (File and Vellucci, 1978) - the behavioural effects of which could be reversed by chlordiazepoxide (ibid.), as has the GABA antagonist picrotoxin (File and Lister, 1983). Furthermore, Gallager et al (1983) demonstrated that the acoustic startle reflex was increased following picrotoxin in post-natal day 15-16 rat pups. Both drugs reduced open to total ratio of arm entries at doses below those significantly affecting enclosed entries. At higher doses, both enclosed and total entries were reduced, but since open entries were affected most the ratio was significantly decreased. No sedation was observed in the home cage - this was also reported by File and Vellucci (1978) with ACTH. The results therefore bear out the hypothesis of Montgomery (1955) that open arms evoke a relatively greater strength of "fear-drive" than do enclosed. The model would therefore appear to be valid for investigation of drug effects on the expression of fear drive.

The α_2-adrenoceptor agonists clonidine, azepexole and guanabenz all produced a relative increase in open arm exploration in doses which caused at the most, mild sedation. For clonidine and guanabenz, increasing the dose of drug resulted in the reversal of the effect with a decrease in open to total ratio. Since this effect occured at doses where a moderate degree of sedation was noted, it is possible that this was due to the interference by the sedative effects of these drugs. The sedative effects of α_2-adrenoceptor agonists have also been reported elsewhere; for example, in chicks, Delbarre and Schmitt (1971) showed that clonidine and a range of other imidazolines had a sedative effect, and in mice, clonidine and guanabenz both potentiated the duration of sleep induced by chloral hydrate (Brown, 1980). However, equisedative doses of diazepam and amylobarbitone still produced an increase in ratio.

The selectivity of these agents for α_2- as opposed to α_1-adrenoceptors is only relative. Although in brain membranes, 3H-clonidine was shown to label α_2-adrenoceptors (Tanaka and Starke, 1980), in the pithed rat clonidine was demonstrated to be equipotent on both presynaptic and postsynaptic α-adrenoceptors (Drew, 1976).

Furthermore, in conscious renal hypertensive rats, results suggested that α_1-adrenoceptors may also mediate the central hypotensive effect of clonidine (Beckett and Finch, 1982). In spontaneously active single neurones of the somatosensory cortex, the effect of clonidine was antagonised by prazosin, and together with other effects, results strongly suggested that centrally, clonidine acts as a partial agonist at α_1-adrenoceptors (Bradshaw et al, 1982). The effect of guanabenz on various autonomic systems in vagotomised anaesthetised cats suggested that guanabenz acted like clonidine in the CNS (Koss, 1983), and in rabbit arterial strips, the effect of guanabenz on adrenergic mechanisms was similar to that of clonidine (Sakakibara et al, 1981). Azepexole on the other hand, has been shown to be more selective than clonidine (van Meel et al, 1981).

In the light of the effects obtained with phenylephrine and St587, and the selectivity of the α_1-adrenoceptor agonists, it is therefore possible that the decrease in the open to total ratio obtained with higher doses of clonidine and guanabenz was due to an effect at α_1-adrenoceptors. This was later confirmed by investigating the effect of 0.075 mg/kg clonidine in the presence of prazosin, where the "anxiogenic-like" effect of the high doses of clonidine was effectively abolished by prazosin.

The four antagonists with selectivity for α_2-adrenoceptors yohimbine, piperoxane (Starke et al, 1975; Drew, 1976), RS21361 (Michel et al, 1981) and idazoxan (Doxey et al, 1983) all reduced the proportion of open to total arm entries at doses below those significantly affecting exploration of the enclosed arms. A similar "anxiogenic-like" effect has also been observed in mice - where it was noted that the yohimbine-treated animals had increased startle, touch and tail pinch responses, very brisk flexor and pinna reflexes, and were hyperreactive to noise or movement (Brown, 1980). At higher doses, all reduced enclosed arm exploration as well, but sedation was never seen in the home cage. This high dose effect may therefore be due to the elevation of fear drive affecting enclosed as well as open arm entries, since the effect of fear drive is only supposed to be "relative" - such that with a high fear drive, both open and enclosed arm entries will be similarly affected.

The effect of an "anxiolytic" dose of an α_2-adrenoceptor agonist in the presence of an α_2-adrenoceptor antagonist was not investigated, since these effects have been reported elsewhere. For example, Kruse et al (1981) showed that the anxiolytic-like effect of clonidine (on punished responding) was antagonised by yohimbine. It was also demonstrated that yohimbine antagonised the suppression of "fixed ratio" responding and increase in tail withdrawal latency induced by clonidine (McCleary et al, 1981). Furthermore, the decrease in acoustic startle response induced by clonidine was blocked by yohimbine (Davis and Astrachan, 1981).

The α_1-adrenoceptor agonists phenylephrine and St587 which have a high degree of selectivity for the α_1-adrenoceptor (Drew, 1976; de Jonge et al, 1981) showed a selective inhibition of open arm entries at doses producing no appreciable degree of sedation. Again, enclosed entries also fell at higher doses although it is noteworthy that the median dose reduced enclosed entries while producing no sedation with St587, and minimal with phenylephrine, bearing out the suggestion that selective effects on the fear drive may influence enclosed arm entries.

The effects of prazosin and thymoxamine were less clear-cut. These agents show considerable selectivity for α_1-adrenoceptors (Cavero et al, 1977; Drew, 1976). As would be predicted from the effects of the corresponding agonists, at low doses both drugs increased the open to total ratio. As the dose was increased however, the ratio fell reversing into an "anxiogenic" picture. This occured at doses of prazosin which caused severe sedation but for thymoxamine, sedation was only minimal at this dose. It would seem unlikely therefore that the differential effects on the open arm entries could be accounted for by the failure of measurement to compensate for sedation.

The overall pattern of results obtained with the α-adrenoceptor ligands was that the α_2-adrenoceptor agonists and the α_1-adrenoceptor antagonists resembled known anxiolytic drugs. Clonidine has been suggested to possess anxiolytic activity (Hoehn-Saric et al, 1981) but the present results suggest that this would be seen only over a narrow dose range. Some patients have indeed been shown to respond to clonidine with an increase in anxiety

(Hoehn-Saric et al, 1981) and it is suggested that this may be due to its α_1-adrenoceptor effect. Prazosin has also been shown to possess "anxiolytic-like" activity - it increased punished drinking in a conditioned suppression of drinking test (Gardner and Piper, 1982).

The α_1-adrenoceptor agonist and the α_2-adrenoceptor antagonists resembled the putative anxiogenic agents ACTH and picrotoxin. Both yohimbine and piperoxone have been reported to produce anxiety in man (Redmond, 1977; Holmberg and Gershon, 1961; Goldenberg, 1947) and phenylephrine increased the acoustic startle amplitude in rats which would be abolished by WB4101 (Davis and Astrachan, 1981), a selective α_1-adrenoceptor antagonist (Drew 1982).

In the current experiments quipazine, a 5-HT receptor agonist (Rodriguez et al, 1973; Barbeau et al, 1980) showed a selective inhibition of open arm entries which was dose related. Sedation was observed at none of the doses used - in fact the animals were hyperreactive. It appears that quipazine exhibited a true "anxiogenic-like" effect since neither total exploratory activity nor enclosed entries were affected.

The selectivity of quipazine for postsynaptic as opposed to presynaptic 5-HT receptors and other systems is well documented. Studies on the antinociceptive action of quipazine suggested that the effect of the drug was due a direct action on postsynaptic 5-HT receptors although a presynaptic component could have also been involved (Samanin et al, 1976). Single cell studies in the rat CNS showed that quipazine had both pre- and postsynaptic 5-HT receptor action, and the post/presynaptic ratio was similar to that of LSD (Blier and de Montigny, 1983). Furthermore, Green et al (1976) demonstrated that quipazine also acts as a weak reversible monoamine oxidase inhibiter both in vivo and in vitro. In rat brain cortical slices preincubated with tritrated NA, quipazine caused an increase in electrically stimulated ^{3}H-overflow (- an effect that was less marked than that on cortical slices preloaded with ^{3}H-5-HT) indicating that quipazine blocks presynaptic inhibitory 5-HT and α-adrenoceptors on monoaminergic neurones of the rat brain cortex (Schlicker and Gothert, 1981).

There is also behavioural evidence suggesting that quipazine possesses some β-adrenergic properties, since the increase in

yohimbine toxicity, antagonism of high dose apomorphine and oxotremorine induced hypothermia were inhbited by d,l,propranolol but not methysergide or d-propranolol (Frances et al, 1980). Quipazine has also been shown to inhibit catalepsy caused by spiroperidol and to counteract reserpine-induced catalepsy suggesting that it may also interact with dopamine receptors (Grabowska et al, 1974b). Hence in order to confirm that the effect of quipazine was due to its action on 5-HT_2 receptors, the effect of quipazine in the presence of the highly selective 5-HT_2 receptor antagonist pirenperone (Colpaert and Janssen, 1983) was investigated. Results showed that 5-HT_2 receptors were involved in the "anxiogenic-like" effect of quipazine.

The effect of the 5-HT_2 receptor antagonists ketanserin and pirenperone (Leysen et al, 1981; Colpaert and Janssen, 1983) were less clear-cut. Pirenperone did not significantly alter the proportion of open arm entries although ketanserin did show an increase in the open to total ratio at 0.05 mg/kg. Total exploratory activity remained unchanged and there was no sedation with either of the antagonists; hence sedation does not appear to be the cause for the inactivity of pirenperone.

In the headtwitch response to 5-HTP, pirenperone exerted behavioural effects that suggested it to be a pure 5-HT antagonist (Colepaert and Janssen, 1983) unlike other putative 5-HT antagonists such as methysergide, pizotifen, metergoline and mianserin which acted complexely as mixed agonists-antagonists. On the other hand, although ketanserin primarily displayed a high binding affinity for 5-HT_2 receptors in comparison to other 5-HT antagonists which either poorly differentiated between 5-HT_1 and 5-HT_2 receptors, showed other primary effects, or were moderately active (Leysen et al, 1981), Fozard (1982) showed that ketanserin also possessed α_1-adrenergic blocking activity. Furthermore, Kalkman et al (1983) demonstrated that the hypotensive activity of ketanserin could be correlated with its affinity for α_1-adrenoceptor and a negative correlation existed between the affinity for 5-HT_2 receptor and the depressor potency. Thus the possibility that the anxiolytic-like effect of ketanserin may be at

least partly due to its α_1-adrenoceptor blocking activity cannot be excluded, since the α_1-adrenoceptor blocking agent prazosin did exhibit an anxiolytic-like effect. The effect of other 5-HT_2 antagonists was not investigated since many of these agents have other actions - for example, Kalkman et al (1983) demonstrated that cyproheptadine, cinanserin methysergide and metergoline also had α_1-adrenolytic activity, the potency of which was important in the hypotensive action of these agents. Furthermore, results from another study suggest that methysergide and cyproheptadine may produce partial agonist effects in addition to their antagonistic action at central 5-HT receptor sites (Colpaert et al, 1979).

In order to investigate whether 5-HT receptors modulated the anxiogenic-like activity of the α_1-adrenoceptor agonists and α_2-adrenoceptor antagonists, the effect of pirenperone on the anxiogenic like activites of phenylephrine and yohimbine was examined. The dose of pirenperone used was that which had previously shown to have blocked the anxiogenic-like effect of quipazine, since because of its action on the quipazine effect, there was reason to suppose that, at least at that dcse, pirenperone possessed 5-HT_2 receptor blocking activity. Moreover, at this dose pirenperone did not affect maze exploration, therefore, any changes in behaviour in these "interaction" experiments would not be due to the action of pirenperone itself on fear-motivated behaviour, but would be due to the presence of pirenperone having a modulatory effect.

The results obtained with phenylephrine in the presence of pirenperone showed that pirenperone failed to modify the anxiogenic-like activity of phenylephrine. This not only suggests that the effect of phenylephrine was not mediated via central 5-HT_2 receptor sites, but also that unlike other 5-HT antagonists, pirenperone does not exhibit α_1-adrenolytic activity at this dose. This effect is comparable to, and in agreement with results from the acoustic startle response where cyproheptadine failed to block the increase in acoustic startle amplitude induced by phenylephrine (Davis and Astrachan, 1981), but was blocked by WB4101, a selective α_1-adrenoceptor blocker (Drew, 1982).

The lack of effect of pirenperone in abolishing the

anxiogenic-like activity of yohimbine also suggest that this effect is not modulated by central 5-HT_2 receptor sites. However, yohimbine has been shown to inhibit 5-HT-induced contraction of large coronary arteries of calf through the blockade of 5-HT_2 receptors (Kaumann, 1983). It appears that at the doses used in these experiments, yohimbine does not exhibit 5-HT_2 blocking activity, since if this was so, then it would be expected that increasing dose of this drug would tend to reverse the anxiogenic-like effect of this drug. In these experiments, such an effect was not observed.

The effect of prazosin on the "anxiogenic-like" activity of quipazine also suggested that this effect involves 5-HT receptor systems, since prazosin failed to modify the effect of quipazine. However, quipazine has also been shown to possess β-adrenergic properties (Frances et al 1980) and therefore the possibility that β-adrenoceptors are involved in the anxiogenic-like action of quipazine cannot be excluded since the use of β-blockers in anxiety is well documented (for review, see Jefferson, 1974) although it is not clear whether the effect of these drugs are central or peripheral. However, it appears that the effect of quipazine is most likely due to its action on 5-HT_2 receptors since pirenperone abolished the anxiogenic-like effect of quipazine.

It thus seems possible from this work that both central noradrenergic and serotonergic systems are involved in fear-motivated behaviour. Further experiments to investigate whether these systems interact in mediating fear and anxiety may be useful in elucidating the mechanisms by which the drugs modulating fear act to produce such effects. A detailed study in the behavioural effects of an α-adrenoceptor ligand, in the presence of a 5-HT receptor ligand and vice-versa, using a wide range of doses for both agents will help to clarify further whether the effect of these drugs are modulated by the interacting neurotransmitter system. It would be interesting to see the effect of specific lesions of various nuclei such as the dorsal raphe and median raphe including the locus coeruleus and also the dorsal bundle, on exploratory activity in the maze - since results in this study have provided evidence that modulating serotonergic and noradrenergic systems of some sort are involved in the expression of fear and anxiety.

Fig 2.1 Effects of anxiolytic and anxiogenic agents on the proportion of open arm entries during maze exploration.

Corresponding * 2p < 0.05 : ** 2p < 0.01 (Mann Whitney 'U' test).
Sedation was measured immediately beforehand in home-cage.

+ Diazepam was administered daily for 10 days. Animals were tested 30 minutes after last injection.

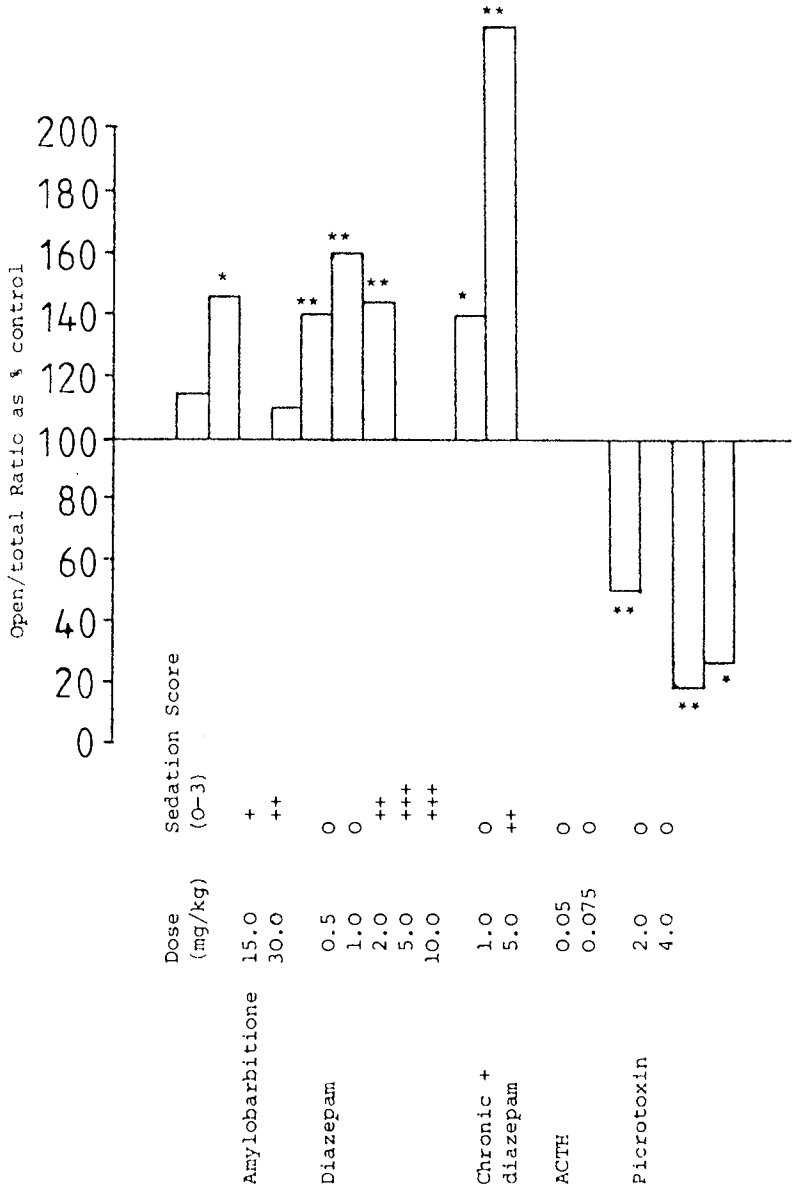


Fig. 2.2 Effects of α_2-adrenoceptor agonists and antagonists on the proportion of open-arm entries during maze exploration.
Statistical comparisons are from raw data:
* 2p ,0.05; ** 2p ,0.01 'Mann Whitney 'U' test)
Sedation was measured immediately beforehand in home cage.

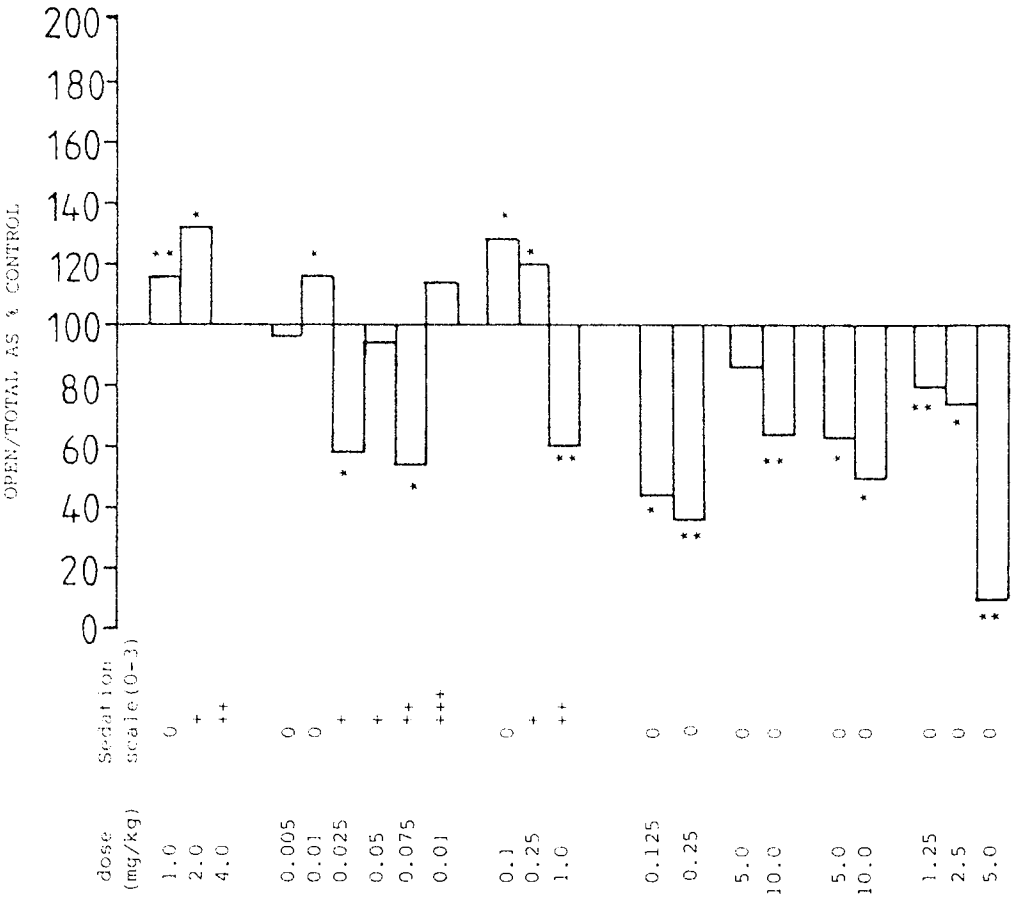


Fig. 2.3 Effects of α_1-adrenoceptor agonists and antagonists on the proportion of open arm entries during maze exploration.
* 2p , 0.05; ** 2p ,0.01 (Mann Whitney 'U' test).
Sedation was measured immediately beforehand in home-cage.

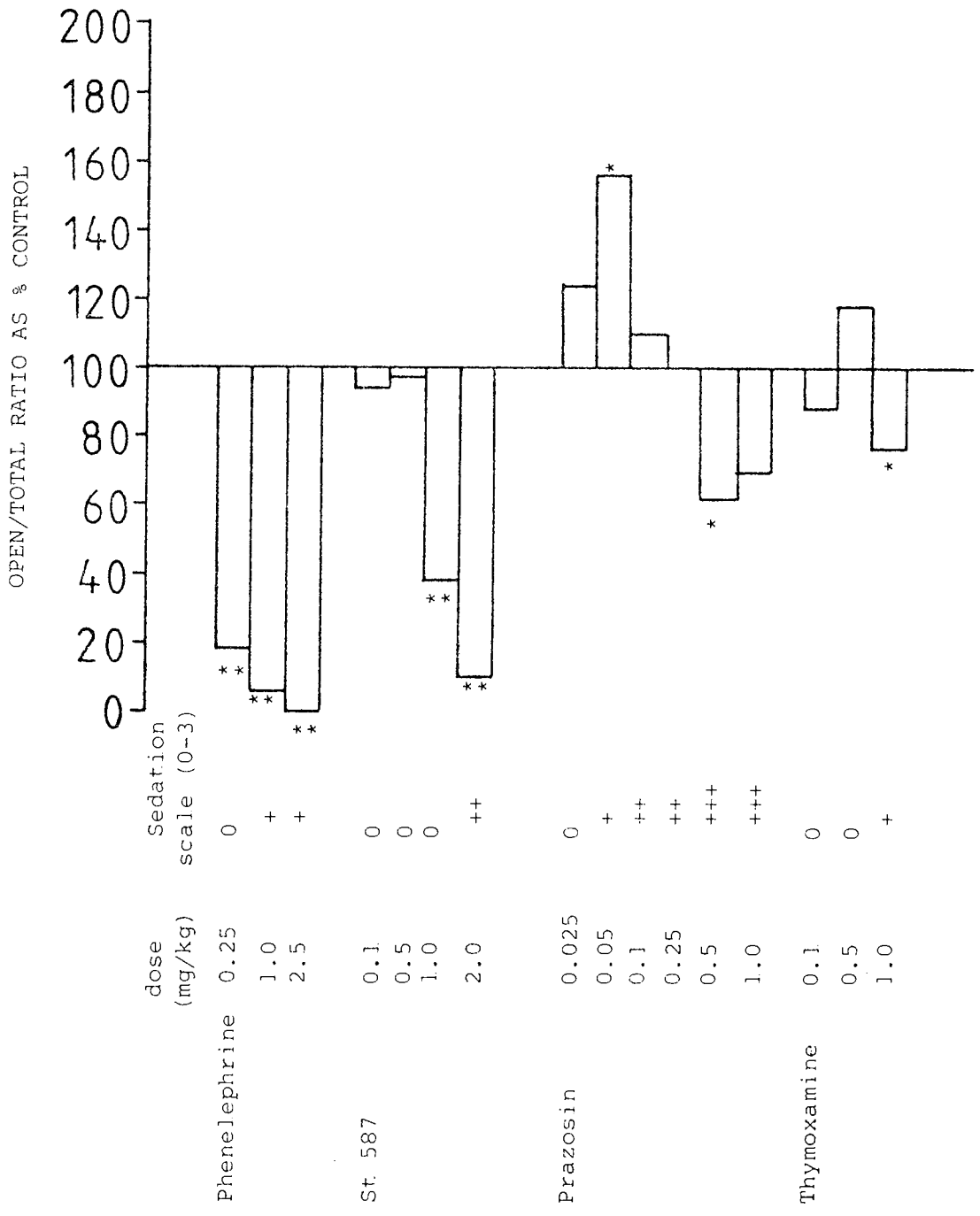
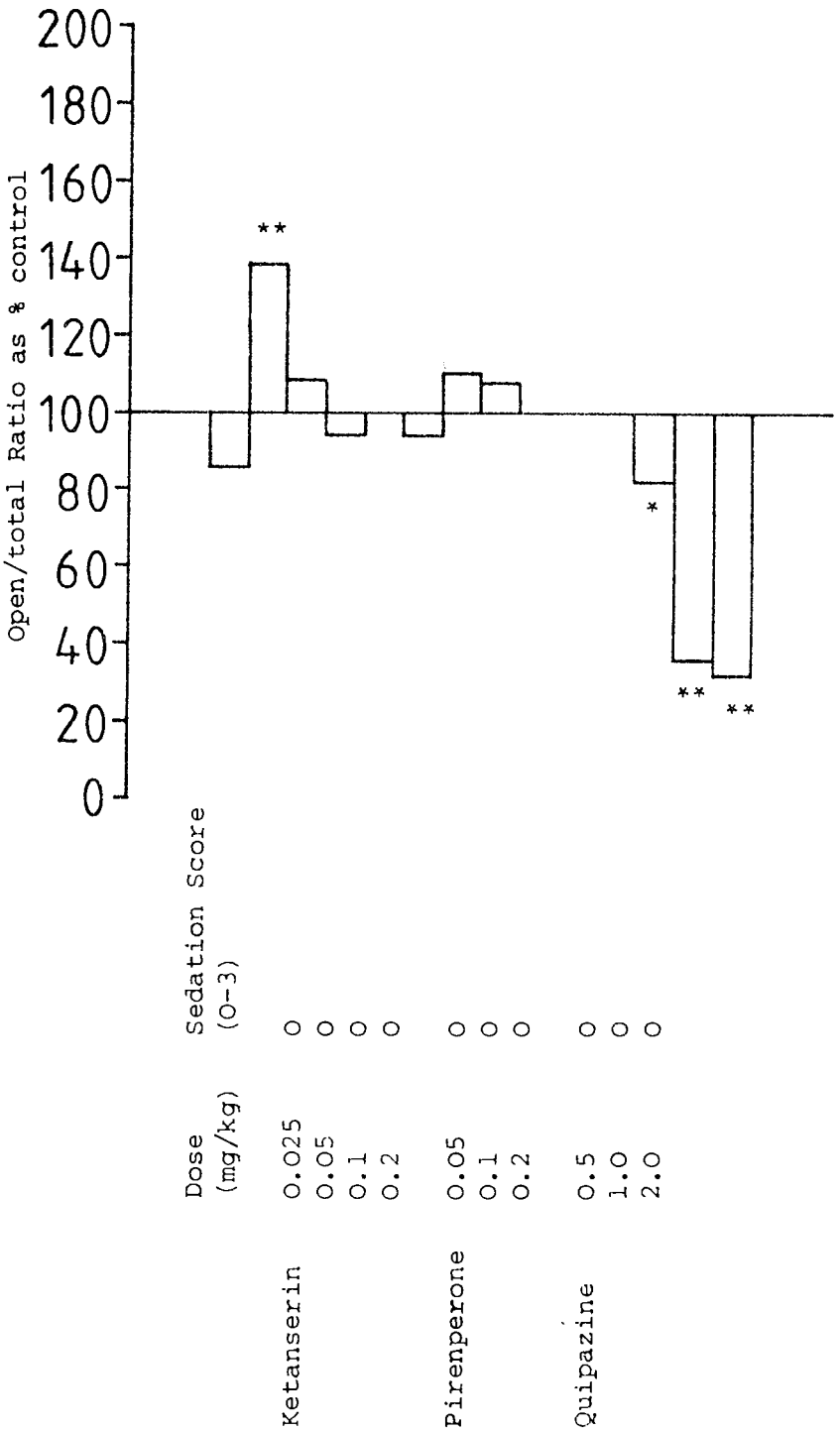


Fig 2.4 Effects of drugs acting at 5-HT_2 receptors on the proportion of open-arm entries during maze exploration

* 2p < 0.05 ** 2p < 0.01 (Mann Whitney 'U' test). Sedation was measured immediately beforehand in homecage.



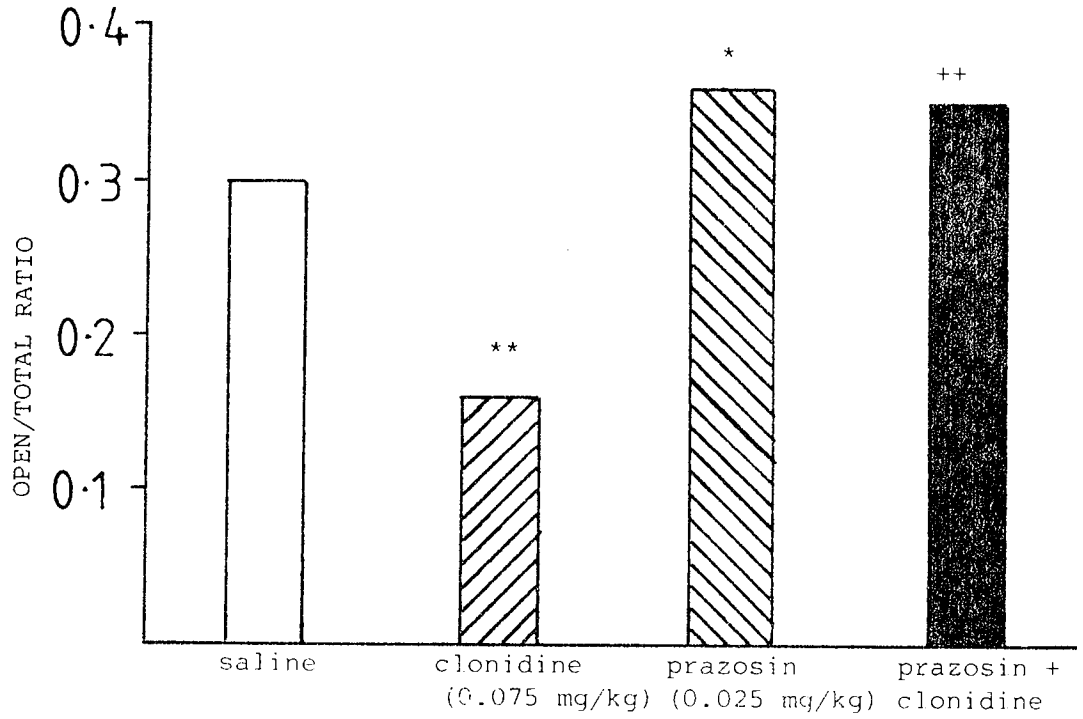


Fig 2.5 Effect of prazosin on the reduction in the proportion of open arm/ total entries induced by high dose clonidine.

Prazosin 0.025 mg/kg injected ip 15 minutes before clonidine 0.075 mg/kg. Remaining groups received prazosin vehicle at this time.

One way analysis of variance (Kruskal Wallis) $p < 0.001$

Significance of differences from saline * $2p < 0.05$; ** $2p < 0.01$

Significance of differences from clonidine - ++ $2p < 0.01$

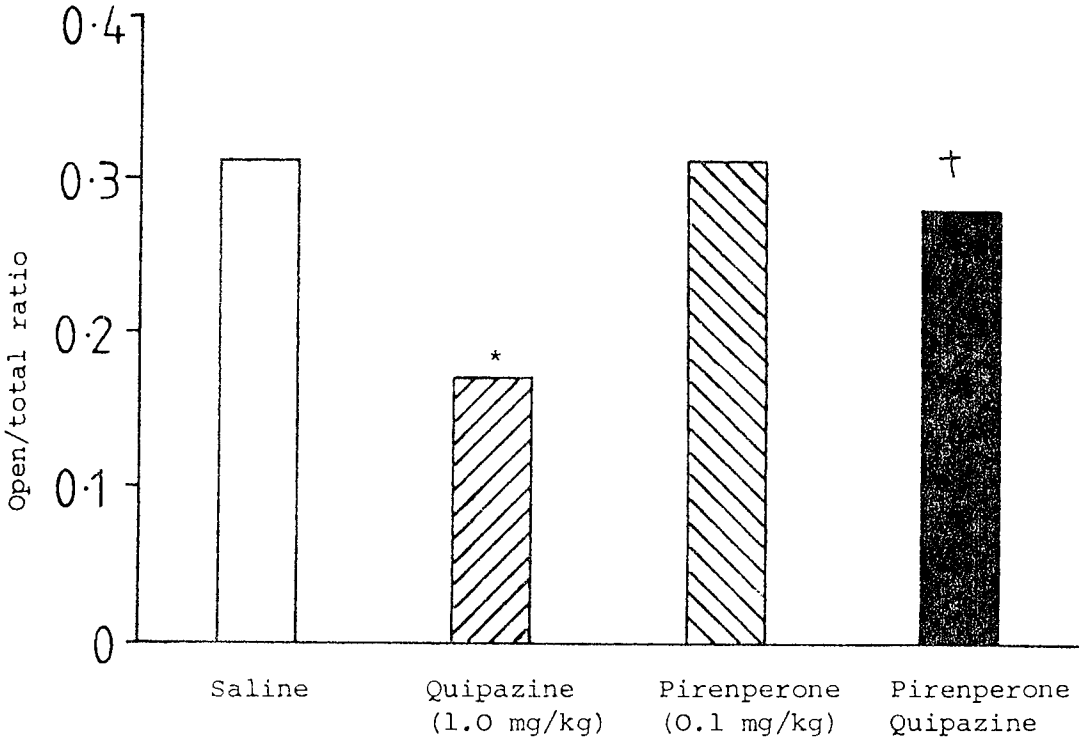
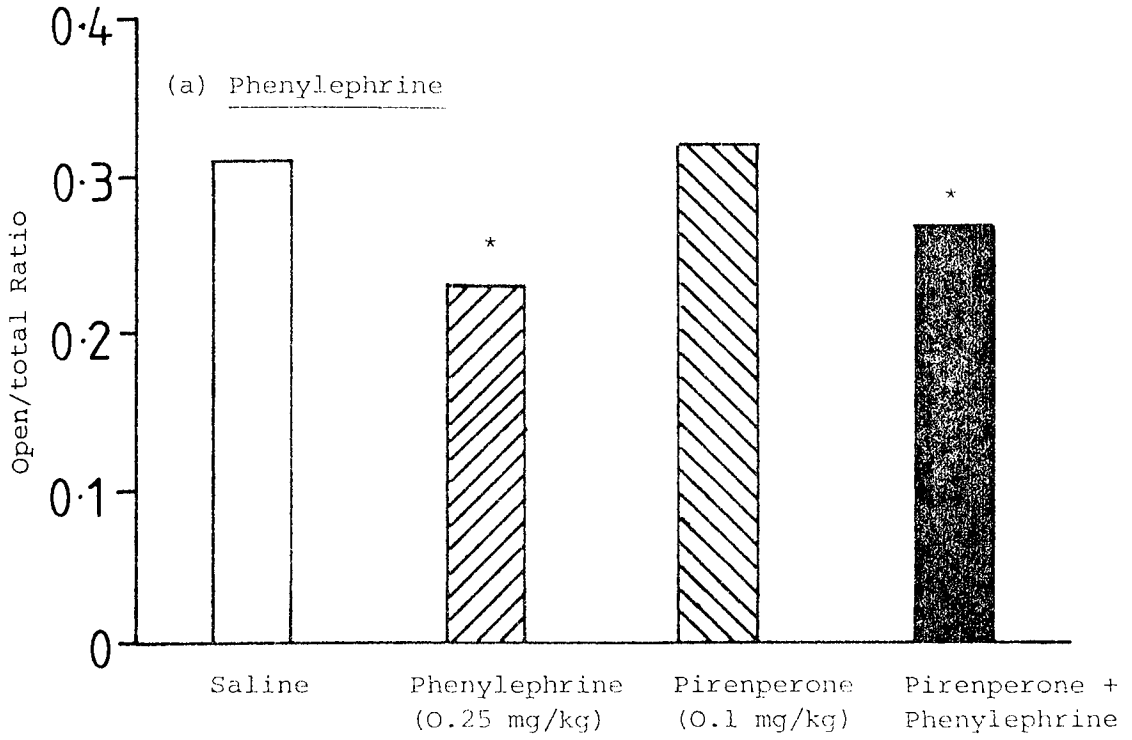


Fig 2.6 Effect of pirenperone on the reduction in the proportion of open arm/total entries induced by quipazine.

Pirenperone 0.1 mg/kg injected up 15 minutes before quipazine 1.0 mg/kg. Remaining groups recieved vehicle at this time.

One way analysis of variance (Kruskal Wallis) $p < 0.001$
Significance of differences from saline * $2p < 0.05$ ** $2p < 0.01$
Significance of difference from quipazine † $2p < 0.05$



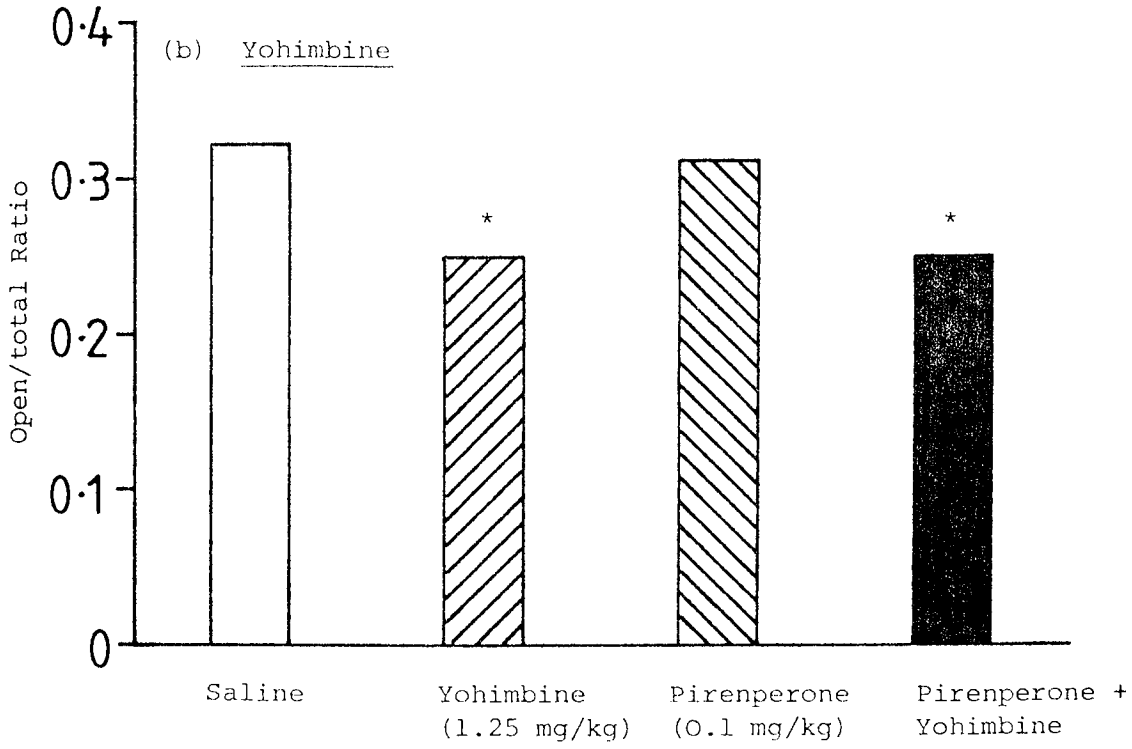
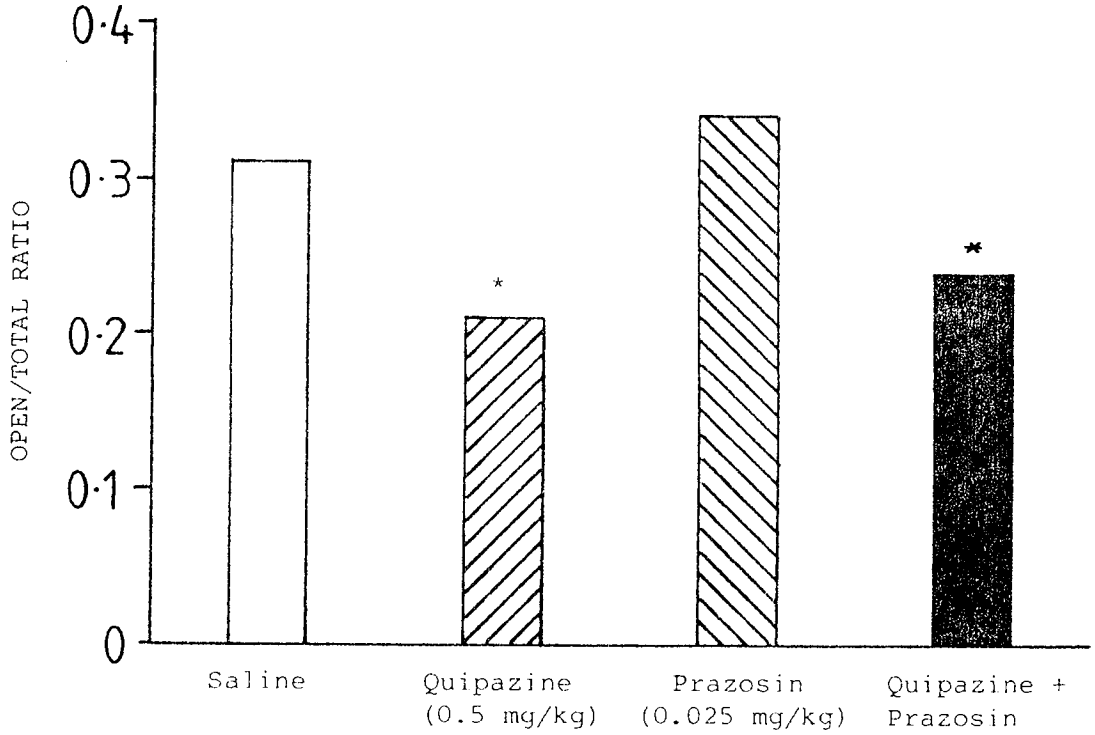


Fig 2.7 Effect of pirenperone on the reduction in the proportion of open arm/total entries induced by the α-adrenoceptor ligands.

* $2p<0.05$ (Mann Whitney 'U' test significance of difference from control)
One way analysis of variance (Kruskal Wallis) $p<0.01$



g 2.8 Effect of prazosin on the reduction in the proportion of open arm/ total entries induced by quipazine.

Prazosin 0.025 mg/kg injected ip 15 minutes before quipazine 0.5 mg/kg Remaining groups received prazosin vehicle at this time.

One way analysis of variance (Kruskal-Wallis) p ,0.001

Significance of differences from saline * 2p ,0.05

Mean number of entries in 10 minutes
(vehicle control values in brackets)

Drug/dose mg/kg		Open		Total		Open/Total	
Amylobarbitone	15.0	3.3 ± 0.6	(3.0 ± 0.6)	10.5 ± 1.8	(10.6 ± 1.7)	.32 ± .02	(.28 ± .01)
	30.0	4.2 ± 1.6	(3.0 ± 0.6)	9.3 ± 3.3	(10.6 ± 1.7)	.41 ± .05	(.28 ± .01)*
Diazepam	0.5	5.8 ± 1.9	(5.2 ± 1.3)	18.6 ± 5.7	(17.3 ± 4.3)	.33 ± .04	(.30 ± .01)
	1.0	4.8 ± 1.5	(4.4 ± 1.5)	9.8 ± 2.3	(12.4 ± 3.8)	.49 ± .03	(.35 ± .01)**
	2.0	9.0 ± 1.5	(3.6 ± 0.5)**	16.5 ± 1.7	(10.8 ± 0.7)**	.54 ± .03	(.34 ± .03)**
	5.0	4.2 ± 0.9	(3.8 ± 0.4)	9.2 ± 1.5	(12.4 ± 1.4)*	.45 ± .03	(.31 ± .01)**
	10.0	0.0 ± 0.0	(2.7 ± 0.5)**	0.0 ± 0.0	(10.0 ± 1.4)**	±	(.27 ± .02)
Chronic diazepam+	1.0	5.8 ± 0.7	(3.4 ± 1.0)	13.8 ± 1.0	(11.0 ± 2.3)	0.42 ± 0.03	(0.3 ± .05)*
	5.0	6.6 ± 1.5	(2.4 ± 0.5)	12.0 ± 2.0	(10.4 ± 0.8)	0.54 ± .04	(.23 ± .03)**
ACTH	0.05	6.6 ± 2.9	(6.0 ± 1.6)	17.6 ± 6.6	(16.0 ± 3.7)	.36 ± 0.2	(.37 ± .02)
	0.075	1.8 ± 0.8	(6.0 ± 1.6)**	9.2 ± 3.9	(16.0 ± 3.7)*	.19 ± .02	(.32 ± .02)**
Picrotoxin	2.0	0.5 ± 0.2	(2.2 ± 0.2)**	5.7 ± 1.2	(7.6 ± 0.7)	.06 ± .03	(.32 ± .06)**
	4.0	0.2 ± 0.2	(2.2 ± 0.2)**	3.0 ± 0.4	(7.6 ± 0.7)	.08 ± .09	(.32 ± .06)*

Table 2.1 Effects of anxiolytic and anxiogenic agents on maze exploration.

Values are expressed as mean ± standard error.

Significance of differences from corresponding vehicle control

* $2p<0.05$ ** $2p<0.01$

+ Diazepam given daily for 10 days - tested 30 mins after last injection.

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

DRUG/dose (mg/kg)		Open		Total		Open/Total	
Idazoxan	0.125	1.0 ± 0.6	(2.7 ± 0.4)	6.2 ± 0.9	(9.0 ± 1.0)*	.13 ± 0.7	(.29 ± .01)*
	0.25	0.7 ± 0.4	(2.7 ± 0.4)	4.7 ± 1.9	(9.0 ± 1.0)**	.10 ± 0.5	(.29 ± .01)**
Piperoxane	5.0	4.8 ± 1.5	(6.6 ± 1.3)	15.4 ± 2.6	(18.0 ± 2.9)	.31 ± 0.3	(.36 ± .02)
	10.0	2.4 ± 2.2	(4.4 ± 1.5)*	9.6 ± 3.8	(12.4 ± 3.8)	.22 ± .06	(.35 ± .01)**
RS 21361	5.0	3.8 ± 2.9	(6.3 ± 2.1)	13.5 ± 8.3	(15.3 ± 2.2)	.26 ± .05	(.31 ± .05)*
	10.0	3.2 ± 3.4	(6.3 ± 2.7)	16.3 ± 7.4	(19.2 ± 6.9)	.16 ± .04	(.32 ± .02)*
Yohimbine	1.25	4.4 ± 0.9	(6.8 ± 0.5)**	15.8 ±3.5	(19.6 ± 1.5)	.28 ± .02	(.135 ± .01)*
	2.5	2.0 ± 1.0	(6.8 ± 0.5)**	8.2 ± 4.9	(19.6 ± 1.5)**	.26 ± .02	(.35 ± .01)**
	5.0	0.2 ± 0.5	(6.8 ± 0.5)**	3.2 ± 1.1	(19.6 ± 1.5)**	.04 ± .05	(.35 ± .01)**
Azepexole	1.0	5.7 ± 2.2	(6.5 ± 2.1	14.7 ± 4.5	(19.0 ± 4.9)	.40 ± .01	(.34 ± 0.1)**
	2.0	6.7 ± 2.7	(4.8 ± 0.8)	17.3 ± 5.4	(17.1 ± 2.6)	.38 ± .02	(.29 ± .02)*
	4.0	1.2 ± 0.2	(3.8 ± 0.4)	3.8 ± 0.4	(12.4 ± 1.4)*	.31 ± .03	(.31 ± .01)
Clonidine	0.005	3.8 ± 2.3	(4.8 ± 1.2)	13.8 ± 3.6	(17.0 ± 2.4)	.27 ± .92	(.28 ± .01)
	0.01	4.2 ± 1.9	(6.0 ± 1.3)*	11.3 ± 2.9	(18.6 ± 3.7)*	.37 ± .07	(.32 ± .02)*
	0.025	1.4 ± 1.1	(5.5 ± 1.3)**	6.2 ± 1.9	(18.9 ± 1.9)**	.21 ± .04	(.36 ± .02)*
	0.05	3.0 ± 1.7	(5.7 ± 1.8)*	9.2 ± 4.1	(16.0 ± 4.3)*	.33 ± .04	(.35 ± .01)
	0.075	1.0 ± 0.8	(5.7 ± 1.8)*	5.7 ± 1.8	(16.0 ± 4.3)**	.19 ± .05	(.35 ± .01)*
	0.1	1.0 ± 0.0	(4.4 ± 1.5)*	2.6 ± 0.5	(12.4 ± 3.8)**	.40 ± .05	(.35 ± .01)
Guanabenz	0.1	6.8 ± 2.1	(4.7 ± 1.8	17.3 ± 5.9	(14.8 ± 4.9)	.40 ± .01	(.31 ± .01)**
	0.25	4.2 ± 1.5	(6.0 ± 2.0)	11.2 ± 4.6	(17.8 ± 3.8)*	.39 ± .02	(.32 ± .04)*
	1.0	0.8 ± 0.4	(5.8 ± 1.0)**	4.0 ± 0.9	(16.8 ± 2.5)*	.21 ± .05	(.35 ± .01)**

TABLE 2.2 Effects of α-adrenoceptor ligands on maze exploration.

Values are expressed as mean ± standard error.

Significance of differences from corresponding vehicle control.
* $2p < 0.05$; ** $2p < 0.01$

DRUG/dose (mg/kg)		Mean number of entries in 10 minutes (Vehicle control values in brackets) Open		Total		Open/Total	
Phenylephrine	0.25	0.5 ± 0.3	(3.0 ± 0.3)**	8.7 ± 1.6	(13.5 ± 1.0)	.04 ± .03	(.22 ± .02)**
	1.0	0.2 ± 0.2	(4.6 ± 0.5)**	5.0 ± 1.3	(15.8 ± 1.5)**	.02 ± .02	(.29 ± .01)**
	2.5	0.0 ± 0.0	(2.3 ± 0.5)**	1.2 ± 0.2	(9.3 ± 1.5)**	.0 ± .0	(.24 ± .01)**
St 587	0.1	5.0 ± 0.9	(4.3 ± 0.9)	15.1 ± 1.4	(12.6 ± 0.2)	.32 ± .04	(.34 ± .04)
	0.5	3.3 ± 1.0	(4.6 ± 0.8)	11.3 ± 2.3	(14.3 ± 2.1)	.29 ± .06	(.30 ± .02)
	1.0	1.5 ± 0.9	(4.6 ± 0.8)**	8.1 ± 2.8	(14.3 ± 2.1)**	.12 ± .06	(.32 ± .02)**
	2.0	0.2 ± 0.2	(3.2 ± 0.8)**	3.2 ± 0.6	(10.1 ± 1.5)**	.03 ± .04	(.27 ± .05)**
Prazosin	0.025	5.2 ± 1.0	(4.3 ± 0.9)	12.3 ± 1.9	(12.6 ± 2.3)	.42 ± .04	(.34 ± .03)
	0.05	3.8 ± 0.7	(2.7 ± 0.5)	9.0 ± 1.1	(10.0 ± 1.4)	.42 ± .06	(.27 ± .02)*
	0.1	1.2 ± 0.2	(1.4 ± 0.2)	10.6 ± 1.6	(12.5 ± 1.4)	.11 ± .02	(.10 ± .01)
	0.25	2.2 ± 0.2	(3.0 ± 0.3)	10.0 ± 1.1	(13.5 ± 1.0)	.22 ± .03	(.22 ± .02)
	0.5	2.2 ± 0.9	(4.6 ± 0.5)*	9.7 ± 1.9	(15.8 ± 1.5)**	.18 ± .05	(.29 ± .01)*
	1.0	1.0 ± 0.4	(2.3 ± 0.5)*	4.7 ± 1.1	(9.3 ± 1.5)**	.17 ± .06	(.24 ± .01)
Thymoxamine	0.1	3.2 ± 0.6	(3.2 ± 0.8)	13.3 ± 1.1	(10.1 ±1.5)	.24 ± .03	(.27 ± .05)
	0.5	2.5 ± 0.5	(3.3 ± 0.5)	6.8 ± 0.8	(10.6 ± 1.3)*	.37 ± .02	(.31 ± .02)
	1.0	1.8 ± 0.3	(3.3 ± 0.5)	7.5 ± 1.0	(10.6 ± 1.3)	.24 ± .02	(.31 ± .02)*

TABLE 2.3 Effects of α-adrenoceptor ligands on maze exploration.

Values are expressed as mean ± standard error.

Significance of differences from corresponding vehicle control.

* $2p < 0.05$ ** $2p < 0.01$

Mean number of entries in 10 minutes
(Saline control values in brackets)

DRUG/dose (mg/kg)		Open		Total		Open/Total	
Ketanserin	0.025	1.5 ± 0.5	(2.5 ± 0.2)	7.3 ± 1.5	(10.7 ± 0.6)	.20 ± .03	(.23 ± .01)
	0.05	2.0 ± 0.5	(2.5 ± 0.2)	6.3 ± 1.4	(10.7 ± 0.6)	.32 ± .03	(.23 ± .01)**
	0.1	5.8 ± 0.5	(4.3 ± 0.6)	19.0 ± 1.0	(14.8 ± 1.8)	.31 ± .02	(.29 ± .01)
	0.2	3.3 ± 0.2	(4.3 ± 0.6)	12.7 ± 1.2	(14.8 ± 1.8)	.27 ± .02	(.29 ± .01)
Pirenperone	0.05	2.2 ± 0.6	(2.5 ± 0.8)	7.7 ± 1.2	(8.0 ± 1.0)	.27 ± .04	(.29 ± .01)
	0.1	4.2 ± 0.8	(5.3 ± 0.6)	13.7 ± 2.6	(18.7 ± 2.2)	.32 ± .04	(.29 ± .02)
	0.2	3.3 ± 0.8	(2.5 ± 0.8)	10.7 ± 3.2	(8.0 ± 1.0)	.31 ± .02	(.29 ± .01)
Quipazine	0.5	4.2 ± 0.7	(5.3 ± 0.6)	17.7 ± 3.3	(18.7 ± 2.2)	.24 ± .03	(.29 ± .02)*
	1.0	1.6 ± 0.9	(4.8 ± 2.3)	13.8 ± 3.6	(17.0 ± 2.4)	.10 ± .04	(.28 ± .01)**
	2.0	2.0 ± 1.3	(4.8 ± 2.3)	14.8 ± 4.5	(17.0 ± 2.4)	.09 ± .03	(.28 ± .01)**

TABLE 2.4 Effect of drugs acting at 5-HT receptors on maze exploration.
Values are expressed as mean ± standard error.
Significance of differences from corresponding vehicle control.
*2p <0.05 ** 2p <0.01

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

Drug/dose mg/kg		Open		Total		Open/Total	
Clonidine	0.075	1.2 ± 0.5	(3.6 ± 0.3)*	6.8 ± 1.2	(12.2 ± 1.1)*	.16 ± .05	(.3 ± .01)*
Prazosin	0.025	6.2 ± 0.7	(3.6 ± 0.3)*	17.0 ± 1.4	(12.2 ± 1.1)	.36 ± .02	(.3 ± .01)*
Clonidine + Prazosin		1.4 ± 0.5	(3.6 ± 0.3)	4.0 ± 0.9	(12.2 ± 1.1)*	.35 ± .06	(.3 ± .01)
Quipazine	1.0	1.6 ± 0.7	(5.2 ± 0.7)*	8.4 ± 1.9	(16.4 ± 1.9)	.17 ± .06	(.31 ±.01)*
Pirenperone	0.1	3.6 ± 0.3		11.8 ± 1.0		.31 ± .02	
Quipazine + Pirenperone		3.2 ± 0.4		11.2 ± 1.0		.28 ± .02	
Phenylephrine	0.25	2.2 ± 0.9	(5.2 ±1.4)	9.4 ± 1.5	(14.2 ± 2.1)	.23 ± .02	(.31 ± .01)*
Pirenperone	0.1	5.0 ± 1.0		15.4 ± 1.2		.32 ±.02	
Phenylephrine + Pirenperone		2.4 ± 0.8		9.8 ± 0.8		.29 ± .02	*
Yohimbine	1.25	3.4 ± 0.6	(4.8 ± 0.7)	13.4 ± 1.7	(15.0± 1.8)	.25 ± .02	(.32 ± .06)*
Pirenperone	0.1	3.4 ± 0.5		10.8 ± 1.0		.31 ± .02	
Yohimbine + Pirenperone		2.4 ± 0.3		9.2 ± 0.4		.25 ± .03	*
Quipazine	0.5	2.6 ± 0.8	(3.6 ± 0.4)	11.4 ± 2.3	(11.6 ± 1.1)	.21 ± .03	(.31 ± .01)*
Prazosin	0.025	4.2 ± 0.7		12.2 ± 1.9		.34 ± .01	(.31 ± .01)
Quipazine + Prazosin		2.6 ± 0.3		11.0 ± 1.3		.24 ± .02	(.31 ± .01)*

Table 2.5 Effects of behavioural interaction between α-adrenoceptor and 5-HT receptor ligands on maze exploration.

*p < 0.05.

CHAPTER 3

THE EFFECT OF α-ADRENOCEPTOR AND 5-HT RECEPTOR LIGANDS ON OPERANT BEHAVIOUR (GELLER-SEIFTER CONFLICT).

Introduction

The Geller-Seifter conflict procedure (Geller and Seifter, 1960) is perhaps the most widely accepted test that has been used to quantify the antianxiety action of drugs (see Cook and Sepinwall, 1975). In this study, the effects of α-adrenoceptor and 5-HT receptor ligands were examined on the Geller-Seifter model of anxiety, since of all the operant behavioural models, this appears to be most widely used to ascertain the anxiolytic and anxiogenic profiles of drugs (see Cook and Sepinwall, 1975). An evaluation of operant conflict as an anxiety model is to be found in the Introduction (Section 10).

These experiments were also performed in an attempt to validate the maze-exploration model (Chapter 2) and to confirm the effects observed in that study. For this purpose the effects of anxiolytic and putative anxiogenic agents were also examined so that a reasonable comparison could be made of the two behavioural tests.

During the training of the animals, it was observed that rates of both punished and unpunished responding tended to increase with time, hence results show slight fluctuations in the control baseline rates of responding depending on the time when the drug was administerd after stabilised rates had been achieved.

1. The Schedule.

Throughout all operant conflict experiments, only the left lever was reinforced. This schedule consisted of a variable interval (VI-2 min) for 10 mins followed by continuous reinforcement with response-contingent footshock for 3 mins (CRFs) which was signalled by the left cue light. The schedule was repeated 4 times, (a total running time for 52 mins). The doses of drugs used were chosen from the results obtained in the maze study (Chapter 2), and sedation was assessed as present or absent by observing the animals in the boxes 30 mins after injection.

Initially, it was attempted to titrate footshock intensity to produce 50% suppression during punished periods. However, this was not successful for all rats since increasing the footshock intensity suppressed punished responding intensely. Two groups of rats were used, and as a result, for Group 1 rats, footshock intensity was titrated to produce >75% suppression, and <25% in Group 2 rats, during the punished periods. Drugs predicted to have an anxiolytic-like activity (from maze study, Chapter 2) were investigated in Group 1 rats, whereas Group 2 rats were used to examine agents that may have an anxiogenic-like effect.

Results are represented as the sum of the total number of lever presses during all 4 periods since this would give a good representation of the overall picture of the effect of drugs on conflict behaviour, and would also compensate for the slight fluctuations in responding in each animal within the 4 periods. Results are presented in order of experimentation thus for any particular drug rats were experienced with those previously mentioned.

2. Group 1 rats

2.1 The effect of diazepam on drug-naive animals.

On the first exposure to diazepam 2.5 mg/kg there was no effect on punished or unpunished behaviour (Fig.3.1). Diazepam was therefore given for the following 3 days to see whether an anxiolytic-effect could be observed. Subsequent administration of diazepam on days 2, 3 and 4 increased punished responding, but unpunished behaviour remained unchanged (Fig.3.1). Sedation was observed on all days of diazepam administration.

In order to confirm that the anxiolytic effect of diazepam could be observed on acute administration after a previous diazepam experience, animals were injected with diazepam 7 days later. Results show that diazepam significantly increased punished responding, but VI responding remained unchanged. (Fig.3.1).

2.2 The effect of clonidine on diazepam-experienced animals.

The first exposure to clonidine (0.1 mg/kg) significantly suppressed unpunished responding, but CRFs responding remained unchanged (Fig.3.2). Saline was injected the following day and this was followed by clonidine (0.025 mg/kg) for 3 consecutive days.

On 2nd exposure to clonidine, punished responding remained unchanged but the 3rd and 4th exposures to clonidine on subsequent days resulted in significant increases in punished responding (Fig.3.2). Unpunished behaviour was significantly reduced on the 2nd exposure, but consequently on the 3rd and 4th exposures to clonidine, the suppression in unpunished responding progressively disappeared (Fig.3.2). However, animals appeared sedated on all days of drug administration.

2.3 The effect of α_2-adrenoceptor agonists.

a. Azepexole.

Azepexole (1.0 and 2.0 mg/kg) increased punished responding although this was not dose-related. Unpunished responding remained unchanged at both doses (Fig.3.3). Sedation was absent at both doses.

b. Clonidine.

An inverted 'U' shaped dose-response relationship was observed with clonidine. Lower doses (0.0125 - 0.025 mg/kg) significantly increased punished responding, whereas at the highest dose, punished responding showed a slight decrease (Fig.3.3).

All doses except 0.01 and 0.0125 mg/kg, significantly reduced unpunished responding - an effect that appeared to be dose-related (Fig.3.3). Sedation was present at doses of 0.025 mg/kg and above.

c. Guanabenz.

0.05 mg/kg guanabenz caused a slight but significant increase in punished responding (Fig.3.3). At all doses (0.025 - 0.5 mg/kg) guanabenz had no effect on punished behaviour.

At lower doses, unpunished responding was unchanged but at higher doses (0.25 and 0.5 mg/kg) it was significantly reduced

(Fig.3.3). Sedation was present at 0.25 and 0.5 mg/kg guanabenz.

The reason for a high punished baseline rate for 0.025 mg/kg was due to the fact that this dose was given after animals had been yohimbine experienced (see Section 2.6) when the rate of responding was increased. It was desirable to look at the effect of this dose on punished responding since 0.05 mg/kg had shown an anticonflict activity.

2.4 The effect of α_1-adrenoceptor antagonists.

It was found that of all the drugs used in this study, prazosin was unique in showing an effect in the third and fourth periods only, and the total number of lever presses during all 4 periods did not show an adequate presentation of the effect of prazosin. For this reason, the effects of prazosin and thymoxamine on the 3rd VI and CRFs periods (Fig.3.4) as well as during all 4 periods (Fig.3.5) have been shown.

a. Prazosin.

A significant anticonflict activity appeared on the first dose of prazosin given (0.05 mg/kg), as well as the slightly higher dose (0.1 mg/kg). VI responding remained unchanged in the third period with all doses of prazosin (0.025 - 0.25 mg/kg) (Fig.3.4).

At the highest dose of prazosin (0.25 mg/kg) CRFs responding was significantly depressed (Fig.3.5).

The control rate of responding was higher with 0.025 mg/kg during the punished period because this dose was also examined after animals were yohimbine experienced (see 2.3.c).

Animals appeared sedated with 0.1 and 0.25 mg/kg prazosin.

b. Thymoxamine.

0.5 and 1.0 mg/kg thymoxamine did not affect punished or unpunished responding during the third period. Total number of lever presses during all 4 CRFs and VI periods also remained unchanged (Figs.3.4 and 3.5). There was no sedation observed with this drug, and 0.5 mg/kg thymoxamine was also examined after animals were exposed to yohimbine, hence the high baseline for punished responses.

2.5 The effect of 5-HT_2 receptor antagonists.

a. Ketanserin.

0.1 mg/kg behaviour increased punished responding (Fig.3.6) although there was no effect on VI-responding. Sedation was absent.

b. Pirenperone.

Pirenperone (0.05 - 0.2 mg/kg) had no effect on the total number of lever presses during all 4 CRFs periods (Fig.3.6). Unpunished responding also remained unchanged at all doses except 0.2 mg/kg where it was significantly reduced. Punished responding remained unchanged at both doses of pirenperone in all 4 individual periods.

2.6 The effect of yohimbine on diazepam-experienced animals.

For these experiments, the baseline for punished responding was altered to between 40-60% suppression, since the effect of yohimbine on punished responding would not be detected if suppression was low, because in the Group 2 rats, yohimbine caused an intense suppression of punished behaviour (Section 3.1).

Owing to the fact that it required seven exposures of yohimbine to drug-naive animals in order to stabilise the response obtained with yohimbine (see 3.1), it was desirable to investigate whether "diazepam-experienced" animals showed similar effects. Results showed that the first exposure to yohimbine induced a suppression in punished responding on drug day and day + 1 (Fig.3.7) whereas unpunished responding remained unchanged on all days (Table 3.1).

One week later, animals were exposed to yohimbine 2.5 mg/kg again. On the second exposure to yohimbine, similar effects were observed as with the first exposure. Punished responding was decreased on drug day and day +1, whereas unpunished responding was unchanged (Fig.3.7, Table 3.1).

3. Group 2 rats.

3.1 The effect of yohimbine on drug naive animals.

On the first exposure to yohimbine (2.5 mg/kg) punished responding was intensely suppressed (maximum on drug day +2) and took two further days to return to pre-injection levels (Fig.3.8)

VI responding was also significantly depressed on the first exposure (Table 3.2). Since yohimbine caused such an intense suppression of both punished and unpunished responding on the first exposure to the drug it was desired to look at the effect of subsequent exposures to yohimbine. Hence, the effect of 6 other exposures were investigated; 2.5 mg/kg ychimbine was injected at weekly intervals and the effects of this were observed up to day +6.

On the second exposure to yohimbine, punished responding was again intensely suppressed (maximum on day +2) and took 3 days to return to baseline (Fig.3.8). VI responding was also suppressed; this took 5 days to return to pre-injection levels (Table 3.2). On the third exposure to yohimbine, unpunished responding remained unaffected even on drug day, but punished responding was depressed on drug day and drug day +1. The fourth exposure to yohimbine caused punished responding to be suppressed up to day +2 whereas VI responding remained unchanged (Fig.3.8; Table 3.2). The fifth exposure to yohimbine decreased both punished and unpunished responding on day +1 but returned to baseline on drug day +2 (Fig.3.8; Table 3.2). The sixth exposure to yohimbine did not affect unpunished responding, but punished responding was still depressed 2 days after yohimbine administration (Fig.3.8). On the seventh exposure to yohimbine, unpunished responding remained unaffected, but punished responding was only suppressed on drug day (Fig.3.8).

3.2 The effect of α_2-adrenoceptor antagonists.

a. Idazoxan.

Idazoxan (0.125 and 0.25 mg/kg) did not alter unpunished responding but punished behaviour was significantly suppressed at both doses (Fig.3.9).

The baseline rate of responding was much higher than that seen with several exposures to yohimbine and idazoxan. This was because following yohimbine and idazoxan animals were reinforced four times a week for 5 weeks but no drugs were administered. This resulted in an increase in both punished and unpunished responding. Therefore in the following experiments, baseline

values are higher than those observed with yohimbine and idazoxan.

b. Piperoxane.

Punished and unpunished responding were both significantly decreased with piperoxane (5.0 mg/kg) (Fig.3.9) although the effect of VI responding was less marked.

Piperoxane also induced a "carry-over" effect. Total lever presses during both VI and CRFs periods were reduced up to day 3 (Fig.3.10). On day 4, responding returned to pre-injection levels.

c. RS 21361.

Punished responding was decreased with RS 21361 (5.0 and 10.0 mg/kg) - an effect that appeared to be dose-dependent (Fig.3.9). Examining the individual periods revealed that maximum effects occurred in the second VI and CRFs periods. Unpunished responding remained unaltered at both doses and also there was no "carry-over" effect. Sedation was absent at both doses.

d. Yohimbine.

Yohimbine (1.25 and 2.5 mg/kg) decreased punished responding (Fig.319) but responding during VI periods remained unchanged at 1.25 mg/kg and decreased at 5.0 mg/kg (Fig.3.9).

A "carry-over" effect was observed at 5.0 mg/kg (Fig.3.10); this effect was less marked than that observed with piperoxane, in that VI-responding returned to baseline on day +1, but CRFs remained significantly suppressed until day +2.

Sedation was absent at both doses of yohimbine.

3.3 The effect of α_1-adrenoceptor agonists.

a. Phenylephrine

Phenylephrine (0.25 - 2.5 mg/kg) reduced responding during both the CRFs and VI periods although VI responding was suppressed to a lesser extent (Fig.3.5). The effects of phenylephrine were partly dose-related, and the lower doses (0.25 and 0.5 mg/kg) caused a "carry-over" effect.

Both doses of phenylephrine (0.25 and 0.5 mg/kg) did not have an effect of VI responding after drug day; however, punished responding was still depressed on drug day +1 with 0.25 mg/kg, and up to drug day +4 with the 0.5 mg/kg dose (Fig.3.10).

At the higher doses of phenylephrine (1.0 and 2.5 mg/kg) sedation was moderate, although animals appeared to be hyperreactive at all doses.

b. St 587.

St 587 1.0 mg/kg showed a significant suppression in punished responding (Fig.3.5) although VI responding remained unchanged. Sedation was absent at this dose of St 587, and there was no "carry-over" effect.

3.4 The effect of quipazine.

Quipazine (1.0 - 4.0 mg/kg) reduced punished responding in a dose-related manner (Fig.3.6). VI-responding was unchanged at 1.0 mg/kg but at 2.0 and 4.0 mg/kg quipazine, responding during this period was also significantly reduced (Fig.3.6). However, the effect of higher doses on unpunished responding was of a smaller magnitude than that of punished responding (Fig.3.6). There was no sedation at any of the doses examined.

3.5 The effect of putative anxiogenics.

a. ACTH.

ACTH (0.075 mg/kg) caused a slight but significant decrease in punished responding although the total number of lever presses during the VI periods remained unchanged (Fig.3.9). There was no sedation observed at this dose of ACTH.

b. Picrotoxin.

The first two animals were administered a dose of 2.0 mg/kg immediately before placement in the box, since this dose was established in the maze-study (Chapter 2) to be a sub-convulsive anxiogenic dose. Eight to nine minutes after picrotoxin injection, both animals started to convulse. The convulsions lasted 3 - 4 mins, after which the animals died.

The remaining animals were subsequently injected with only 0.5 mg/kg picrotoxin. Hence statistical evaluation with picrotoxin where n = 3 was not possible. Individual results for the 3 animals showed that the lever presses during punished periods decreased for one rat, but remained unchanged for the other two (Fig.3.9).

Discussion

The anxiolytic effect of diazepam was not observed on the first exposure to the drug, but required two further exposures for the maximum effect on anticonflict activity to be achieved. Margules and Stein (1968) reported a similar effect with oxazepam on drug-naive animals. On the first exposure, there was a marked reduction in unpunished responding and only a partial anti-punishment effect. After 4 to 5 days of consecutive daily drug administration there was a tolerance to the depression of unpunished behaviour while the anticonflict activity actually increased to an asymptotic level. They proposed that the anticonflict effect was unmasked as the sedative effect disappeared.

Although it is possible that the sedative effect of diazepam inhibited the anticonflict activity to be expressed on the first exposure, sedation may only be party responsible for this effect, since unpunished responding remained unaffected on the first exposure to diazepam in the present experiments where the equivalent dose was much lower. Sepinwall and Cook (1978) have shown that chlordiazepoxide produced a large anticonflict effect and no alteration in unpunished behaviour in drug-naive animals that had been given a large dose of chlordiazepoxide 27 days earlier, after the training session. Animals behaved as though they were "drug-experienced". With this 27 day interval between treatments, it was highly unlikely that the immediate appearance of the "drug-sophisticated" anticonflict profile was due to the disappearance of the sedative effect or the presence of residual traces of chlordiazepoxide or its metabolites. Further, the immediate appearance of the drug-sophisticated profile during the first test session that was conducted in the drug-state made it highly unlikely that a drug-behaviour interaction was required for the full anticonflict effect to occur.

The effect of clonidine on "diazepam-experienced" animals was similar to that obtained with diazepam on "drug-naive" animals. However in this case unpunished responding was intensely suppressed on the first treatment. After 3 days of consecutive daily clonidine

administration, unpunished responding was only slightly suppressed and a significant increase in punished responding was observed.

The results obtained with clonidine show that the "drug-experience" phenomenon also occurs with clonidine in animals that were "diazepam-experienced". This suggests that the effects produced by the first and subsequent exposures to drugs having an anticonflict activity is not a generalised "anticonflict- experience" phenomenon but was specific to the drug used.

Yohimbine produced an intense suppression of punished responding with a "carry-over" effect at the first exposure, of 3 days post-drug experience in drug-naive animals. Yohimbine administration at weekly intervals consistently showed a decrease in punished responding. The effect on drug day was maintained, while the time taken to return to baseline generally decreased with increasing number of exposures to the drug. In diazepam-experienced animals however, the "carry-over" effect on post-drug days was not as intense. Although the first exposure to yohimbine suppressed punished responding by day+2, responding had returned to pre-injection levels. Furthermore, by the second exposure to yohimbine in diazepam experienced animals, the "carry-over" effect was virtually abolished.

The anticonflict activity of yohimbine on post-drug days suggest that tolerance occurs with increasing number of exposures. It is possible that the initial carry-over effect is due to "drug-day" experience producing a classic aversive conditioning to the box environment. However, this does not explain why an intense "carry-over" effect was also observed with phenylephrine, piperoxane as well as a higher dose of yohimbine in the same animals after the effects of yohimbine had been investigated. However it is interesting to note that although the "carry-over" effect was decreased with increasing number of exposures, punished responding was intensely suppressed on drug-day irrespective of the number of exposures. Furthermore other α-adrenoceptor ligands examined in these animals also produced significant "anxiogenic-like" activity. This also suggests that although post-drug day effects may be subjected to tolerance, aversive events occuring while the drug is present may be subjected to sensitisation. However, if sensitisation did occur the suppression of

punished responding on drug-day would be expected to increase, but results with yohimbine show that there is no increase in suppression induced by yohimbine with increasing number of exposures.

The effects of yohimbine on drug-naive and diazepam-experienced animals also emphasize the importance of previous drug-exposure an drug history of animals used in conflict behaviour. In this case, pre-exposure to diazepam altered the effect of the first exposure to yohimbine as post-drug days although in punished responding on drug day was intensely suppressed. The reason for this effect is not clear although it may be suggested that a previous "anxiolytic" experience induces a "damping effect" on post anxiogenic drug days, such that post-drug behaviour is considerably modified. It is possible that diazepam-experienced animals were experiment sophisticated (since a number of other agents were investigated) at the time when yohimbine was administered, and owing to this the "novelty" of "drug injection" may have become less.

The effect of ACTH on the Geller-Seifter conflict test was as expected - punished responding was suppressed with no effect on unpunished behaviour. However, Sahgal et al (1979) failed to show an anxiogenic-like effect with ACTH 4-10 in a behavioural conflict schedule in pigeons, whereas in that schedule amylobarbitone released punished responding.

It is possible that the failure to detect an anxiogenic-like effect with ACTH $_{4-10}$ (Sahgal et al 1979) was due to the doses used and the time at which behaviour was investigated. Sahgal et al (1979) used 0.1 - 0.3 mg/kg of ACTH $_{4-10}$ (as compared to 0.075 mg/kg in this study) and injections were given 1 hr prior to testing whereas in this study, animals were placed in the box immediately after injection. Furthermore, pigeons were used in the study by Sahgal et al (1979) as compared to rats in this study, hence the possibility that failure to detect an "anxiogenic-like" effect with ACTH$_{4-10}$ may also have been due to a species difference as well as a difference in schedule, cannot be excluded.

The effect of picrotoxin in this study could not be adequately determined because the first two animals convulsed and subsequently died although the dose used was one that had been shown to be a

subconvulsive anxiogenic dose in the maze study (Chapter 2) as well as the social interaction test (File and Lister, 1983). Results suggest that these animals were more sensitive to the convulsive effects of picrotoxin than drug-naive animals used in the maze study (Chapter 2) perhaps because at the time of picrotoxin injection in this study, animals were already "drug-experienced". Drugs that had been administered previously included the α_2-adrenoceptor antagonists, α_1-adrenoceptor agonists and quipazine.

The involvement of central monoamines in convulsive threshold has been reported by Kilian et al (1973) who showed that the threshold for maximal electro-convulsions were lowered by the 5-HT and α_1-adrenoceptor antagonists cyproheptadine and phentolamine, whereas L-dopa and 5-HTP raised the threshold. Clonidine was shown to cause a dose-dependent decrease in the duration of pentylenetetrazole (PT2)-induced seizures and the anticonvulsant activity of clonidine was antagonised by yohimbine (Papanicolaou et al, 1982). Furthermore piperoxane significantly potentiated PTZ-induced tonic-seizures and mortality, whereas prazosin and propranolol alone did not have an effect and did not alter the potentiation of tonic-seizures induced by piperoxane (Lazarova et al, 1983), suggesting that α_2-adrenoceptors were involved in the control of these seizures. LLoyd and Worms (1982) also showed that yohimbine potentiated the clonic and tonic seizures induced by picrotoxin, it is possible therefore that pre-exposure to the α-adrenoceptor ligands may have resulted in the animals being more sensitive to the convulsant effects of picrotoxin.

The role of GABA in the convulsant action of picrotoxin has been suggested on the basis of the effect of iontophoretic application of picrotoxin where the antagonism of the inhibitory action of GABA was demonstrated, and on membrane binding studies (see Meldrum, 1979). It has also been reported that prolonged social isolation has the effect of lowering brain GABA content in rats (Bolin and Davanzo, 1981) and Arbilla et al (1982) showed that several α_2-adrenoceptor antagonists inhibited the electrically-evoked release of ^{3}H-GABA. Hence the supersensitivity of these animals to picrotoxin may also in part be due

to the effect of prolonged isolation on brain GABA content therefore exaggerating the effect of a small dose of picrotoxin, which would otherwise have been subconvulsive.

The effect of α_2-adrenoceptor ligands on conflict behaviour generally paralleled those obtained in the maze exploration study (Chapter 2). The α_2-adrenoceptor agonists consistently released punished behaviour, even at doses where unpunished behaviour was significantly depressed, whereas the antagonists caused an intense selective suppression of punished behaviour. However, at doses where a general depressant activity was observed, sedation was absent (which was also confirmed with the doses used in the maze exploration study). Hence, it is unlikely that at doses where both punished and unpunished behaviour was suppressed, this was due to a sedative effect of the agent.

Results obtained with the α_2-adrenoceptor agonists are consistent with those of Bullock et al (1978) who demonstrated that clonidine increased punished responding whereas unpunished behaviour was unaffected at lower doses, and markedly suppressed at higher doses at which sedation was observed. Yohimbine was shown to antagonise the anticonflict activity of clonidine although phenoxybenzamine had no effect at a lower dose, but partly antagonised the effect of clonidine at a higher dose (Kruse et al, 1981). The inhibiting effect of phenoxybenzamine at higher dose on clonidine's anticonflict activity may reflect α_2-adrenoceptor blockade at the dose used, since although an α_1-adrenoceptor blocking effect has been established (Drew, 1976) peripherally, a presynaptic action of phenoxybenzamine has also been suggested (Starke et al, 1975).

The α_2-adrenoceptor antagonists showed an anxiogenic-like effect in this behavioural model. Kruse et al (1981) however, failed to show any effect with yohimbine as punished responding, although the release in punished behaviour induced by clonidine was antagonised by yohimbine. Sepinwall and Cook (1981) demonstrated that although yohimbine antagonised the rate depressing effects of clonidine, by itself yohimbine exerted an anticonflict effect. On the basis of the differential effects observed with yohimbine in "drug-naive" and "diazepam-experienced" rats, it is possible that the discrepancies in

the effect of yohimbine observed in other studies is due to the influence of previous drug experience, since these studies do not specify the order in which drugs were administrated.

The effect of α_1-adrenoceptor agonists was also as predicted. Phenylephrine and St587 both suppressed punished behaviour, with lesser effect on unpunished responding. The effect of these agents have not been previously examined in Geller-Seifter conflict, although in the potentiated startle paradigm phenylephrine increased the amplitude of potentiated startle (Davis and Astrachan, 1981).

The effects of α_1-adrenoceptor antagonists were less clear cut. Prazosin increased punished responding at lower doses, whereas increasing the dose caused a decrease in punished behaviour. Prazosin did not have an effect on the first and second CRFs periods, but both the third and fourth punished periods increased responding rate although total lever presses during all 4 periods did not show any significant changes in responding, increases im punished responding were observed in the third and fourth periods with the lower doses of prazosin. This may be due to the rate of absorption of prazosin, in that it is not until the third period that a sufficient amount of drug is present in the brain. Similar results were obtained with prazosin in the maze exploration study (Chapter 2) where the "anxiolytic-like" effect was demonstrated 30 mins after injection.

In this behavioural model thymoxamine had no effect. Two doses of thymoxamine were investigated and the punished responding baseline was high for one dose and low for another, such that if the effect of thymoxamine was dependent upon the rate of punished responding (as has been demonstrated with clonidine by Sepinwall and Cook (1981)), then this effect would be detected. However, at neither doses did thymoxamine have any effect on punished or unpunished responding.

The effect of prazosin or thymoxamine have not been previously examined in the Geller-Seifter conflict schedule, although in a conditioned suppression of drinking test, prazosin increased drinking - an effect that was of a similar magnitude to that of diazepam (Gardner and Piper, 1982). The reason for the inactivity of thymoxamine in this study is not clear, although results are consistent with those obtained

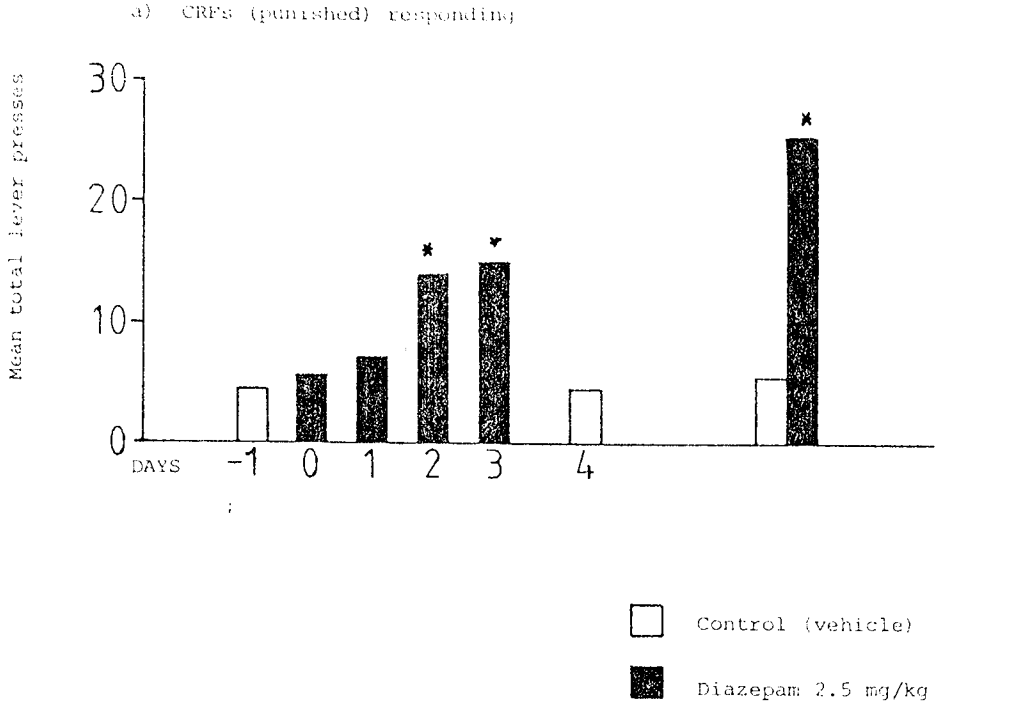
in the maze exploration study (Chapter 2), where thymoxamine did not have an effect, although prazosin exhibited "anxiolytic-like" activity.

Quipazine was shown to suppress both punished and unpunished behaviour although the effect on unpunished responding was considerably less. Conversely ketanserin and pirenperone released punished responding (although the effect of the latter was not significant), suggesting that serotonergic systems also modulate anticonflict activity.

The effect of quipazine, ketanserin and pirenperone on punished responding have not been previously examined although the effects of other serotonergic agents have been reported. Aprison and Ferster (1961) demonstrated a marked suppression of food rewarded behaviour after a combined administration of 5-HTP and a monoamine oxidase inhibitor, and cyproheptadine was shown to increase response rates suppressed by punishment in drinking behaviour (Graeff, 1974). However, it is not clear whether the action of these agents in moderating anticonflict effects is mediated via 5-HT_1 or 5-HT_2 receptors since although Cerritto and Raiteri (1979) have suggested that cyproheptadine and methysergide may act out preferentially at post-synaptic receptors, both these agents may also act as antagonists at 5-HT_1 receptors (Peroutka and Snyder, 1979). Further work requires to be done on investigating the effect of 5-HT_{1A} receptor agonist 8-OHDPAT (Cortes et al, 1984) in order to establish whether the effects of cyproheptadine and methysergide were mediated via 5-HT_2 receptors.

In view of the results obtained in this study, all of these are consistent with the notion that central noradrenergic and serotonergic systems modulate anticonflict activity and are therefore at least in part involved in the modulation of anxiety. However, in the Geller-Seifter conflict schedule, rate-dependency may have also influenced the effects of drugs observed. (A detailed explanation of this phenomenon is to be found in the introduction - Section 10.3d). In addition, in the study presented in the present chapter, two groups of animals with different rates of responding were used. Owing to this, drug effects observed may have been influenced by the rate of responding. In the following study, using a modified schedule to that used by de Carvalho et al (1983), rate dependency effects have also

been considered. Furthermore, "carry-over" effects produced by the α_2-adrenoceptor antagonists have also been investigated.



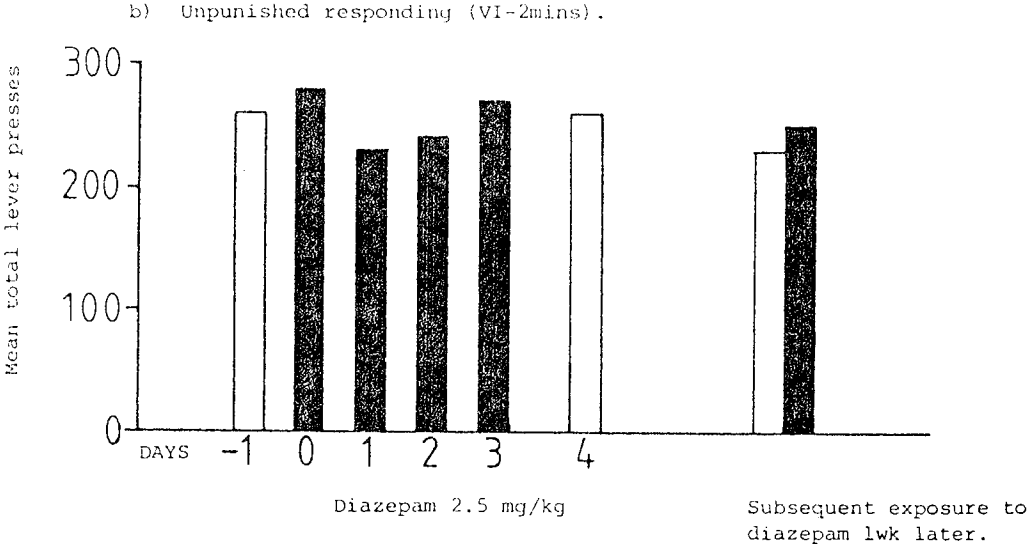


Fig. 3.1 The effect of diazepam 2.5 mg/kg on a) punished and b) unpunished responding on drug-naive animals.

*p <0.05 Wilcoxon matched pairs - signed ranks test.

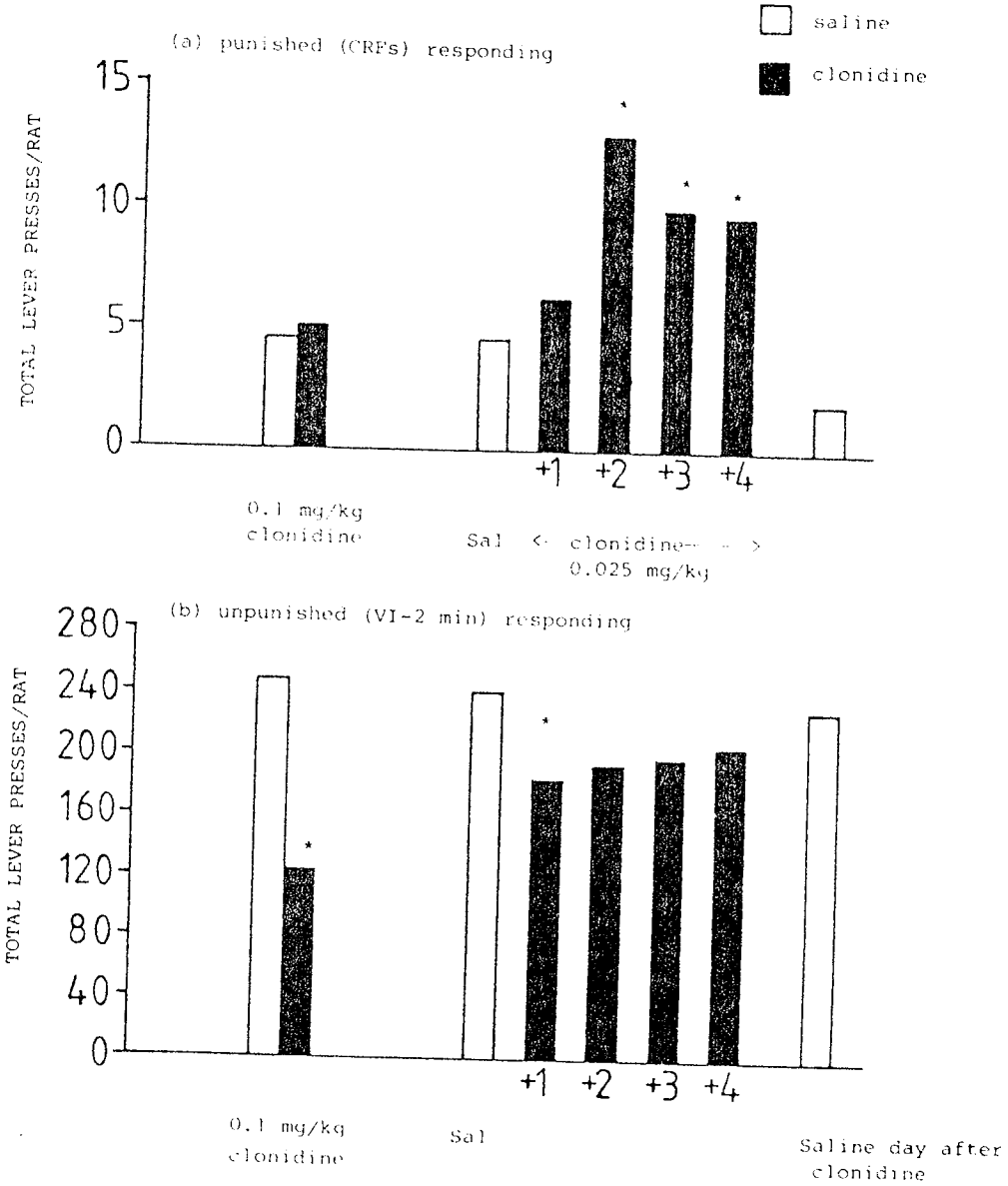


Fig 3.2 The effect of first exposure to clonidine on (a)punished(CRFs) and (b)unpunished (VI-2min) responding in diazepam experienced animals.
Results are expressed as the mean of the total number of lever presses/rat in all 4 periods.

* $p < 0.05$ Wilcoxon matched pairs signed ranks test.

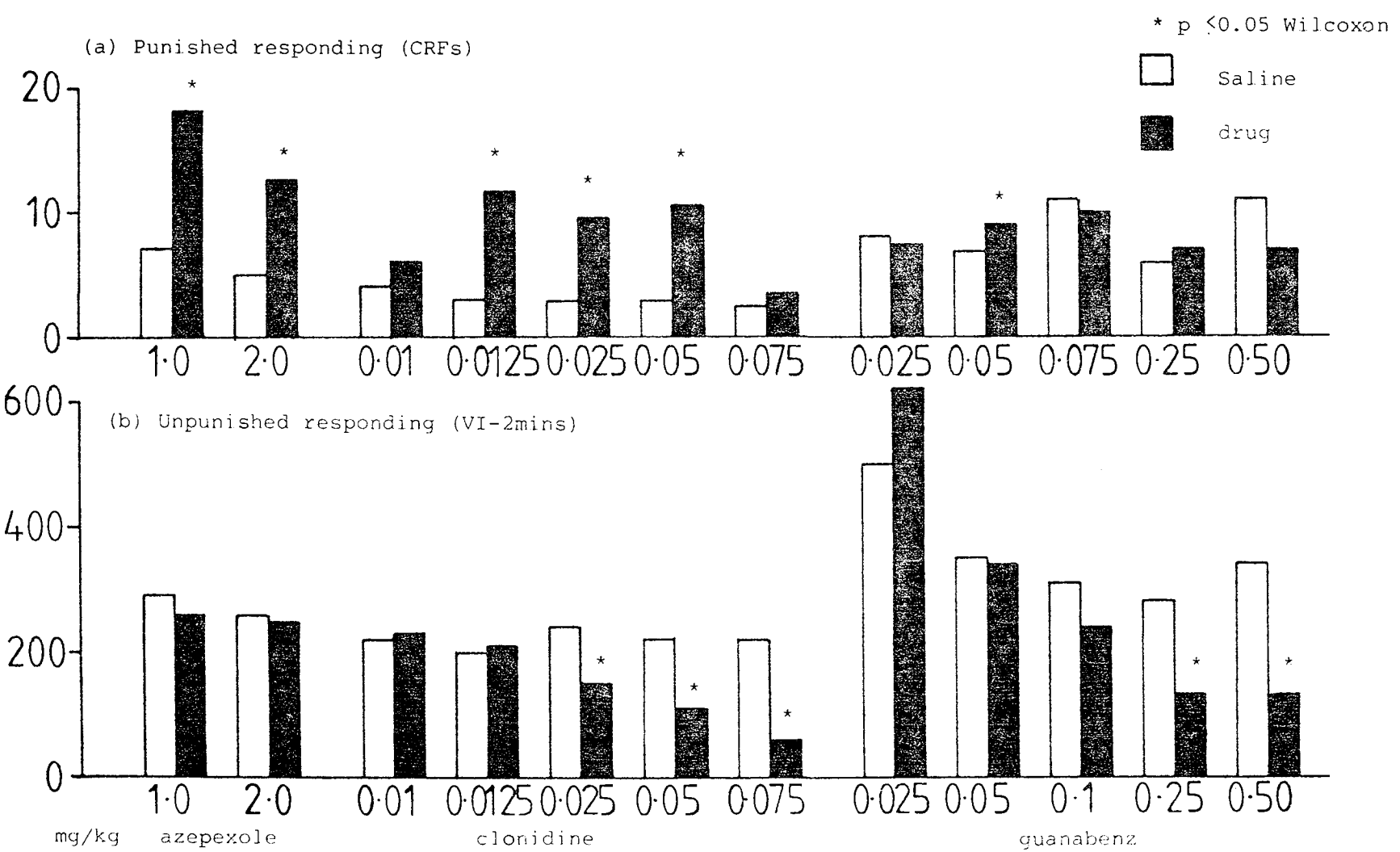
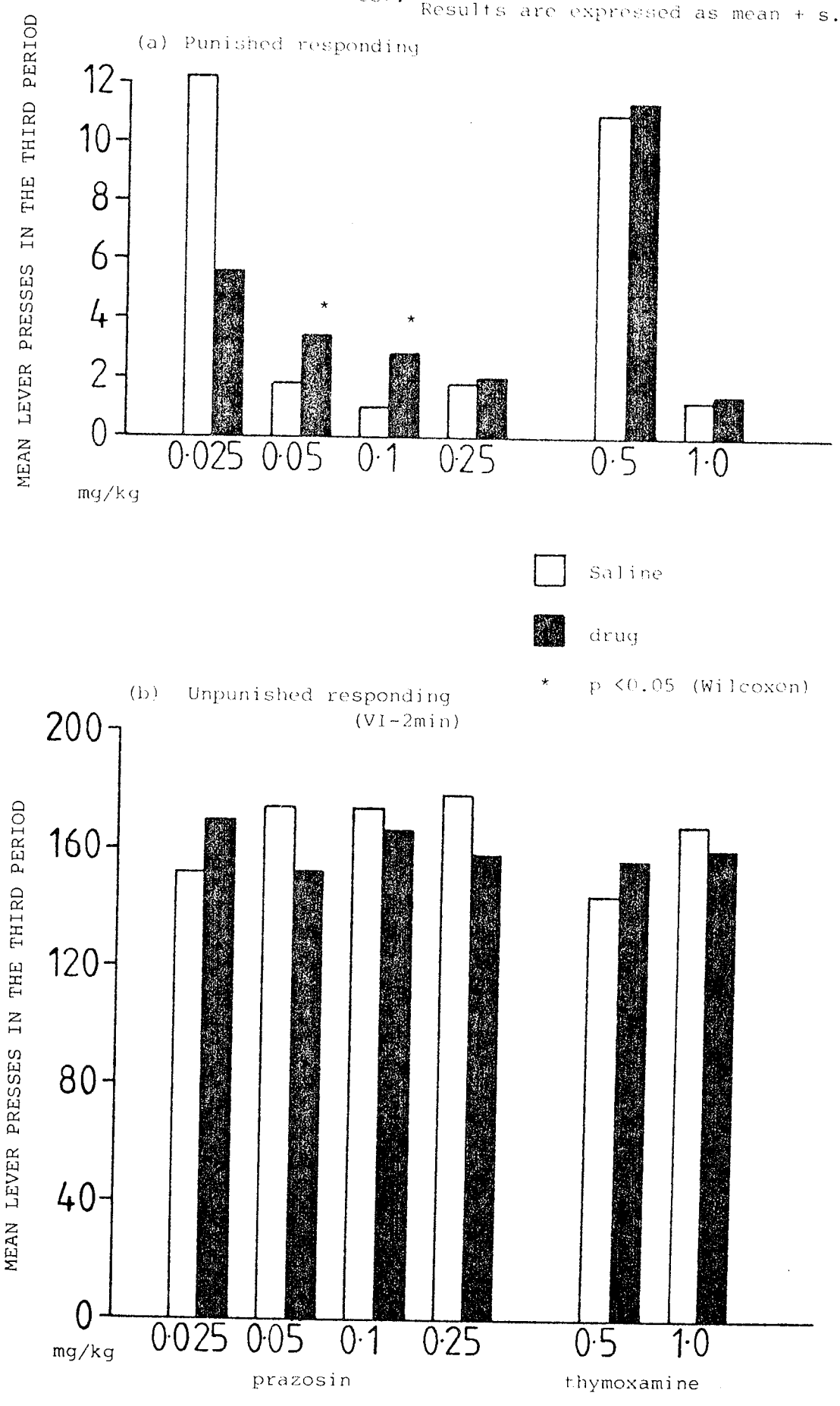


Fig 3.3 The effect of α_2-adrenoceptor agonists on (a)punished and (b)unpunished responding. Results are expressed as the mean of the total number of lever presses in all 4 periods.

Fig. 3.4 The effect of prazosin and thymoxamine on (a)punished and (b)unpunished responding in the third period of the Geller-Seifter conflict test. Results are expressed as mean + s.e.m.



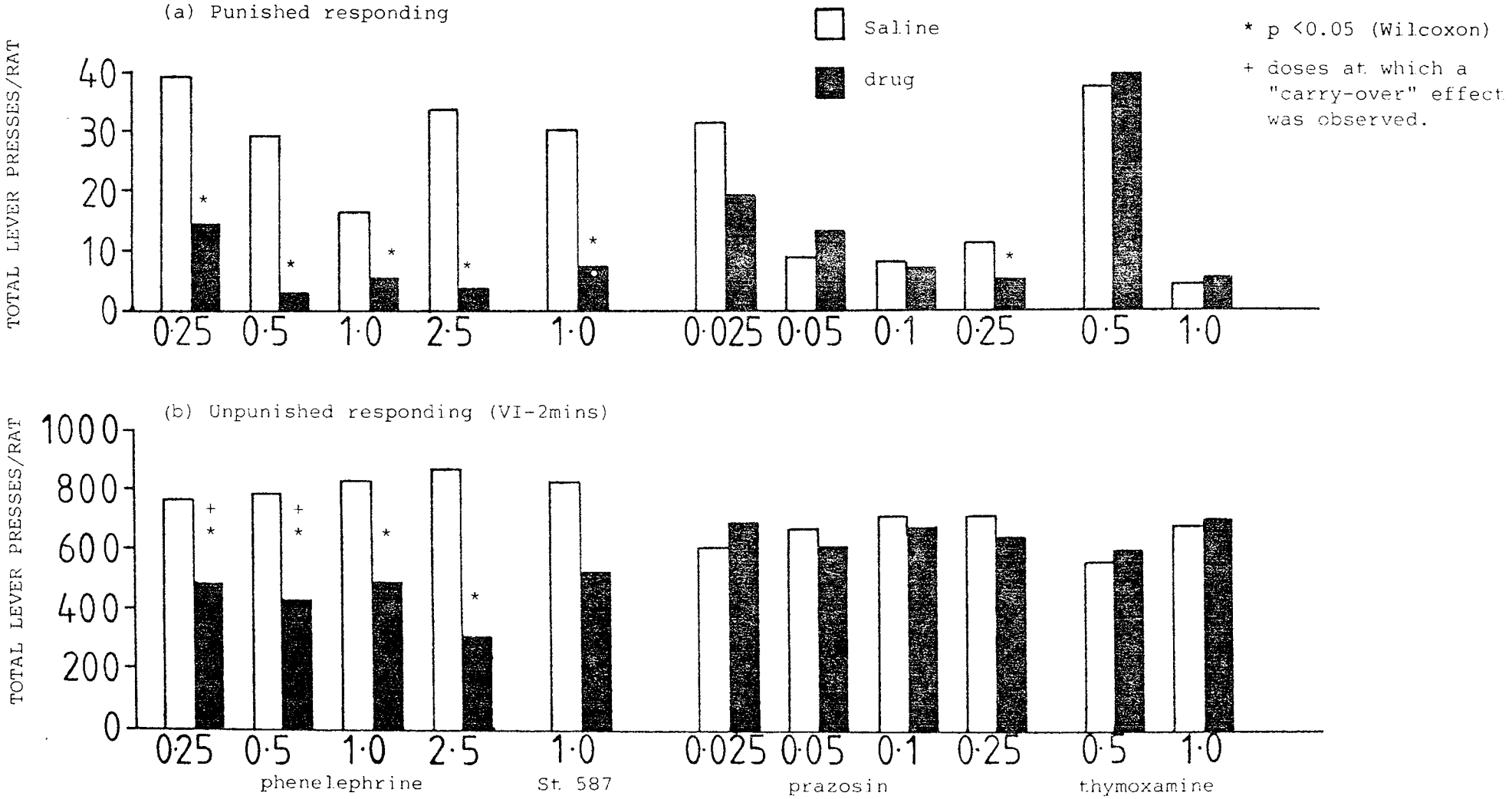
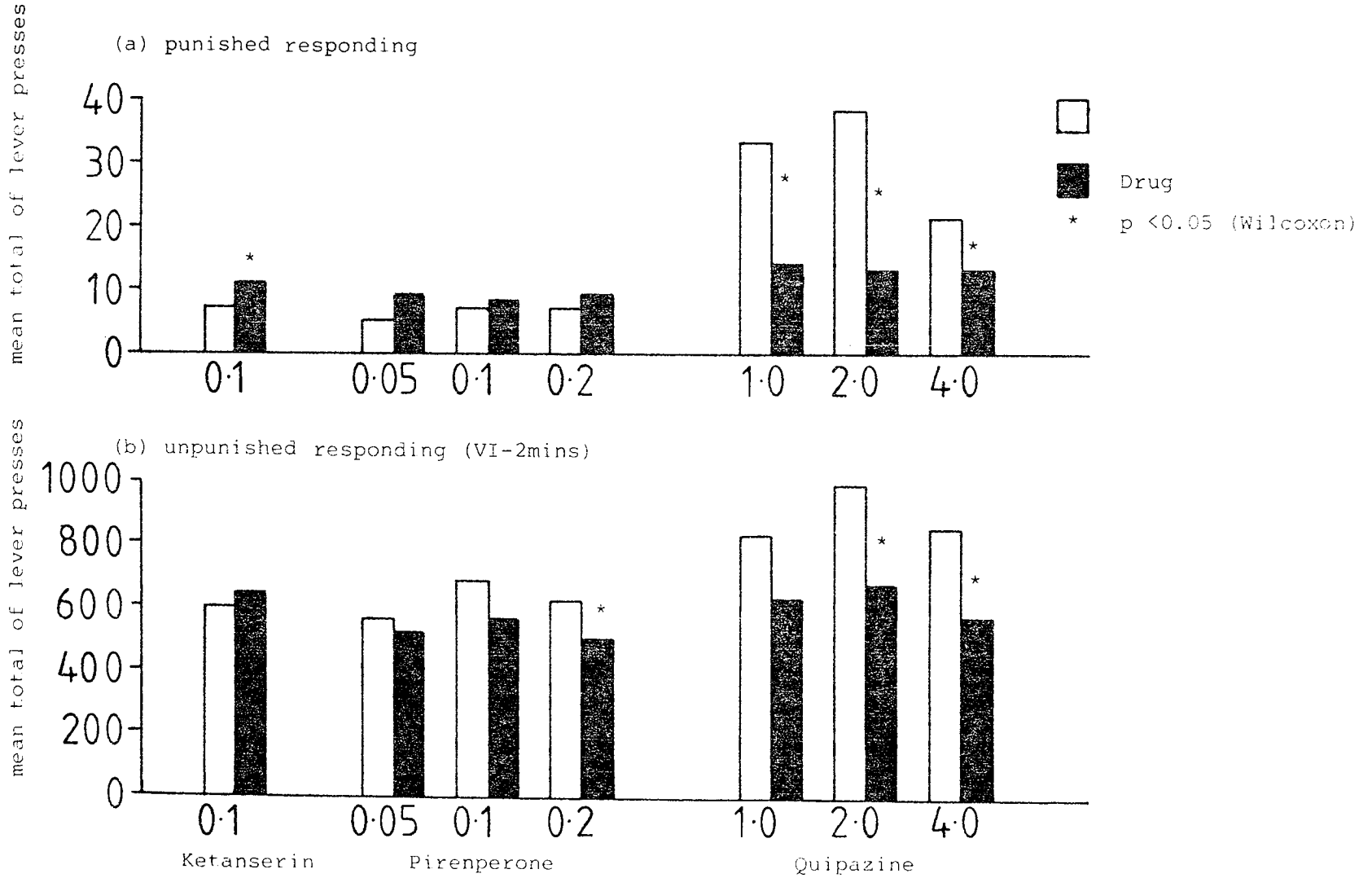


Fig 3.5 The effect of α_1-adrenoceptor agonists and antagonists on (a)punished and (b)unpunished responding. Results are expressed as the mean of the total number of lever presses in all 4 periods.

Results are expressed as mean of the total number of lever presses in all 4 periods.



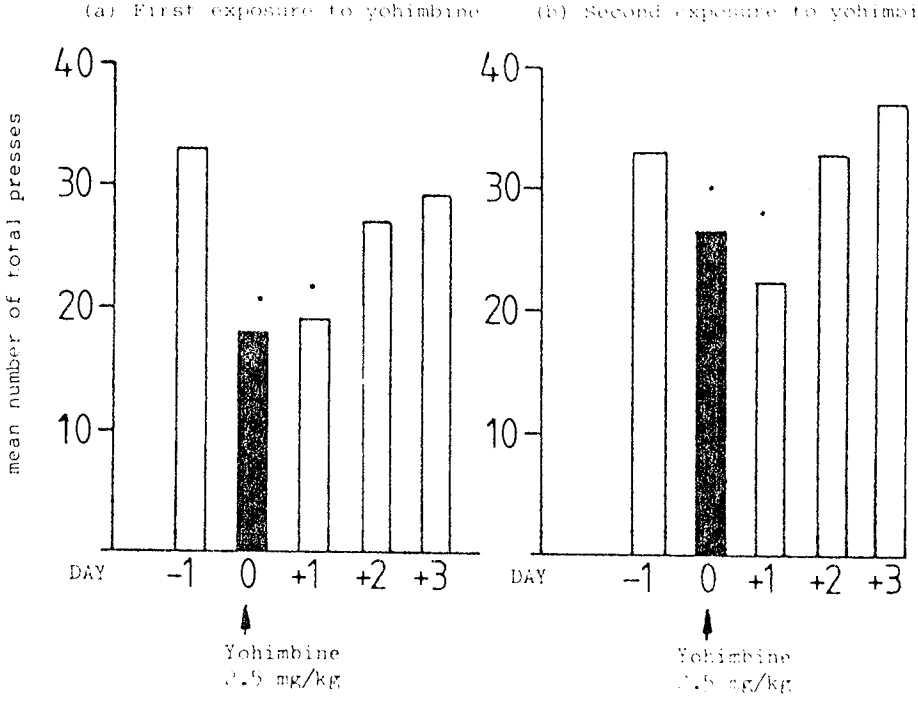


Fig. 3.7 The effect of (a) first and (b) second exposure to yohimbine (2.5 mg/kg) on punished responding in diazepam-experienced animals.

	Previous day Saline	Yohimbine 2.5 mg/kg	DAY + 1	DAY + 2	DAY + 3
1st exposure to Yohimbine	466 ± 53	443 ± 61	497 ± 55	482 ± 52	487 ± 55
2nd exposure to Yohimbine	476 ± 51	503 ± 30	494 ± 29	477 ± 42	472 ± 44

Table 3.1 The effect of first and second exposure to yohimbine 2.5 mg/kg on VI responding (VI-2min) in diazepam-experienced animals.

Results are expressed as the mean of the total number of lever presses/ratduring all 4 VI periods. (mean ± s.e.m.)

* $p < 0.05$ Wilcoxon matched pairs signed ranks test

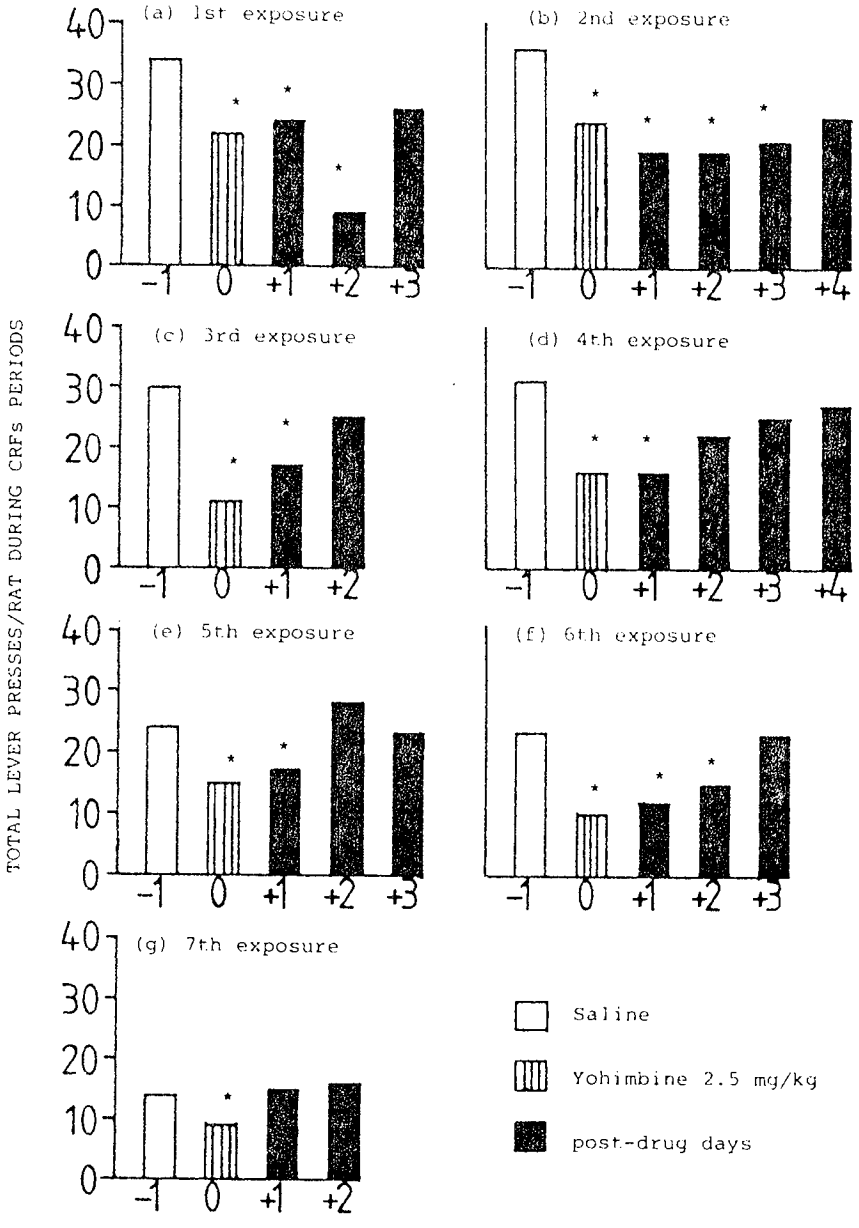


Fig 3.8 The effect of number of exposures to yohimbine in previously drug-naive animals punished responding.

On Day -1, saline was administered. Yohimbine was administered on day 0. Days following drug day are represented by +1, +2, +3 etc.

* $p < 0.05$ Wilcoxon matched pairs signed ranks test.

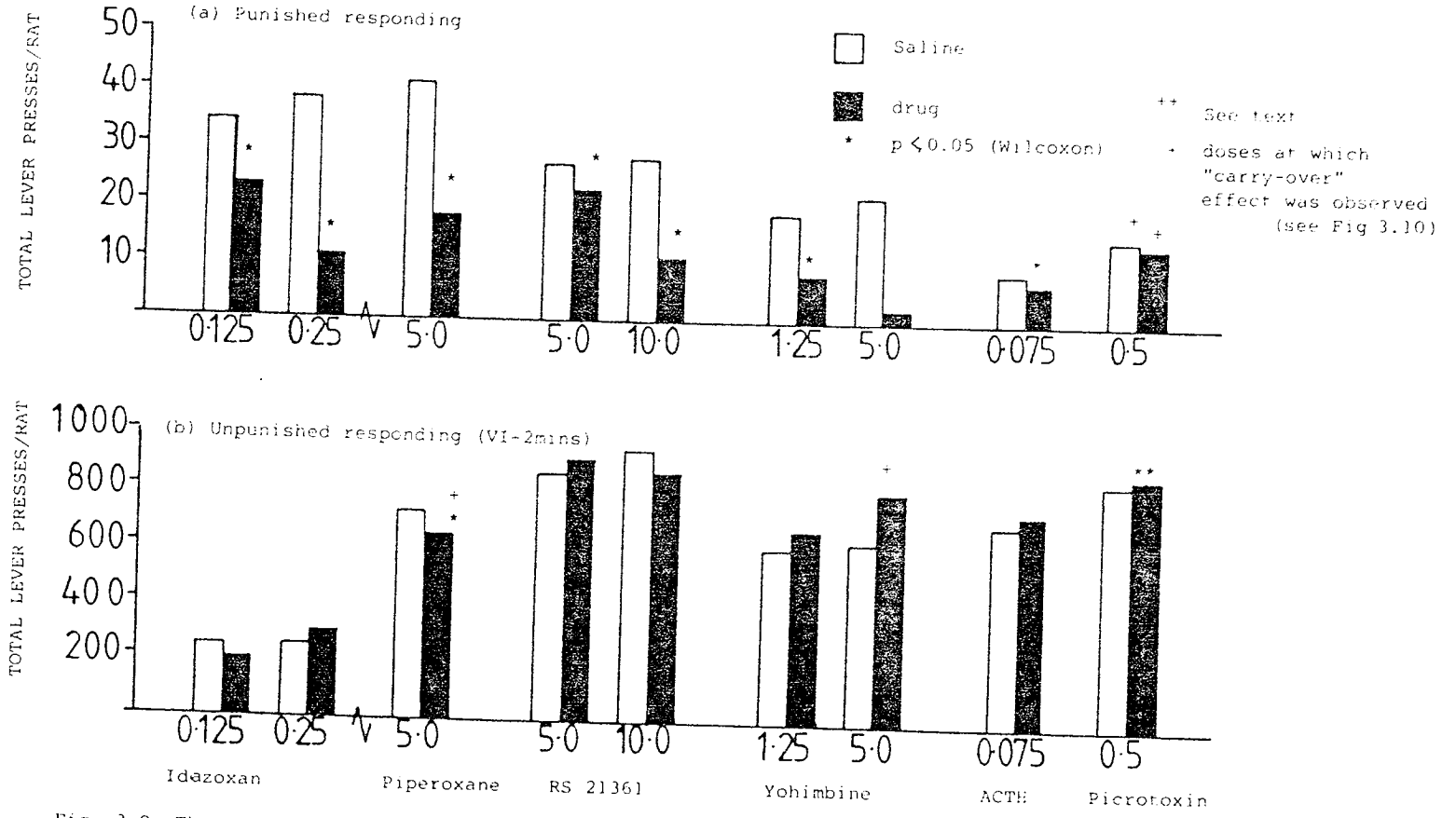


Fig. 3.9 The effect of α_2-adrenoceptor antagonists and putative anxiogenics on (a) punished and (b) unpunished responding.

Results are expressed as mean of the total number of lever presses in all 4 periods.

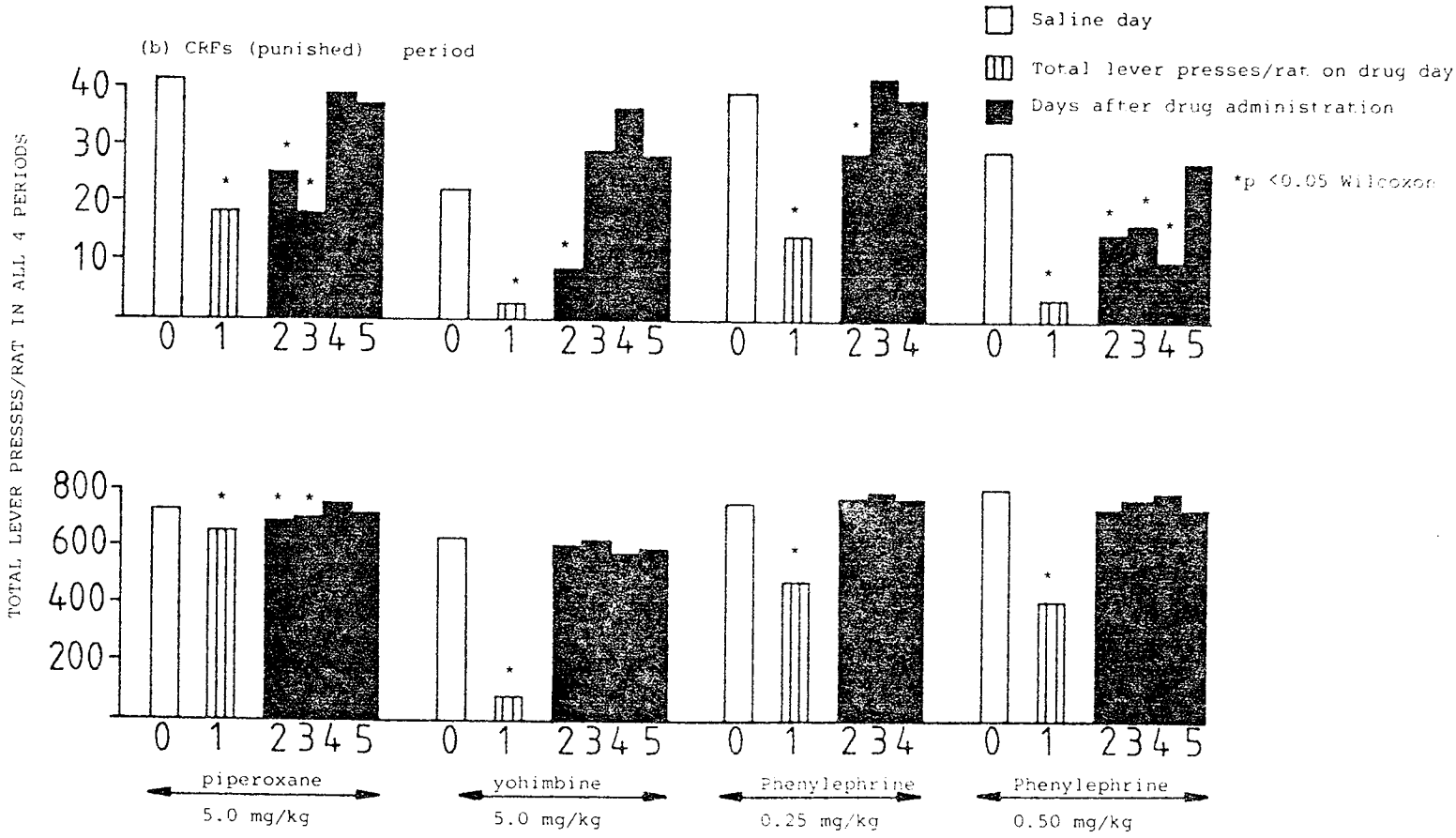


Fig. 3.10 The effect of drugs showing a "carry-over" effect on (a)punished and (b)unpunished responding on subsequent days after drug administration.

	Previous Saline control day	Yohimbine 2.5 mg/kg	Day 1 after yohimbine	Day 2 after yohimbine	Day 3 after yohimbine	Day 4 after yohimbine
1st exposure	304 ± 41	266 ± 47*	279 ± 42	187 ± 36*	233 ± 25*	299 ± 31
2nd exposure	248 ± 30	243 ± 30*	223 ± 27*	196 ± 28*	208 ± 22	235 ± 47
3rd exposure	349 ± 65	306 ± 83	350 ± 65	340 ± 47	378 ± 46	383 ± 39
4th exposure	371 ± 53	376 ± 68	354 ± 55	351 ± 42	305 ± 38	390 ± 34
5th exposure	295 ± 36	373 ± 54	263 ± 25*	338 ± 35	294 ± 32	
6th exposure	641 ± 52	632 ± 78	633 ± 63	621 ± 61	661 ± 56	
7th exposure	550 ± 30	537 ± 71	599 ± 58	544 ± 49	614 ± 50	

Table 3.2 The effect of various exposures to yohimbine 2.5 mg/kg in previously drug-naive animals, on unpunished (VI-2 min) responding.

Results are expressed as mean ± s.c.m.

Animals were injected with yohimbine 2.5 mg/kg at weekly intervals.

*$p<0.05$ Wilcoson matched pairs signed values test.

CHAPTER 4

THE EFFECT OF CHRONIC DIAZEPAM AND THE α-ADRENOCEPTOR LIGANDS ON CONFLICT BEHAVIOUR.

Introduction.

Although the Geller-Seifter conflict test is a widely accepted test for investigating antianxiety actions of drugs it has certain drawbacks. For instance it consists of a low density positive reinforcement (VI) without punishment that alternates with periods of relatively high density positive reinforcement (CRF) coupled with punishment. In a schedule of this kind, responding during the punishment period cannot be adequately compared with unpunished behaviour on the basis of the rate of responding since there are different baselines because the schedule consists of two different components. Therefore, a schedule was required which would take into account this variable and would be such that both unpunished and punished behaviour could be paralleled as the basis of the components - the only difference being that of the presence of a contingent footshock.

Also in the Geller-Seifter conflict schedule, phenylephrine, yohimbine and piperoxane showed "carry-over" effects and it was desirable to investigate these effects further. In the experiments of de Carvalho et al (1983), a more simple schedule was used. It consisted of three CRF segments of 5 mins each, the middle one having response-contingent footshock. In this behavioural model, the suppression of behaviour in the first unpunished and the second punished period with no effect on the third unpunished behaviour component was attributed to "anticipatory" anxiety (de Carvalho et al, 1983). On this basis it was felt necessary to examine whether these agents affect anticipatory anxiety, since in the Geller-Seifter schedule, unpunished VI responding was also affected on post-drug days (see Chapter 3). Furthermore, the effect of chronic adminstration of yohimbine could also be investigated, which would help to establish whether the punishment-induced suppression of behaviour undergoes tolerance, or that stimulus sensitisation occurs. It would also ascertain whether habituation occurs with the "anxiogenic" effect.

The effects of clonidine and diazepam in the Geller-Seifter conflict schedule were not apparent on the first exposure, but required further exposures for a release in punishment-induced suppression to

occur. In the schedule used by de Carvalho et al (1983), diazepam showed an effect although it is not clear whether this effect was on the first exposure; hence this schedule was considered further.

In this study, initially the schedule described by de Carvalho et al (1983) was used with some modifications. A "Time-Out" period of 5 mins was incorporated in this schedule at the end of the third unpunished segment. This was done so that the effect of drugs on responding during the "Time-Out" period would help to distinguish specific effects from non-specific ones. For instance, amylobarbitone was shown to increase punished responding as well as unpunished responding with no effect on Time-Out indicating that this agent does not release punished responding in a non-specific manner (Sahgal et al, 1979).

Initially, the effects of diazepam and clonidine were investigated in this study to examine whether there was any difference in the effects of these agents on drug-naive and drug-experienced animals. Rate dependency effects were also examined with clonidine and diazepam. Following this, various modifications of the unpunished and punished segments were carried out. For example, some segments were changed to a variable interval (VI-30 secs) component, whereas others were on a continuous reinforcement (CRF) schedule, etc. The effect of clonidine and diazepam were investigated on these different schedules in order to examine which of these schedules showed a maximum effect with clonidine and diazepam so that it could be used for subsequent experiments to confirm the results obtained in the Geller-Seifter study (Chapter 3) and to look at noradrenergic and sertonergic interactions in modulating conflict behaviour. This schedule would also be used to examine the effects of α-adrenoceptor and 5-HT receptor ligands on L.C. lesioned animals.

1. The effect of chronic diazepam in "drug-naive" and "clonidine-experienced" animals on the CRF schedule (Groups A and B).

In this experiment, diazepam (2.5 mg/kg) was given for 11 consecutive days and behavioural effects were observed 30 mins after injection on each day. Responding in the conflict period was suppressed to 50%.

Both groups of animals showed a decrease in unpunished responding during both segments (Fig.4.1). Clonidine-experienced animals did not respond differently from drug-naive group (Fig.4.1). Punished responding increased significantly on the first exposure to diazepam in both groups. (Fig.4.2).

Diazepam significantly elevated time-out responding on the 1st exposure in the clonidine-experienced animals, whereas an increase was observed on the 2nd exposure to diazepam in the drug-naive group. Subsequent exposure to diazepam appeared to cause tolerance on time-out - as from day 3, responding in this period remained unchanged. (Fig.4.2).

2. The effect of chronic clonidine in "drug-naive" and "diazepam-experienced" animals on the CRF schedule (Groups A and B).

Clonidine (0.025 mg/kg) was given for 11 consecutive days and behavioural effects observed 30 mins after injection. Baseline responding in the conflict period was 50%.

In the 1st and 3rd unpunished periods, clonidine decreased responding in both groups of rats, although in the diazepam-experienced animals the first exposure to clonidine did not significantly decrease unpunished responding (Fig.4.3).

In the drug-naive group, punished responding was significantly decreased on the 1st and 2nd exposure to clonidine, and on subsequent days punished responding returned to baseline levels. Diazepam-experienced animals however did not show a difference in punished responding throughout chronic exposure to clonidine (Fig.4.4). Responses in the Time-Out period remained unchanged in both groups throughout the 11 day exposure to clonidine. (Fig.4.4).

3. The effect of chronic clonidine on a VI/CRF schedule (Group C).

Clonidine did not release punished responding in the CRF schedule in either "drug-naive" or "diazepam-experienced" animals. In this schedule, a VI-30sec component was incorporated, to examine whether the lack of effect in the CRF schedule was due to the absence of a VI-component. At the time of this experiment, animals were drug-naive (Groups C rats) and the effect of clonidine (0.025 mg/kg) for 4 days was investigated, since in the previous conflict test (Chapter 3), clonidine released punished responding by drug day 2. Responding during conflict period was suppressed to 50%.

The schedule comprised a first VI-30 sec unpunished period which was followed by a CRFs punished period, and then a CRF (unpunished) period. After this, animals were subjected to Time-Out for 5 mins.

The effect of clonidine on the VI-30 sec and CRF unpunished periods are shown in Fig.4.5. Responding was significantly decreased during all 4 days of treatment with the VI-30 sec period, whereas CRF responding decreased significantly on days 1 and 4. CRFs responding remained unchanged on all 4 days, whereas Time-Out increased on days 1 and 2, but returned to baseline thereafter (Fig 4.5).

4. Rate dependency effects of diazepam and clonidine.

The effect of diazepam 2.5 mg/kg and clonidine (0.025 mg/kg) for 4 days on the VI/CRF schedule (see above) was examined, and the effect of these agents on individual animals during the punished periods was analysed.

Results showed that with diazepam, punished responding was released irrespective of the baseline responding rate of the animal. With clonidine however, "high-responders" (>6 responses during the punished period) did not increase punished responding whereas "low-responders" (<5 responses in punished period) consistently released punished responding on all 4 days of treatment.

5. The effect of chronic clonidine in a CRF schedule with an altered punished responding baseline.

In this schedule, all 3 segments (two unpunished, and a middle punished segment) were based on a CRF responding, which was followed by a time-out period. Punished responding was suppressed to give ⟨6 responses/rat.

Clonidine (0.025 mg/kg) was administered for 4 days, and Fig.4.6 shows that both unpunished periods included a decrease in responding during all 4 days. Responses in the punished period were significantly increased on all drug days (maximum effect on day 3), and responding returned to baseline upon withdrawal of clonidine (Fig.4.6). Time-Out was unaffected on all drug days.

6. The effect of clonidine and diazepam on four conflict schedules.

The effect of clonidine (0.025 mg/kg) and diazepam (2.5 mg/kg) was investigated on 4 behavioural schedules in order to detect which one shows a maximum effect on punished responding but unpunished responding is least affected. The conflict schedules used were as follows:-

(a) FFF-T - all 3 5 min segments were based on CRF responding, of which the second period was punished.

(b) VFF-T - the first unpunished segment comprised a VI-30 sec component which was followed by a punished period (CRFs) and then a CRF (unpunished) period.

(c) VVsV-T - all 3 segments were based on a VI-30 sec schedule - the middle one being punished. However, in the punished period a shock was delivered at every lever press.

(d) VVV-T - a schedule similar to that described in (c), but during the punished period, a shock was only delivered when a food reward was obtained.

In all four schedules, the 3 5 min segments were followed by a 5 min Time-Out period. Baseline suppression level for punished responding was 6 responses during that period.

For clonidine, a significant decrease in responding in the first unpunished period was observed in all 4 schedules. Similar effects were observed in the third unpunished periods, although schedules incorporating a VI-30 sec component showed a greater decrease

in unpunished responding (Fig.4.7).

In the V-FFT schedule, punished responding remained unchanged, whereas all other schedules caused a significant increase in punished responding, (maximum effect being obtained with VVV-T) (Fig.4.7). A significant decrease in responding during Time-Out was observed with VVV-T and VVsV-T, whereas VFF-T caused a significant rise in responding during time-out (Fig.4.7). The FFF-T schedule did not affect Time-Out responding.

With diazepam, unpunished responding in the first and third periods decreased in all 4 schedules, however only the first unpunished period of the VFF-T schedule caused a significant fall (Fig.4.8). Punished responding was significantly increased with all 4 schedules; the maximum effect was observed with VVV-T (Fig.4.8). Time-Out responding was similarly increased in all 4 schedules, the maximum effect being observed withVVVs-T (Fig 4.8.).

7. The effect of acute and chronic RS 21361 on VVV-T schedule.

The effect of Rs 21361 (10 mg/kg) was examined 15 mins after injection, since in the Geller-Seifter conflict test (Chapter 3), RS 21361 produced a maximal effect in the second period (between 13 and 26 mins post-injection).

RS 21361 given acutely did not affect unpunished VI-responding in either period, although responding was slightly decreased. A significant fall in punished responding was observed at this dose (Fig.4.9).

The effect of chronic RS 21361 (10 mg/kg for 5 days) showed that unpunished responding was not significantly altered on drug days whereas punished responding was significantly depressed on all drug days with the maximum effect on day 4 (Fig.4.9). However, upon withdrawal of drug, punished responding returned to baseline immediately, although responding in this period on day 1 after withdrawal was slightly depressed (Fig.4.9).

Responding during the time-out period remained unchanged during acute and chronic exposure to RS 21361 (Fig.4.9).

8. The effect of acute and chronic yohimbine on VVV-T schedule.

The effect of yohimbine (2.5 mg/kg) given acutely showed that unpunished responding as well as Time-Out remained unchanged, whereas punished responding was significantly decreased (Fig.4.10).

Chronic administration of yohimbine (2.5 mg/kg for 5 days) showed similar effects. A consistent fall in punished responding was observed on all drug days, which returned to pre-injection baseline upon withdrawal of yohimbine (Fig.4.10). Unpunished responding in both periods, as well as Time-Out responding remained unchanged during all days of drug treatment. (Fig.4.10).

Discussion

The effects of chronic diazepam (11 days) on a CRF schedule of conflict behaviour in drug-naive and clonidine-experienced animals showed that unpunished responding during both periods was initially suppressed. This effect was maintained until day 8 after which suppression of behaviour in both unpunished periods decreased and responding had returned to pre-injection levels.

The effect of diazepam on unpunished responding may have been due to the development of tolerance to the sedative effect of the drug Margules and Stein (1968) reported that the decreases in unpunished behaviour (perhaps due to sedation) underwent tolerance after 3 - 4 doses of 20 mg/kg oxazepam. In this study unpunished responding took 8 days to return to baseline levels. The anomoly in these results may be due to the difference in drug used and also the change in schedule, since the Geller-Seifter conflict schedule was used in the study of Margules and Stein (1968) which consists of a VI-2 min component during the unpunished segments.

The release of punished responding in clonidine-experienced animals was maintained on all 11 days of diazepam administration. However, in drug-naive animals although diazepam increased punished responding on all days, significant increases were not obtained on 3 out of 11 days. Also, neither of the groups showed a stable increase in punished responding until after day 9, the increase had stabilised to a large extent. However, in general the effect on punished responding was maintained with both groups of animals.

In drug-naive animals, diazepam showed a release in punished responding on the 1st exposure. These results imply that the lack of effect of diazepam on the 1st exposure in the Geller-Seifter test (Chapter 3) was not due to an initial sedative effect (since the same dose was examined in this schedule) and also not entirely due to the absence of a previous diazepam experience.

The discrepancy in the results obtained with diazepam on 1st exposure could be due to the difference in schedule. For instance, in the Geller-Seifter test, a VI component was included whereas this schedule consisted of CRF components. However, results confirm the findings that there is no cross-sensitisation between clonidine and

diazepam, that is, the effects of diazepam on punished responding are not dependent upon previous clonidine experience.

Responding during the time-out period showed that although both groups of animals showed an initial increase in responding, this returned to baseline immediately after the increase. Results therefore confirm that diazepam released punishment induced suppression in a specific manner, since responding during punished periods was maintained throughout chronic diazepam administration whereas time-out responding to normal pre-injection levels returned immediately after the initial increase.

Clonidine reduced unpunished responding in both drug-naive and diazepam-experienced animals throughout the 11 days of administration. Responding during the time-out period remained unaffected on all days of clonidine administration with both groups of animals. The decrease in unpunished responding appeared to stabilise after 2 - 3 administrations, and showed no tendency to increase levels of responding with an increase in the number of exposures to clonidine. However, the decrease in unpunished responding in diazepam experienced animals was less marked. This may have been due to the previous exposure to the sedative effects of diazepam suggesting that the animals were less sensitive to the sedative effects of clonidine.

Punished responding was suppressed on the first and second exposures to clonidine with drug-naive animals, which then returned to pre-injection levels on the third day. However, on days 10 and 11 of administration punished responding was slightly depressed and this lower baseline was maintained even after clonidine was withdrawn. Diazepam-experienced animals had a slightly different effect. Punished responding remained unchanged on all days of clonidine administration and although on Day 2 there was a slight decrease, the response had stabilised thereafter on a slightly lower baseline. However, upon withdrawal of clonidine punished responding was slightly increased and a higher baseline was maintained.

The differences in response during punished periods obtained with clonidine in drug-naive and diazepam-experienced animals indicate the importance of previous drug-experience in operant behaviour. In this case the sedative effects of clonidine may have been at least in

part, responsible for the difference in the effects of clonidine on drug-naive and diazepam-experienced animals. In the latter group, owing to the previous chronic exposure to diazepam, tolerance to the sedative effects may have occurred, causing a smaller depression in punished responding on the first two exposures to clonidine. It appears therefore that there is cross tolerance to the sedative effects of clonidine by diazepam. However, this does not explain why a release in punished responding was not observed with either groups of animals on chronic clonidine exposure as was seen in the Geller-Seifter study (Chapter 3).

The lack of effect of clonidine in this schedule could have been due to the absence of a VI-component, since in the Geller-Seifter schedule, clonidine was shown to release punished responding on the 3rd exposure. It is possible that the extent to which clonidine can release punished responding is in part dependent upon the rate of responding during the initial unpunished period. For instance, if the rate of responding is higher, then the effect of clonidine on punished responding would be greater, since there would be a larger difference between the two baselines. One way of increasing the rate of responding during the unpunished period is to introduce a VI component.

Results from the study which incorporated a VI-30sec component in the first unpunished period (where conflict responding was still suppressed to 50%) showed that clonidine had no effect on punished responding on all 4 drugs days. Unpunished behaviour during the VI component was consistently suppressed whereas in the third CRF period, significant decreases in responding were only observed on days 1 and 4 respectively. Time-Out responding increased on day 1 but subsequently returned to baseline levels. Results therefore suggest that the anticonflict activity of clonidine is not dependent upon the rate of responding in the initial unpunished period, hence it is unlikely that the release of punished responding with clonidine in the Geller-Seifter schedule was due to the VI-component and the high responding rate during this period.

The lack of effect of clonidine could have been due to rate-dependency. An analysis of interindividual variability with clonidine

and diazepam administered for 4 days in drug- sophisticated animals revealed that diazepam released punished responding in a specific manner irrespective of the pre-existing response rates. Clonidine on the other hand only increased punished responding in "low responders" (animals having a conflict baseline of <5 responses) whereas "high responders" (>6 responses) did not increase punished responding.

The analysis of the interindividual variability with clonidine also confirms the findings of Sepinwall and Cook (1981) who demonstrated that the anticonflict activity of clonidine was only observed if the number of responses during the punished periods was less than 8 responses per minute. The results obtained in this study show a greater sensitivity of clonidine to the rate of punished responding - but this may be due to the dose or the difference in schedule in this study. In order to confirm the lack of effect of clonidine was due to the relatively "high" punished responding rate, the effect of clonidine (for 4 days) on the CRF schedule was investigated. A period of 4 days was chosen since in the Geller-Seifter schedule, initially, clonidine and diazepam were both given for 4 days when their anticonflict effects were examined. The CRF schedule was used so that it could be established that the anticonflict effect of clonidine is not dependent upon a VI-component. Furthermore, for every animal, the rate of punished responding was suppressed so that all the animals had a responding rate of <6 during the punished period.

Clonidine appeared to have a significant anticonflict effect on all 4 days of administration in this CRF schedule which had an altered rate of punished responding. Time-Out responding remained unchanged whereas unpunished responding was intensely suppressed on all 4 days, but returned to pre-injection levels after clonidine was withdrawn. However, it was not possible to reduce time out responding on control days to <6 responses.

The effect of clonidine and diazepam on various schedules showed that the extent to which responding was affected in an unpunished or punished period, also depended upon whether the period was based on a VI or a CRF schedule. For instance, although clonidine reduced unpunished responding in all 4 schedules tested, during both the first and third period, diazepam decreased unpunished responding in

the first period which was based on a VI-30secs, but did not affect the second unpunished period which was on a CRF schedule.

Diazepam increased punished responding in all 4 schedules of conflict behaviour - the largest increase being observed in the schedule when both punished and unpunished periods were on VI-30 secs, and in the former period shock was only delivered on obtaining a reward. In the same schedule clonidine also exhibited an anticonflict activity which was of a higher magnitude than that observed with the schedule comprising a CRF in all periods. For this reason, the schedule consisting of the VI-30 secs component for all 3 periods and that which delivered shock only when the reward was obtainable, was used for subsequent experiments.

The effect of clonidine on responding during the time-out period in all four schedules further confirmed the choice of the schedule to be used for subsequent experiments. Although diazepam increased time-out responding in all four schedules, clonidine had different effects. An increase in time-out responding was observed in the schedule comprising a VI-30 secs for the first unpunished period only this would therefore not be a useful schedule since it would imply that the release of punished responding by clonidine is not a specific effect because time-out has also increased. The CRF schedule showed that clonidine had no effect on time-out responding, whereas both VI-30 sec schedules decreased responding.

The effect of clonidine on time-out responding in the VI-30 sec schedule (where shock was delivered in the punished period only with a reward) indicates that clonidine releases punishment in a specific manner since although time-out responding is reduced, punished responding is significantly increased further confirming the choice of this schedule for subsequent experiments. However, time-out responding rate was not <6 responses. Therefore it is possible that the decrease in time-out responding may have been due to the relative high response rate during this period.

On the VI-30 secs schedule of conflict behaviour, acute RS 21361 caused a suppression of punished responding whereas unpunished responding (in the first and third period) was unchanged as was responding in the time-out. Chronic administration of RS 21361 (for 5

days) showed slight fluctuations in unpunished responding during both periods (although generally unpunished and time-out responding remained unchanged) whereas the reduction in punishment-induced suppression was maintained throughout the 5 days. Results with yohimbine show a similar effect, suggesting that the effect of these α-adrenoceptor antagonists on punished behaviour is a selective one because time-out responding remained unaffected during both acute and chronic administration of these agents.

Both yohimbine and RS 21361 did not alter responding during the first unpunished period. On the basis of the effects of β-CCM in a similar schedule (de Carvalho et al, 1983), this indicated that neither of the agents have an effect of "anticipatory anxiety". It is possible that the failure to detect a decrease in unpunished responding in the first segment with yohimbine and RS 21361 may be due to the difference in time-course of administration as compared to the administration of βCCM (de Carvalho et al, 1983), since behaviour was investigated 3 mins after βCCM injection. However, in general the lack of effect of both RS 21361 and yohimbine on the first and third unpunished periods further confirm the likelihood that these agents do not affect anticipatory anxiety since if they did, a decrease in unpunished responding on 1st exposure in the first period would be expected, whereas in the third period, unpunished responding would remain unchanged.

Results from this study have emphasised the importance of previous drug history in investigating the effects of drugs modulating conflict behaviour. Whereas acute and chronic administration of RS 21361 and yohimbine have shown a lack of effect on anticipatory anxiety, further work is required to establish whether tolerance occurs on a long-term basis. Results have also shown that the VVV-T schedule was the best in yielding maximum effects on punished responding with clonidine, with the least effect on time-out and unpunished responding. For behavioural studies reported in Chapters 5 and 6, this schedule has been used.

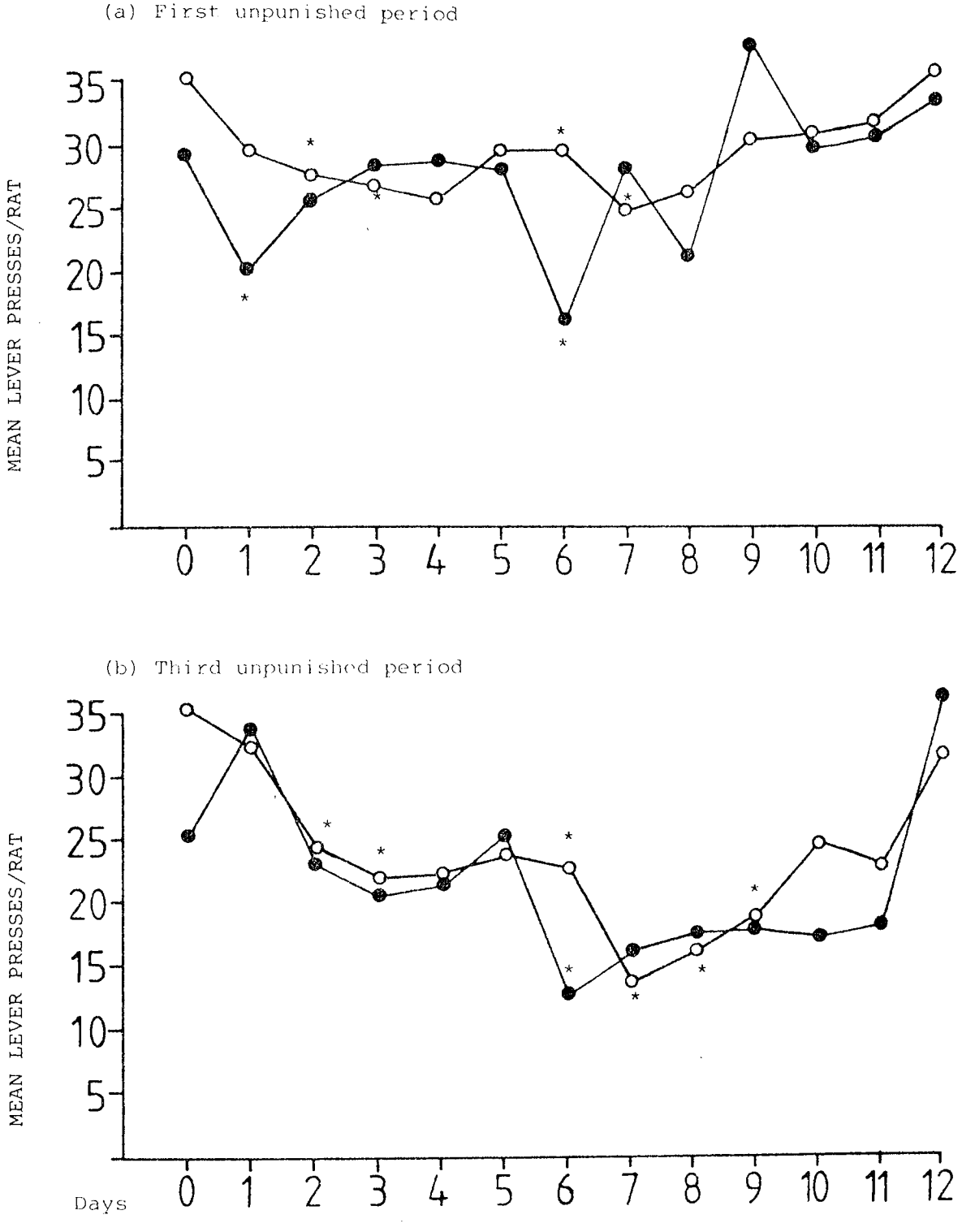


Fig. 4.1 The effect of diazepam 2.5 mg/kg naive and clonidine experienced animals on unpunished responding in a CRF schedule.

(○) Drug naive animals (first experience is diazepam
(●) Clonidine experienced animals.

Diazepam 2.5 mg/kg was injected on Days 1 to 11 (inclusive and saline was given on Days 1 and 12 respectively. Behaviour was looked at 30 mins after injection.

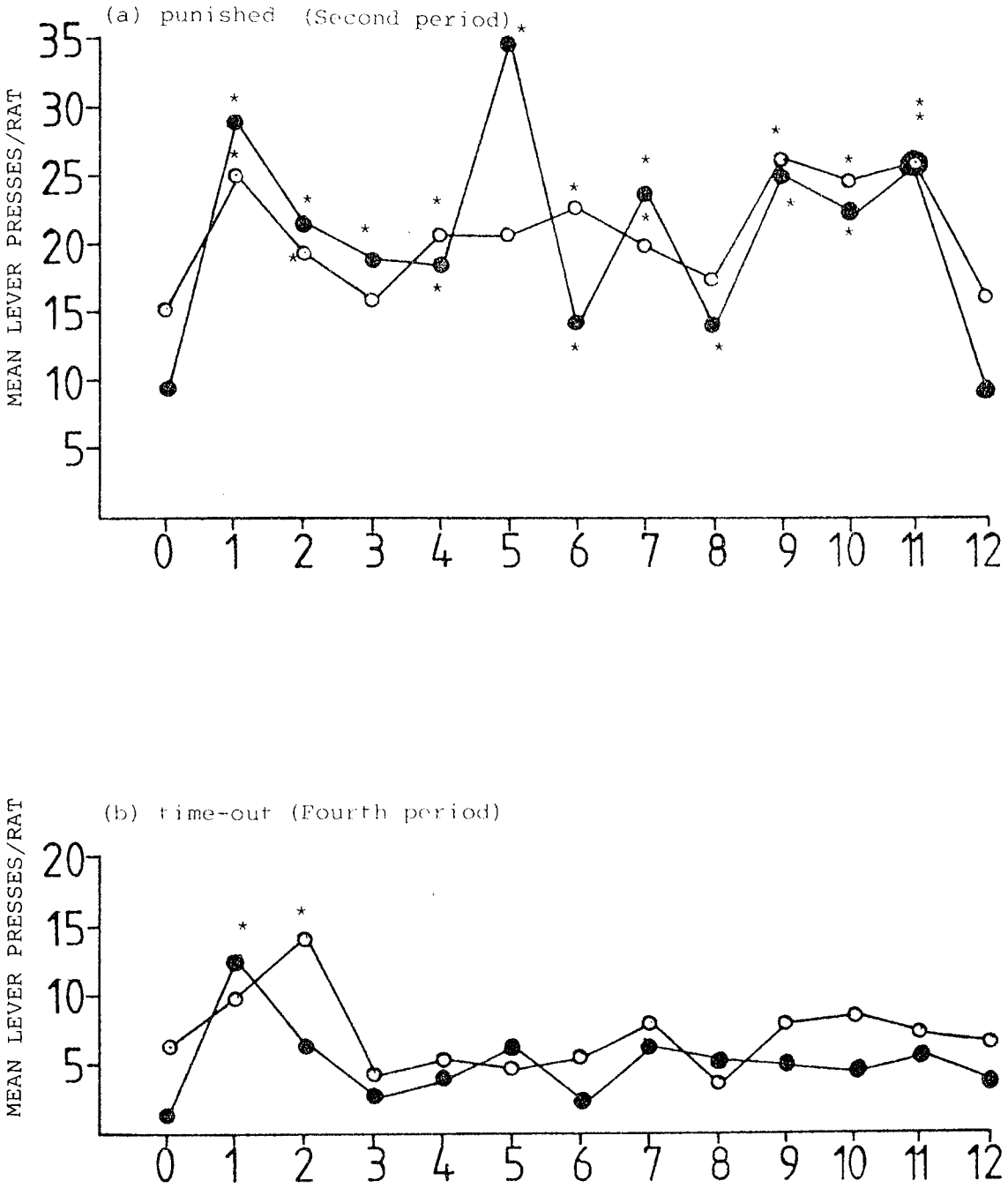


Fig. 4.2 The effect of diazepam 2.5mg/kg in naive and clonidine-experienced animals on (a) punished and (b) time out responding in a CRF schedule

(0) Drug naive animals (first experience in diazepam)
(●) Clonidine experienced animals
Diazepam (2.5 mg/kg) was injected on days 1 to 11 (inclusive) and saline was given on days 1 and 12 respectively. Behaviour was investigated 30 mins after injection.

(○) Drug naive animals (first experience is clonidine);
(●) diazepam experienced animals

Clonidine 0.025 mg/kg was injected on days 1 to 11 (inclusive)
Saline was administered on days 0 and 12 respectively.
Animals were subjected to the test 30 mins after injection.

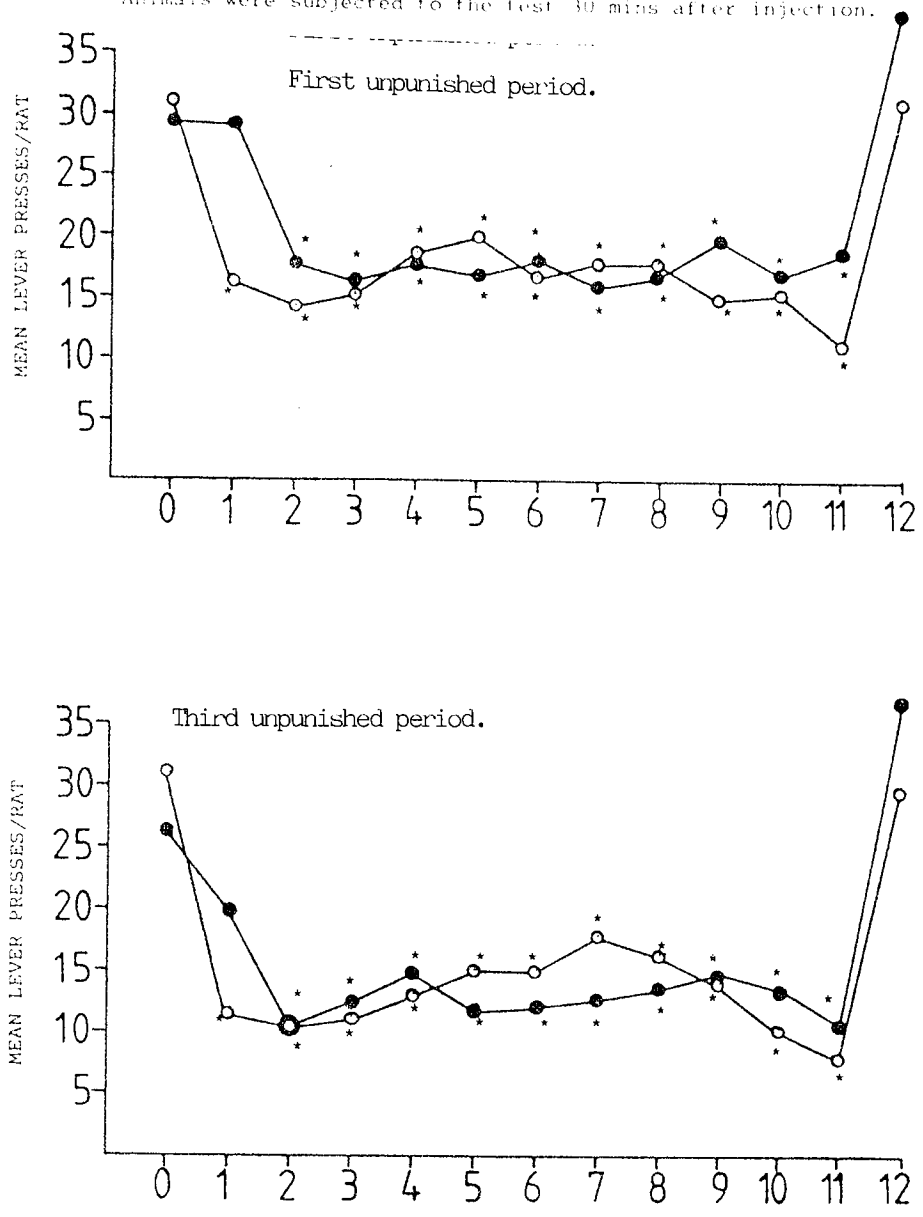


Fig.4.3 The effect of clonidine 0.025mg/kg in naive and diazepam-experienced animals on unpunished responding in a CRF schedule.

(O) Drug naive animals (first experience is clonidine);
(●) diazepam experienced animals

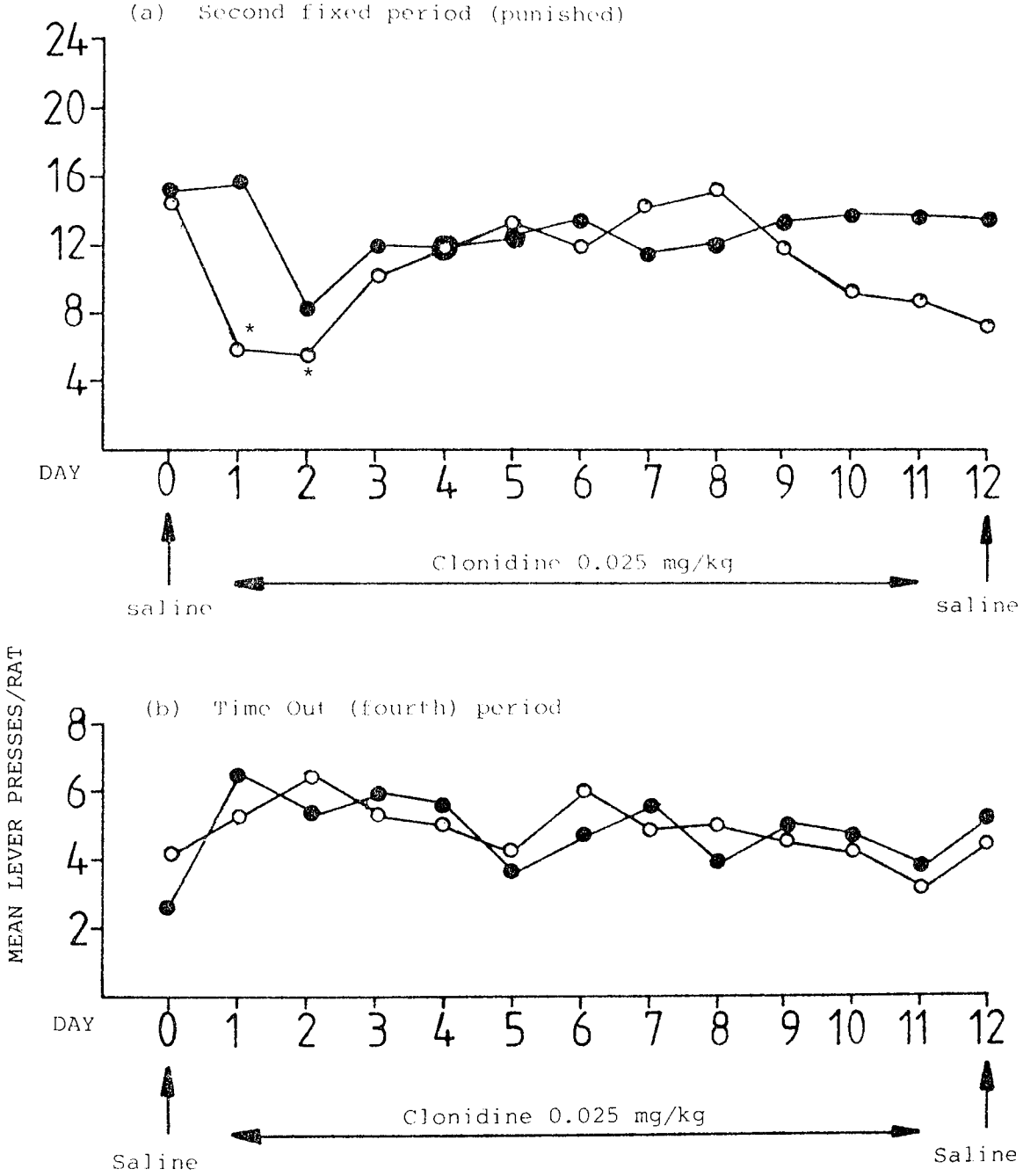


Fig 4.4 The effect of clonidine 0.025 mg/kg in naive and diazepam experienced animals on (a) punished and (b) time out responding in a CRF schedule.

Clonidine 0.025 mg/kg was injected on days 1 to 11 (inclusive), and saline administered on Days 0 and 12 respectively. Behaviour in the Skinner box was investigated 30 mins after injection.

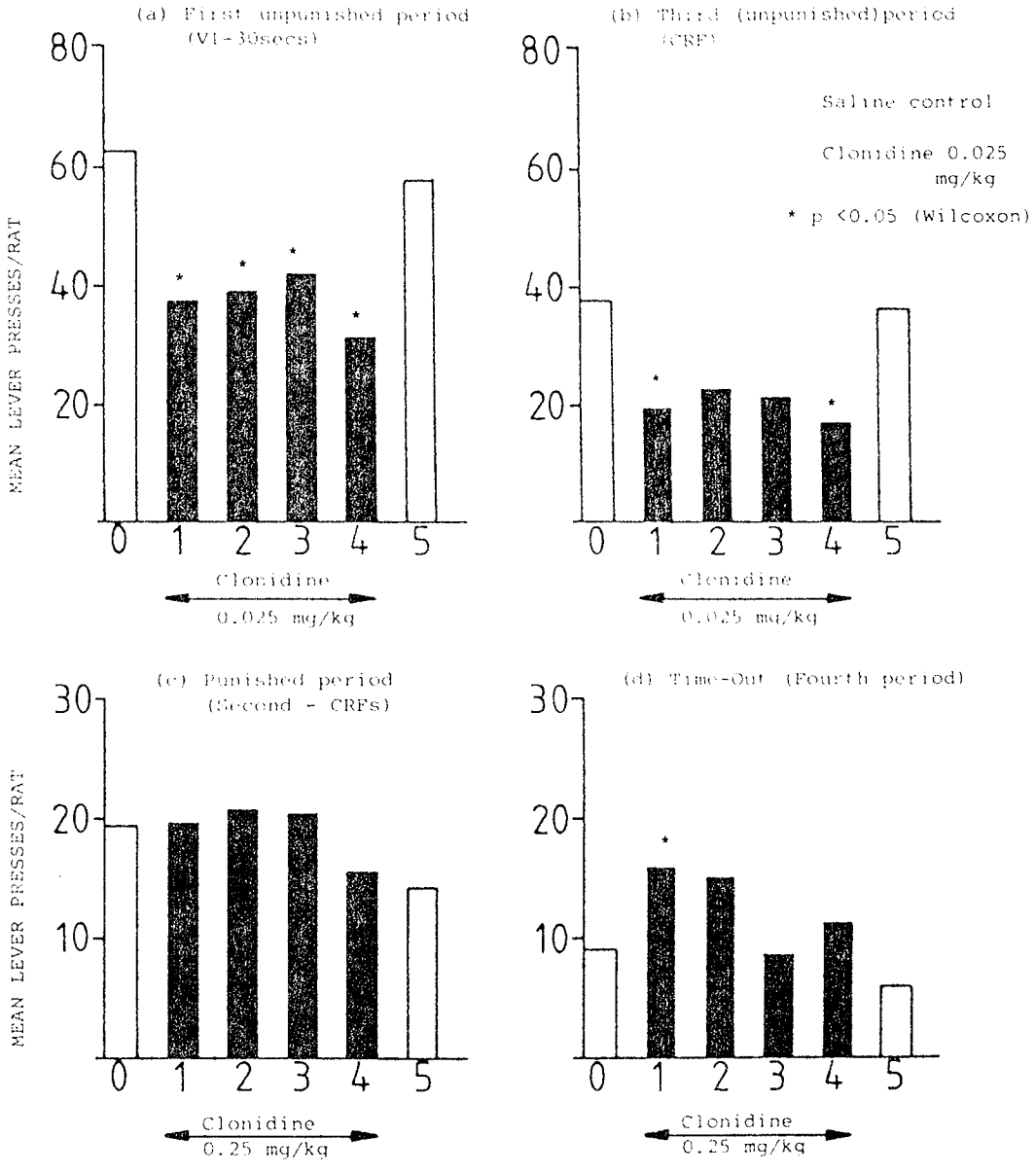


Fig. 4.5 The effect of clonidine 0.025 mg/kg for 4 consecutive days on a variable/fixed schedule of conflict behaviour.
Animals were injected 30 mins before placement in the box.

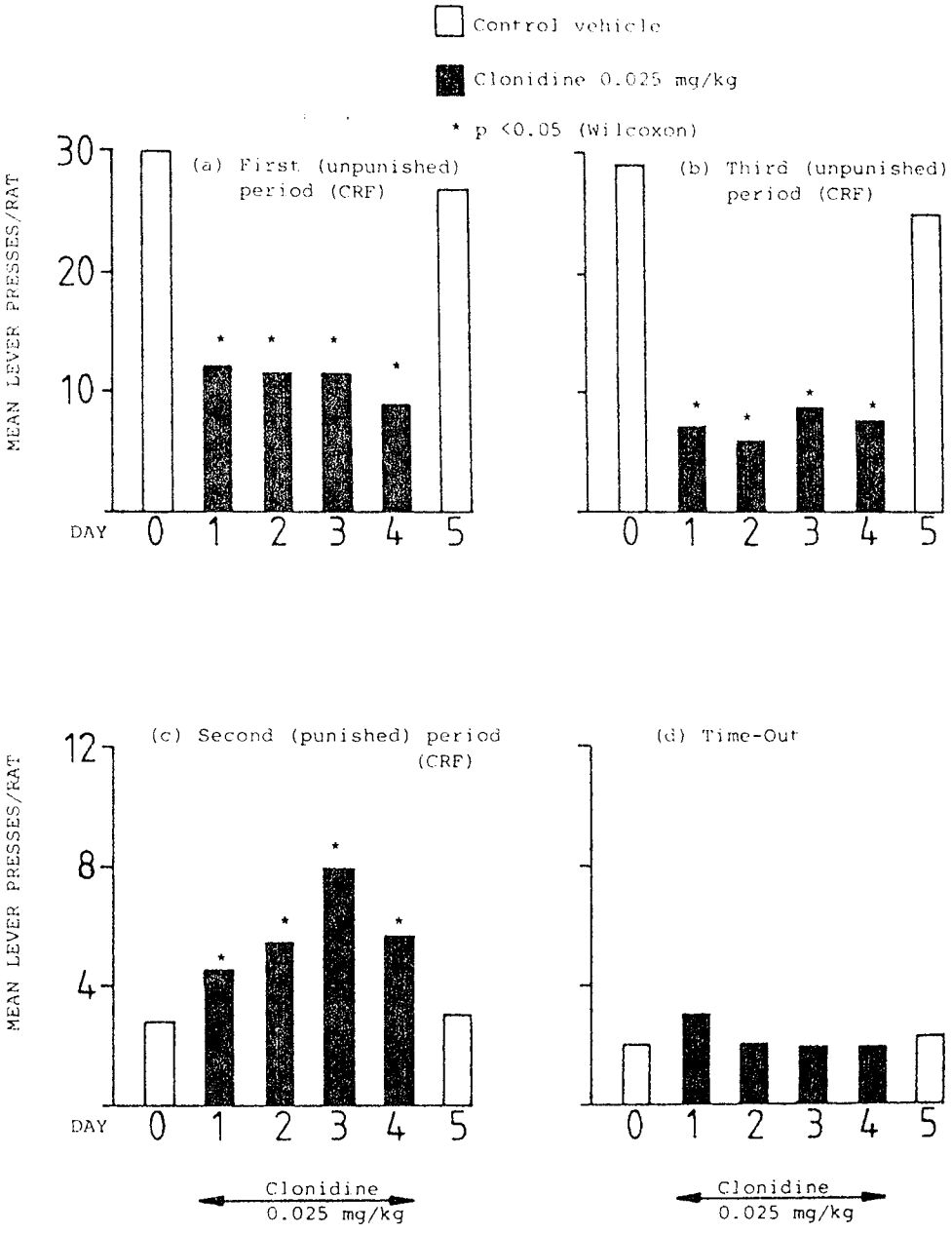


Fig. 4.6 The effect of chronic clonidine in a CRF schedule of conflict behaviour with an altered rate of punished responding.

Animals were injected 30 mins before placement in the box. Punished responding was suppressed so that not more than 6 responses were made during the punished period.

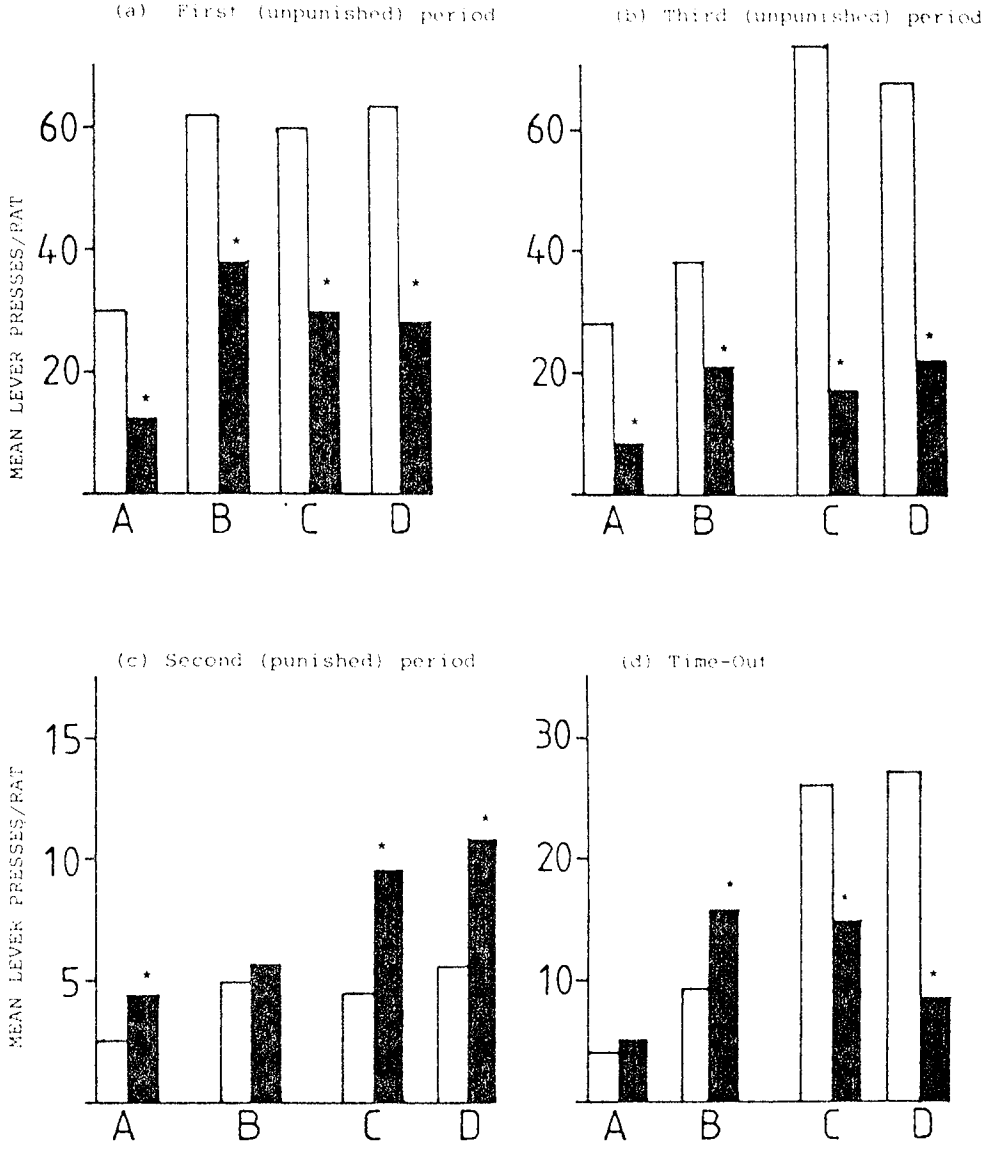


Fig 4.7 The effect of clonidine 0.025 mg/kg in 4 conflict behavioural schedules.

Behaviour was investigated 30 mins post-injection.
(A) FFT-T (B) VFF-T (C) VVsV-T (D) VVV-T

Saline Clonidine 0.025 mg/kg

* $p<0.05$ (Wilcoxon)

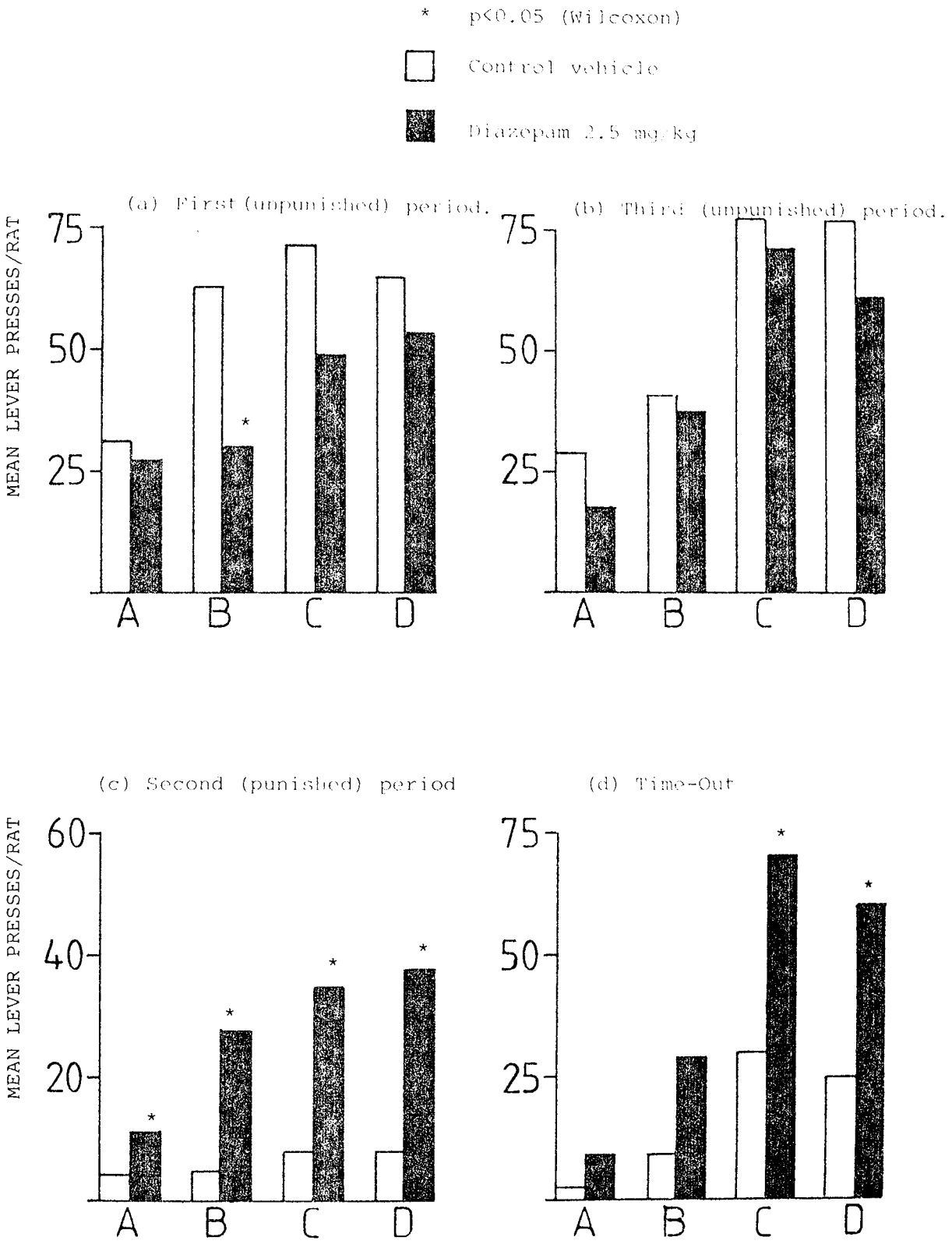
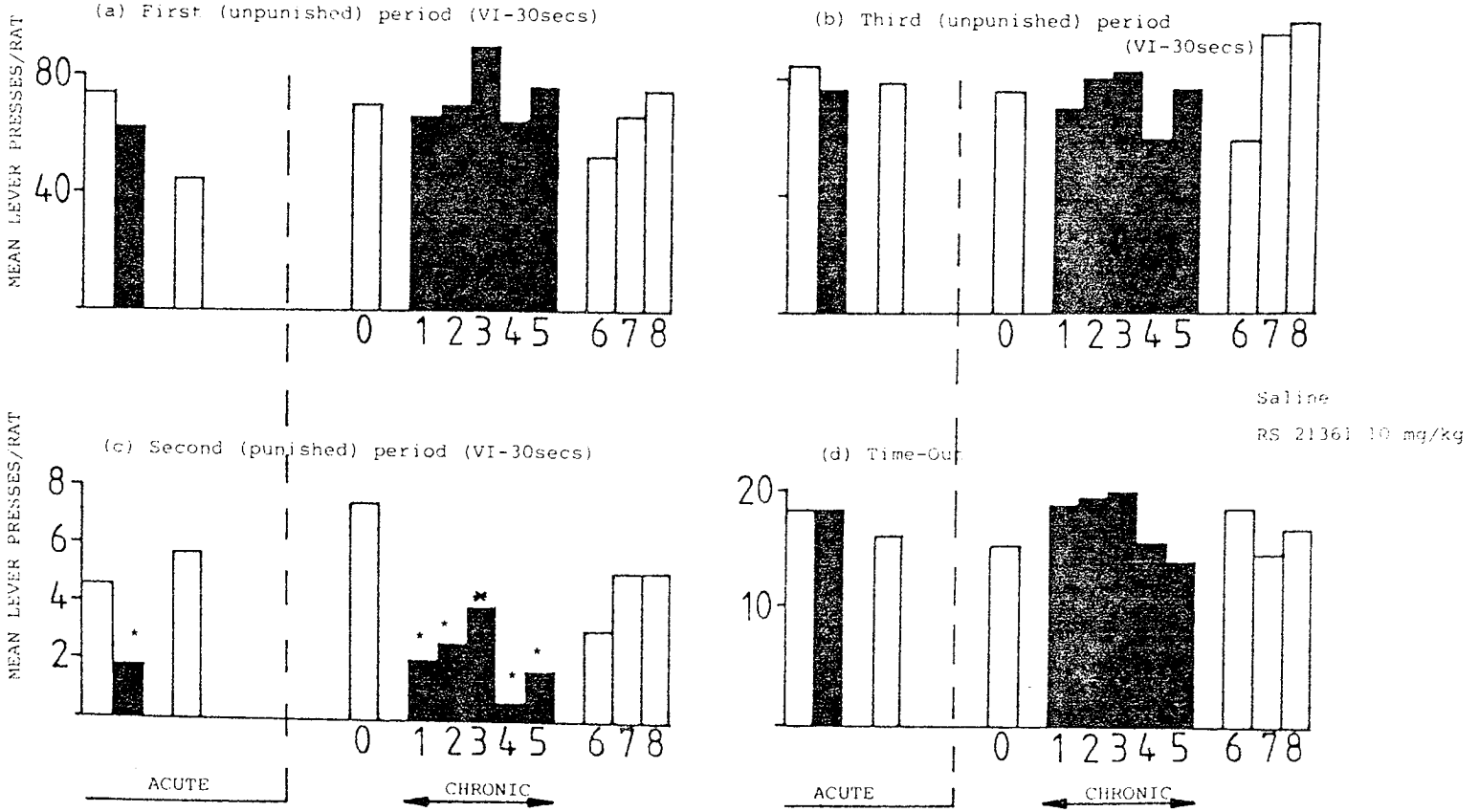


Fig. 4.8 The effect of diazepam 2.5 mg/kg in 4 conflict behavioural schedules. Behaviour was investigated 30 mins after injection.

(A) FFF-T (B) VFF-T (C) VVsV-T (D) VVV-T (see text)

Vehicle control diazepam 2.5 mg/kg

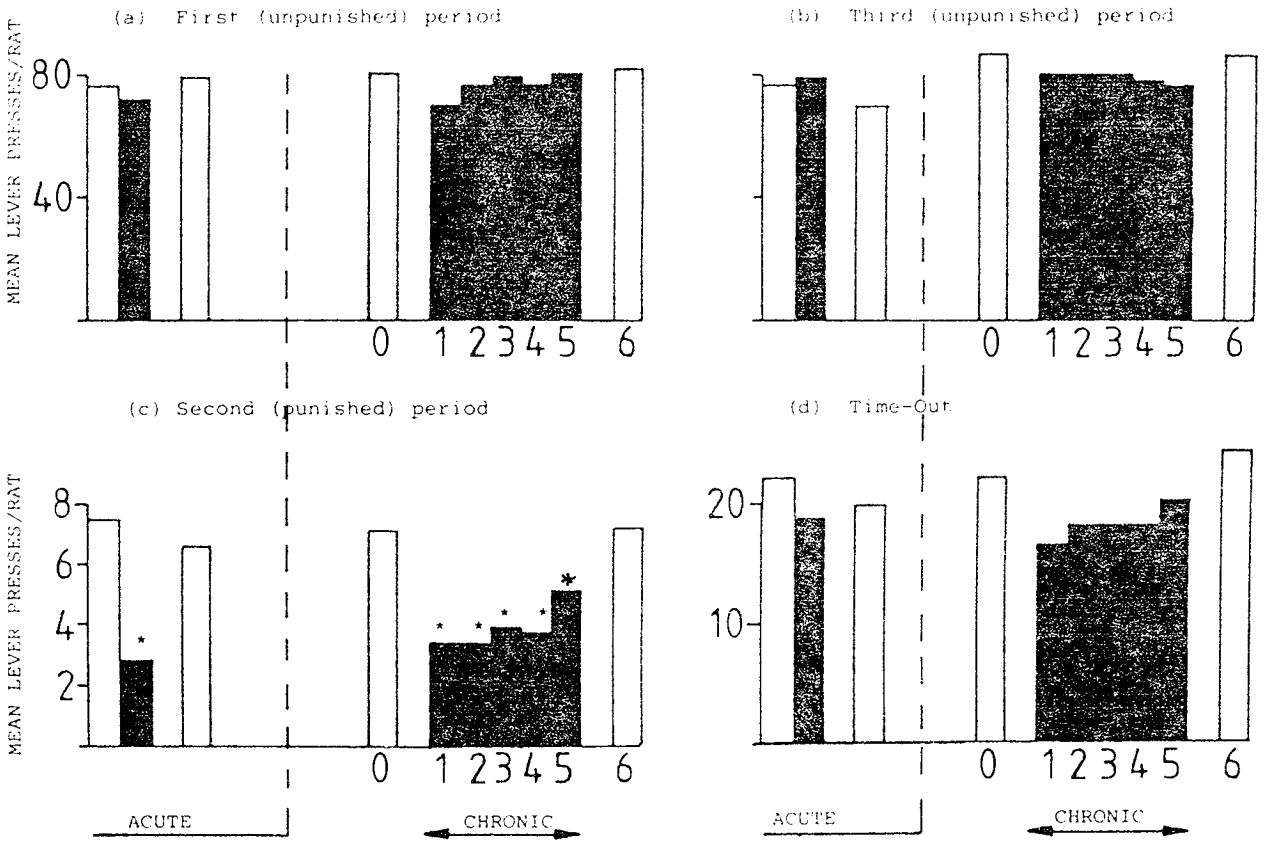
Fig 4.9 The effect of acute and chronic RS 21361 10 mg/kg in a VI-30 sec schedule of conflict behaviour.



Animals were injected 15 mins before placment in the box.

Day 0 - Saline. Days 1 -5 -RS 21361 (10 mg/kg). Days 6 - 8 - Days after withdrawal

Fig 4.10 The effect of acute and chronic yohimbine 2.5 mg/kg in a VI-30 sec schedule of conflict behaviour.



Animals were injected 30 mins before placement in the box.
Day 0 - Saline. Day 1-5 - Yohimbine 2.5 mg/kg. Days 6 - Day after withdrawal

Saline. Yohimbine 2.5 mg/kg

CHAPTER 5

INVESTIGATION OF THE INTERACTIONS BETWEEN α-ADRENOCEPTOR AND 5-HT RECEPTOR LIGANDS IN CONFLICT BEHAVIOUR.

Introduction

The modulation of conflict behaviour by drugs acting at α-adrenoceptors and 5-HT receptors has been established previously in the Geller-Seifter conflict test (Chapter 3), suggesting the involvement of both noradrenergic and serotogenic systems in conflict behaviour. The aim of this study was to examine whether the observed effects of α-adrenoceptor ligands (as shown in the Geller-Seifter test, Chapter 3) could be due to an interaction with 5-HT systems and conversely, whether the observed effects of 5-HT receptor ligands could be due to an interaction with noradrenergic systems.

In this study the schedule similar to that of de Carvalho et al (1983) was used which was slightly modified (see Chapter 4). It consisted of 3 VI-30sec segments of 5 min each, (the middle one having reward-contingent footshock) followed by a 5 min Time-Out period. By examining the effects of α-adrenoceptor agents alone and in combination with 5-HT receptor ligands (and vice versa) in this schedule it would also help in confirming the effects of these agents observed in the Geller-Seifter conflict schedule. Furthermore, it would help distinguish whether anticipatory anxiety was occuring with agents that have not been examined in the previous study (see Chapter 4).

The interaction between α-adrenoceptor and 5-HT receptor ligands in modulating "fear-motivated" behaviour has been examined in a previous study (Chapter 2). Results from this study would help in comparing interactions between these two type of agents in different behavioural models. Furthermore, in this study the interaction of diazepam and ACTH with α-adrenoceptor and 5-HT receptor ligands was also investigated in order to determine whether these agents modulate the effects of ACTH and diazepam.

The doses of the drugs used were chosen from the effects obtained in the Geller-Seifter conflict test (Chapter 3). In every case, the dose of drug used for pretreatment was a sub-anxiolytic or subanxiogenic one (as observed in the study reported in Chapter 3) so that the anxiolytic or anxiogenic profile of the drug used for pretreatment would not affect the action of subsequent drug administration.

1. General comments on inter action in conflict behaviour

In these interaction experiments, two drugs were given. The first one was injected 15 mins before the second, and behaviour was investigated 30 mins after the second injection.

Statistical evaluation for the effect of each drug (compared to previous day control values) was done by means of the Wilcoxan matched pairs-signed ranks test. For comparing the effect of pretreatment with a particular drug, for every animal, the ratio of the control:drug day was calculated. Comparisons were then made using the Mann Whitney 'U' test between the ratios of the values obtained, with the effect of the drug, and the effect of the drug in the presence of another agent.

2. The effect of α-adrenoceptor ligands and quipazine on the antipunishment effect of diazepam.

a. The effect of diazepam in the presence of yohimbine.

Diazepam 1.0 mg/kg increased punished responding with no effect on unpunished behaviour (Table 5.1). Although the dose of yohimbine used did show a slight but significant effect on punished responding, it failed to alter the release of punishment-induced suppression caused by diazepam (Table 5.1). Yohimbine also did not significantly affect the increase in Time-Out responding caused by diazepam. Unpunished responding remained unaltered both in the presence and absence of yohimbine (Table 5.2).

b. The effect of diazepam in the presence of phenylephrine.

Diazepam 1.0 mg/kg increased the rate of responding during the second punished period, and although responding during time-out was increased, it was not statistically significant (Table 5.2). Phenylephrine failed to abolish the effect of diazepam on punished responding, and the presence of phenylephrine did not have an effect on unpunished responding (Table 5.2).

Phenylephrine 0.1 mg/kg alone did not affect responding in any of the periods.

c. The effect of diazepam in the presence of quipazine.

Diazepam released punishment-induced suppression at 1.0 mg/kg and also significantly increased time-out responding (Table 5.1). In the presence of quipazine 1.0 mg/kg, the release in punished

responding was still maintained (Table 5.1) Neither agents (alone or in conbination) had an effect on unpunished reponding.

3. The effect of ACTH in the presence of α-adrenoceptor ligands and ketanserin.

a. The effect of ACTH in the presence of ketanserin.

ACTH alone (0.075 mg/kg) suppressed further responses during the second punished period, but did not alter unpunished or time-out responding (Table 5.3). Pretreatment with ketanserin (0.05 mg/kg) which alone did not have an effect, failed to abolish the reduction in punished responding induced by ACTH (Table 5.3).

b. The effect of ACTH in the presence of clonidine.

Although the significant depressant effect of ACTH on punished reponding in the presence of clonidine (0.005 mg/kg) was observed, this effect appeared to be less marked than that obtained with ACTH alone (Table 5.3). Neither agents (alone or in combination) affected unpunished responding or responses during the time-out period.

c. The effect of ACTH in the presence of prazosin.

Neither prazosin (0.05 mg/kg) nor ACTH (0.075 mg/kg) had any significant effect of time-out or unpunished responding. ACTH significantly decreased punished responding; however, this effect was not blocked by prazosin (Table 5.3).

4. The effect of α-adrenoceptor ligands in the presence of ketanserin.

a. The effect of clonidine.

Clonidine (0.025 mg/kg) markedly suppressed unpunished reponding (in both periods) as well as the Time-Out period (Tables 5.5 and 5.6).

However, punished responding was significantly increased (Table 5.5). At the dose used (i.e. 0.05 mg/kg) ketanserin had no effect.

Table 5.5 shows that ketanserin failed to alter the release of punishment-induced suppression caused by clonidine. The effects of clonidine on unpunished responding and time-out remained unchanged in the presence of ketanserin.

b. The effect of yohimbine.

Yohimbine (2.5 mg/kg) caused a small but significant decrease in punished responding with no effect on unpunished responding or Time-Out (Table 5.5; 5.6). Ketanserin did not alter the effect of

yohimbine on punished responding. The number of lever presses during the punished period still remained suppressed (Table 5.5). However, responding during the Time-Out period was increased in the presence of ketanserin (Table 5.5).

Ketanserin 0.05 mg/kg did not significantly affect responding in any of the periods (Table 5.5; 5.6).

c. The effect of phenylephrine.

0.25 mg/kg phenylephrine significantly decreased both punished and unpunished responding, but Time-Out responding remained unaffected (Table 5.5). Ketanserin 0.05 mg/kg alone had no effect but when the effect of phenylephrine was investigated in the presence of ketanserin, the latter abolished the suppression of unpunished-responding induced by phenylephrine (Table 5.6). Although punished responding was still significantly decreased in the presence of ketanserin, the effect was markedly reduced (as compared to the effect of phenylepherine alone).

The number of lever presses during the time-out period remained unchanged at all times (Table 5.5).

d. The effect of prazosin.

Prazosin 0.05 mg/kg increased punished responding, with no effect on the unpunished periods. In the presence of ketanserin, the increase in punished responding was still maintained, and this increase was potentiated by ketanserin (Table 5.5). Unpunished responding and time-out remained unaffected in the presence of both agents.

Ketanserin alone (0.05 mg/kg) had no effect on punished responding, whereas in the third unpunished period, responding was significantly depressed (Table 5.6).

5. The effect of quipazine in the presence of α-adrenoceptor ligands.

a. The effect of quipazine in the presence of clonidine.

Quipazine 2.0 mg/kg alone significantly decreased punished responding and also decreased the number of lever presses in the third unpunished period (Table 5.7). Since a sub-anxiolytic dose of clonidine was used (0.005 mg/kg), clonidine did not significantly affect responding in any of the periods.

The effect of quipazine in the presence of clonidine showed that both punished, and unpunished responding in the third period were

still significantly depressed (Table 5.7). The effect of quipazine on punished responding in the presence of clonidine was not different to that obtained with quipazine alone (Kruskal-Wallis test). However, responding during the time-out period was significantly increased when both agents were administered (Table 5.7).

b. The effect of quipazine in the presence of prazosin.

Quipazine 2.0 mg/kg markedly depressed responding during the third unpunished and the second punished periods (Table 5.7). The responding during the time-out period was increased by quipazine, although this was not statistically significant.

The effect of prazosin (0.05 mg/kg) in the presence of quipazine is shown in Table 5.7. Although prazosin abolished the effect of quipazine in the third unpunished period, punished responding was still significantly depressed.

Prazosin alone, did not have an effect on unpunished responding, although a slight (but significant) release in punishment induced suppression was shown to occur (Table 5.7).

Discussion

The α-adrenoceptor and 5-HT receptor ligands failed to modify the anticonflict activity of diazepam or the increase in punishment induced suppression produced by ACTH. Although the effect of α-adrenoceptor and 5-HT receptor ligands in the anxiogenic-like activity of ACTH has not been previously examined, in the squirrel monkey, yohimbine appeared to partially block the anticonflict activity of chlordiazepoxide (Sepinwall and Cook, 1981) and intraventricular 5-HT in the rat was shown to decrease the anxiety reducing effect of oxazepam (Stein et al, 1975). In the light of these effects of yohimbine and 5-HT on chlordiazpoxide and oxazepam respectively, both yohimbine and quipazine might be expected to block the anticonflict activity of diazepam.

The lack of effect of yohimbine, phenylephrine and quipazine could have been due to the doses used in this study. For instance, it is possible that at the doses used, both quipazine and yohimbine may have had weak blocking effects on the anticonflict activity of diazepam; however, these effects could not be detected, owing to the massive increase in punished responding produced by 2.5 mg/kg diazepam. Sepinwall and Cook (1981) have reported that yohimbine was able to block the anticonflict effects only at certain dose levels of chlordiazepoxide, which supports the suggestion for the lack of effect of yohimbine, phenylephrine and quipazine.

Another reason for the failure to detect any changes in anticonflict activity of diazepam with yohimbine and quipazine could be due to the schedule used. In the previous studies (Sepinwall and Cook, 1981; Stein et al, 1975) the Geller-Seifter conflict schedule was used, therefore the difference in schedule as well as a species difference (in the case of Sepinwall and Cook (1981), where behaviour was examined in squirrel monkeys) may account for the lack of effect of quipazine and yohimbine in this study.

The failure to modify ACTH induced suppression of punished responding by clonidine, prazosin and ketanserin suggest that the anxiogenic-like activity of ACTH is not influenced by α-adrenoceptor or 5-HT mechanisms. These results are inconsistent with those

of File and Vellucci (1978) who demonstrated changes in 5-HT and 5-HIAA following ACTH administration. However, it is important to note that only one dose of each of these agents have been examined and have been examined and therefore further work will be required to determine whether or not these agents modify the effects of ACTH in conflict behaviaour at a range of doses.

In this behavioural schedule, clonidine alone decreased unpunished responding in both periods as well as the Time-Out, and as expected, released the punishment-induced suppression. On the other hand, yohimbine did not affect unpunished or Time-Out responding, but significantly reduced punished responding. Ketanserin appeared to have no influence on the increase of punishment-induced suppression of yohimbine, or the release of punished responding induced by clonidine. The decrease in unpunished and time-out responding due to clonidine also remained unaffected by the presence of ketanserin.

Although the effect of a 5-HT receptor antagonist on the activity of yohimbine in conflict behaviour has not been previously examined, results in this study are consistent with those of Kruse et al (1981) who demonstrated that in the Geller-Seifter conflict schedule, methysergide was unable to alter the release in punishment-induced suppression caused by clonidine.

Phenylephrine alone caused an intense suppression of both punished and unpunished behaviour in this conflict schedule. However there was no significant effect of time-out responding, suggesting that it had a selective depressant action which was not due to sedation. Ketanserin antagonised the decrease in unpunished responding induced by phenylephrine, and although punished behaviour was still suppressed, the effect appeared to be less marked in the presence of ketanserin. On the basis of this effect, a sub-anxiolytic dose of ketanserin might be expected to significantly potentiate the anticonflict activity of prazosin, but results show that ketanserin failed to influence the increase in punished responding induced by prazosin.

Recent studies have shown that ketanserin possesses α_1-adrenoceptor blocking activity (Kalkman et al, 1983; Fozard, 1982), and the modulating effect of ketanserin on phenylephrine-induced behaviour may be due to its α_1-adrenoceptor effect. Further experiments are required

to determine whether or not ketanserin is able to antagonise the effect of phenylephrine at a range of doses, and whether it modifies the effect of prazosin at doses other than the one used in this study.

The effect of quipazine in this schedule was as expected - punished responding was suppressed and although unpunished behavior was unchanged in the first period, the third period produced a significant decrease in unpunished responding. This decrease may have been a consequence of the occurence of stimulus sensitisation in the punished period. It is possible that quipazine induced a stimulus sensitivation effect, which lasted even after the stimulus was withdrawn (at the end of the punished period), resulting in a reluctance to respond in the third unpunished segment which followed on from the punished period. However, it is also possible that the absorption of quipazine is slow and the anxiogenic-like effect may still be present during the third period, therefore causing a decrease in unpunished behaviour.

Clonidine had no effect on the reduction in punished or unpunished behaviour induced by quipazine, although the combination of clonidine and quipazine increased Time-Out responding. Results demonstrated that at that dose, clonidine does not alter the effect of quipazine although it is not clear why an increase in time-out responding was observed since if anything, a decrease would be expected because clonidine has shown to decrease time-out responding at higher doses (Chapter 4).

Prazosin appeared to have a selective effect on changes in conflict behaviour induced by quipazine. Although the suppression of punished responding was unaffected by the presence of prazosin, the decrease in unpunished responding in the third segment caused by quipazine was abolished. On the basis of the effect of quipazine on unpunished behaviour and the suggestion that it may cause stimulus sensitisation, the effect of which is maintained even after the stimulus is withdrawn, prazosin appears to modify this behaviour. The results suggest that prazosin may be acting by overcoming the anxiogenic-like effect produced by quipazine after stimulus is withdrawn.

From this study it appears that 5-HT mechanisms do not modulate the anxiolytic- and anxiogenic profiles of α-adrenoceptor ligands;

conversely, the anxiogenic-like effect of quipazine is not modified by α-adrenoceptor ligands. It is also apparent that the anticonflict activity of diazepam and the anxiogenic-like effect of ACTH is not influenced by α-adrenoceptor or 5-HT receptor mechanisms. However only one dose of each drug has been investigated and further experiments examining these interactions using a range of doses with both types of drugs would help to clarify whether noradrenergic and serotonergic systems act independently or whether an interaction exists between these two systems in modulating conflict behaviour.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m.			
		Punished responding		Time-Out	
Diazepam	1.0	12.3 ± 1.0	(3.5 ± 0.9)	36.0 ± 6.9	(16.4 ± 6.3)*
Phenylephrine	0.1	2.5 ± 0.6	(4.0 ± 0.6)	19.5 ± 3.2	(16.3 ± 4.1)
Yohimbine	2.5	1.9 ± 0.2	(4.9 ± 0.4)	16.5 ± 1.2	(18.0 ± 3.6)
Quipazine	0.5	3.2 ± 0.4	(4.6 ± 0.8)	16.4 ± 8.1	(26.3 ± 5.5)
Diazepam + Phenylephrine		14.5 ± 2.1	(5.0 ± 1.0)*	30.6 ± 1.8	(17.5 ± 3.1)*
Diazepam + Yohimbine		15.5 ± 2.9	(4.6 ± 1.5)*	48.6 ± 9.3	(18.9 ± 3.3)*
Diazepam+ Quipazine		14.0 ± 3.3	(4.6 ± 2.1)	33.1 ± 3.8	(20.3 ± 4.9)

Table 5.1 The effect of phenylephrine, yohimbine and quipazine on the effect of diazepam on punished responding and time-out. Previous saline control day values are shown in brackets. Phenylephrine, yohimbine and quipazine were injected 15 mins before diazepam, and animals were placed in the box, 30 mins after diazepam injection.

* $p<0.05$ (Wilcoxon) compared to previous saline control day.

† $p<0.05$ (Mann-Whitney-'U' test) compared to diazepam alone.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m. First unpunished period	Third unpunished period
Diazepam	1.0	63.6 ± 3.4 (76.3 ± 5.2)	86.3 ± 6.1 (74.1 ± 3.8)
Phenylephrine	0.1	63.8 ± 4.2 (61.9 ± 3.3)	65.0 ± 4.4 (67.3 ± 2.8)
Yohimbine	2.5	78.3 ± 2.5 (62.0 ± 3.6)	78.0 ± 7.1 (65.4 ± 6.3)
Quipazine	0.5	69.0 ± 6.3 (73.0 ± 8.1)	64.2 ± 3.6 (68.0 ± 6.1)
Diazepam + Phenephrine		71.3 ± 5.4 (70.2 ± 6.5)	76.9 ± 3.3 (84.3 ± 4.8)
Diazepam + Yohimbine		81.3 ± 2.4 (78.4 ± 6.3)	98.6 ± 6.5 (91.1 ± 6.3)
Diazepam + Quipazine		67.3 ± 6.4 (65.0 ± 7.5)	71.6 ± 4.5 (70.1 ± 3.6)

Table 5.2 The effect of phenylephrine, yohimbine and quipazine on the effect of diazepam on unpunished responding.

Previous saline control day values are shown in brackets. Phenylephrine, yohimbine and quipazine were injected 15 mins before diazepam, and animals were placed in the box 30 mins after diazepam injection.

*$p < 0.05$ (Wilcoxon) compared to previous saline control day.

†$p < 0.05$ (Mann-Whitney 'U' test) compared to diazepam alone.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m.	
		Punished responding	Time-Out
ACTH	0.075	0.5 ± 0.3 (4.6 ± 0.2)*	22.0 ± 6.8 (17.0 ± 3.6)
Prazosin	0.05	3.2 ± 0.4 (2.5 ± 0.6)*	16.3 ± 3.9 (20.5 ± 6.4)
Clonidine	0.005	2.7 ± 0.6 (2.3 ± 0.8)	24.5 ± 3.3 (22.3 ± 6.7)
Ketanserin	0.05	3.4 ± 0.3 (3.0 ± 0.3)	23.5 ± 7.5 (26.3 ± 6.8)
ACTH + Prazosin		0.9 ± 0.4 (4.1± 0.3)*	23.3 ± 3.2 (19.2 ± 1.9)
ACTH + Clonidine		1.0 ± 0.6 (4.9 ± 0.4)*	18.4 ± 2.3 (16.6 ± 1.9)
ACTH + Ketanserin		0.6 ± 0.1 (4.2 ± 3.3)*	19.2 ± 3.9 (17.8 ± 3.3)

Table 5.3 The effect of prazosin, clonidine and ketanserin on the effect of ACTH on punished responding and time-out. Previous saline control day values are shown in brackets. Prazosin clonidine and ketanserin were injected 15 minutes before ACTH, and animals were placed in the box 30 minutes after ACTH injection.

*$p < 0.05$ (Wilcoxon) compared to previous saline control day.

†$p < 0.05$ (Mann-Whitney 'U' test) compared to ACTH alone.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m. First unpunished period	Third unpunished period
ACTH	0.075	59.4 ± 3.9 (55.2 ± 8.4)	60.2 ± 5.8 (59.1 ± 6.6)
Prazosin	0.05	57.1 ± 3.3 (62.5 ± 6.7)	62.7 ± 3.0 (64.1 ± 4.3)
Clonidine	0.005	66.3 ± 6.1 (64.1 ± 6.3)	81.4 ± 9.4 (80.3 ± 7.5)
Ketanserin	0.05	70.4 ± 3.9 (66.3 ± 7.2)	63.6 ± 8.5 (70.3 ± 6.7)
ACTH + Prazosin		61.3 ± 3.7 (66.4 ± 5.4)	69.4 ± 7.6 (64.3 ± 3.9)
ACTH + Clonidine		68.3 ± 8.5 (70.4 ± 9.3)	63.7 ± 4.9 (67.1 ± 4.8)
ACTH + Ketanserin		60.8 ± 3.5 (62.7 ± 4.9)	64.6 ± 5.8 (65.0 ± 3.6)

Table 5.4 The effect of prazosin, clonidine and ketanserin on the effect of ACTH on unpunished responding.
Previous saline control day values are shown in brackets.
Prazosin, clonidine and ketanserin were injected 15 mins before ACTH, and animals were placed in the box 30 mins after ACTH injection.

* $p < 0.05$ (Wilcoxon) compared to previous saline control day

† $p < 0.05$ (Mann-Whitney 'U' test) compared to ACTH alone.

DRUG	mg/kg	Mean no.of lever presses ± s.e.m. Punished responding	Time-Out
Phenylephrine	0.5	0.2 ± 0.2 (3.8 ± 0.6)*	16.3 ± 1.7 (18.0 ± 2.8)
Prazosin	0.05	5.9 ± 0.8 (2.2 ± 0.9)*	23.6 ± 6.3 (20.0 ± 4.1)
Clonidine	0.025	7.9 ± 0.4(2.8 ± 0.5)*	8.2 ± 0.9 (26.4 ± 3.1)*
Yohimbine	2.5	1.9 ± 2.1 (5.9 ± 0.4)*	26.3 ± 3.8 (18.4 ± 1.9)
Ketanserin	0.05	3.0 ± 0.3 (3.6 ± 0.4)	28.5 ± 3.8 (26.2 ± 5.9)
Phenylephrine + Ketanserin		1.4 ± 1.0 (5.2 ± 1.0)*	15.4 ± 4.9 (18.9 ± 3.6)
Prazosin + Ketanserin		6.2 ± 1.3 (2.0 ± 0.9)*	22.4 ± 3.1 (18.3 ± 1.8)
Clonidine + Ketanserin		7.9 ± 2.0 (3.9 ± 0.6)*	4.5 ± 0.9 (22.3 ± 4.3)*
Yohimbine + Ketanserin		1.4 ± 0.6 (4.2 ± 0.3)	33.9 ± 8.5 (22.3 ± 6.4)*

Table 5.5 The effect of ketanserin on changes in punished responding and time-out induced by the α-adrenoceptor ligands. Previous saline control day values are shown in brackets. Ketanserin was injected 15 mins before the α-adrenoceptor ligand, and behaviour was investigated 30 mins after last injection.

* $p < 0.05$ (Wilcoxon) compared to previous saline control day.

† $p < 0.05$ (Mann-Whitney 'U' test) compared to α-adrenoceptor ligand alone.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m. First unpunished period	Third unpunished period
Phenylephrine	0.25	38.6 ± 3.4 (60.0 ± 5.7)*	42.3 ± 6.2 (75.4 ± 7.1)*
Prazosin	0.05	66.4 ± 8.5 (59.5 ± 7.4)	61.0 ± 6.4 (63.3 ± 5.8)
Clonidine	0.025	19.5 ± 3.4 (62.4 ± 6.6)*	21.8 ± 3.7 (65.6 ± 3.9)*
Yohimbine	2.5	75.2 ± 6.8 (63.9 ± 4.8)	72.3 ± 6.6 (65.4 ± 3.6)
Ketanserin	0.05	49.8 ± 4.3 (62.3 ± 3.5)	54.3 ± 3.8 (69.1 ± 7.2)
Phenylephrine + Ketanserin		41.3 ± 5.7 (76.3 ± 9.4)*	62.3 ± 6.3 (78.4 ± 3.2)
Prazosin + Ketanserin		60.1 ± 3.9 (63.3 ± 5.4)	60.7 ± 3.4 (68.3 ± 6.7)
Clonidine + Ketanserin		65.1 ± 6.8 (21.3 ± 1.4)*	16.2 ± 1.9 (60.1 ± 6.9)*
Yohimbine + Ketanserin		66.1 ± 3.9 (66.3 ± 4.8)	75.1 ± 3.9 (60.8 ± 5.4)

Table 5.6 The effect of ketanserin on changes in unpunished behaviour induced by the α-adrenoceptor ligands.
Previous saline control day values are shown in brackets.
Ketanserin was injected 15 mins before the α-adrenoceptor ligand, and behaviour was investigated 30 mins after last injection.

* $p < 0.05$ (Wilcoxon) compared to previous saline control day.

† $p < 0.05$ (Mann-Whitney 'U' test) compared to α-adrenoceptor ligand alone.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m.	
		Punished responding	Time-Out
Quipazine	2.0	0.8 ± 0.4 (4.5 ± 0.6)*	28.4 ± 3.8 (19.3 ± 4.2)
Prazosin	0.05	3.9 ± 0.6 (2.3 ± 0.5)*	11.2 ± 1.0 (15.4 ± 1.8)
Clonidine	0.005	2.9 ± 1.0 (3.6 ± 1.2)	26.5 ± 3.9 (22.6 ± 5.1)
Quipazine + Prazosin		0.0 ± 0.0 (3.9 ± 0.4)*	24.5 ± 1.7 (20.8 ± 3.2)
Quipazine + Clonidine		0.4 ± 0.2 (3.0 ± 0.8)*	38.2 ± 6.7 (18.1 ± 3.1)*
		First unpunished period	Third unpunished period
Quipazine	2.0	56.1 ± 3.6 (62.3 ± 4.9)	58.4 ± 8.6 (80.0 ± 9.8)*
Prazosin	0.05	58.4 ± 5.9 (60.3 ± 4.2)	58.1 ± 4.9 (64.3 ± 3.6)
Clonidine	0.005	69.4 ± 3.9 (66.6 ± 5.2)	80.6 ± 7.6 (82.1 ± 9.4)
Quipazine + Prazosin		50.1 ± 8.4 (64.3 ± 7.2)	59.4 ± 5.8 (63.9 ± 7.6)
Quipazine + Clonidine		63.4 ± 6.4 (78.4± 9.1)	64.1 ± 7.9 (80.1 ± 8.5)*

Table 5.7 The effect of prazosin and clonidine on the effect of quipazine in a VI - 30 sec schedule of conflict behaviour. Previous day saline control values are shown in brackets. Clonidine/prazosin was injected 15 mins before quipazine, and behaviour was investigated 30 mins after quipazine injection.

* $p < 0.05$ (Wilcoxon) compared to previous day saline control

† $p < 0.05$ (Mann-Whitney 'U' test) compared to quipazine alone.

CHAPTER 6

BEHAVIOURAL EFFECTS OF L.C. LESIONS.

Introduction

The involvement of the L.C. in aversive-motivated behaviour has been previously examined, yielding inconsistent results (see Introduction - Section 11.1b). It was therefore desirable to investigate the effect of L.C. lesions in maze exploration and conflict behaviour in an attempt to evaluate the role of the L.C. in such behaviour.

Operant conditioning training commenced 2 days after operation although food restriction was initiated 7 days after operation in order to allow the animals to recover. Rats were trained on a VI-30 sec schedule consisting of three VI-30 sec segments of 5 mins each, the middle one having reward-contingent footshock, followed by a time-out period. The effects of L.C. lesions on maze exploration was examined 18 days after operation at the time when animals were "drug naive".

Initially it was hoped that pre-trained animals used in the previous study (Chapters 4 and 5 - obtained from Bantin and Kingman Limited) would be lesioned and that effects of conflict behaviour could be examined on "drug-experienced" animals. When these rats were operated on using pentobarbitone anaesthesia (60 mg/kg ip) death rate of 3/4 occured during operation. When halothane (0.5 - 2.0%) in oxygen/nitrous oxide 20/80 was substituted, 7/10 deaths occured during the operation. Untreated animals showed a death rate of 50% and 40% respectively in this procedure. There are two possible explanations. Firstly, that the trained rats were "drug-experienced" and this resulted in increased sensitivity to the effects of anaesthetics. Secondly, the fact that previously in these laboratories, lesions had only been performed on male hooded rats obtained from Glaxo Research Laboratories, hence animals from Bantin and Kingman may have been more sensitive than those of the Glaxo strain. Consequently, Glaxo strain rats were used for lesioning. Following this animals were trained, exposed to the maze and the effects of α-adrenoceptor and 5-HT receptor ligands were examined starting 21 days after lesioning.

1. L.C. lesioned animals.

Bilateral lesions of the L.C. (with 6-OHDA) were performed in 10 animals and 8 animals were sham-operated. After behavioural experiments were completed various brain regions of 6 of the sham-operated controls and all 10 treated animals were biochemically assayed in order to confirm that treated animals had a sustained depletion of NA in the cortex and hippocampus, and cerebellum with no effect on the hypothalamus, and that DA remained unchanged in all these regions including the striatum (see Table 6.1).

Of the 10 6-OHDA treated animals, 6 showed a severe depletion of NA in the hippocampus and cortex, and cerebellum while having no change in striatal DA and hypothalamic NA. Therefore, statistical evaluation of the behavioural results obtained was carried out using values from the 6 treated animals in which L.C. lesions had been confirmed biochemically.

In the maze exploration study, statistical evaluation was carried out using the Mann-Whitney 'U' test. The Wilcoxon matched pairs-signed ranks test was used to confirm the effects of drugs (as compared to the previous day control values) in conflict behaviour in both groups of animals. The L.C. lesioned animals were compared with the sham-operated for each drug by calculating the ratio of the number of levers pressed on drug day to previous saline control day in each period and for every animal. The ratios of the L.C. lesioned animals were then compared to the sham-operated controls using the Mann-Whitney 'U' test. Statistical evaluation (using the ratios) was also done using the Kruskal Wallis Anova test, and both methods of statistical evaluation gave a similar significant level.

2. The effect of L.C. lesions on maze exploration.

There were no differences between L.C. lesioned animals and sham-operated controls in the open to total ratios (Table 6.2). The total number of entries in the L.C. lesioned animals remained unchanged compared to sham-operated controls. However, in both groups of animals, the open to total ratio as well as the total number of entries was low (compared to control values obtained in the previous study - Chapter 2).

3. The development of conflict behaviour in L.C. lesioned animals.

During the initial magazine training, it was observed that the sham-operated controls learned to associate a lever press with the condensed milk reward within 3 days of daily training for 30 minutes. The L.C. lesioned animals however appeared very frightened when placed

in the box. Three out of the 6 lesioned animals had to be left in the box overnight before they showed any exploration within the box. Subsequently, the lesioned animals were then trained, and it was found that 5 out of 6 animals took 5 days of daily training for 30 minutes before they learned to associate a lever press with the condensed milk reward. In these 5 animals, owing to the slower rate of training, a small container with condensed milk was placed in their cages overnight, so that they could be familiarised with the reward obtainable at every lever response. Animals were subsequently trained as usual. When shock was introduced the L.C. lesioned animals appeared to take a longer time before unpunished responding in the third period was comparable to the first unpunished period.

3.1 The effect of drugs on conflict behaviour.

The effect of diazepam, ACTH and the α-adrenoceptor and 5-HT receptor ligands were examined on conflict behaviour. The doses of the drugs used were chosen from the previous study (Chapter 5) where they were shown to alter conflict behaviour. The schedule used was that described in Chapter 5 - it consisted of three VI-30 sec segments of 5 mins each followed by a 5 min time-out period. Initially, footshock intensity was titrated to produce <6 responses during the second punished period because the effects of anxiolytic-like agents were determined first. Baseline responding was then increased to between 6 and 10 responses thereafter so that the effects of anxiogenic-like agents could be determined.

The effect of drugs were investigated 30 mins post-injection; results are reported in the order of experimentation, such that previous drug experience can be seen by looking at the effects reported earlier.

3.2 The effect of diazepam.

Punished responding was significantly increased in both groups of rats (Table 6.3) with diazepam 2.5 mg/kg, as was responding in the Time-Out period although the increase in the latter for both groups of animals was not as intense (Table 6.3). Unpunished responding was significantly reduced in the first period, whereas the third unpunished period was unaffected. Behaviour in the L.C. lesioned animals was no different from the sham-operated controls in all 4 periods (Tables 6.3; 6.4).

3.3 The effect of clonidine.

Clonidine (0.025 mg/kg) suppressed unpunished reponding in both periods as well as the Time-Out. Punished responding was significantly increased in both groups of animals. Responding during all 4 periods in

the L.C. lesioned animals did not appear to be different from sham-operated controls (Tables 6.3; 6.4).

3.4 The effect of prazosin.

Both groups of animals increased punished responding to a similar extent with prazosin 0.05 mg/kg. Unpunished as well as Time-Out responding were unaffected at this dose of prazosin in both groups of animals (Tables 6.3; 6.4).

3.5 The effect of ketanserin.

Unpunished and Time-Out responding remained unchanged in both groups of animals. Punished responding was significantly increased with ketanserin (0.10 mg/kg). L.C. lesions did not interfere with the increase in punished responding induced by ketanserin (Tables 6.3; 6.4).

3.6 The effect of yohimbine.

Punished responding was intensely suppressed with 2.5 mg/kg yohimbine. Responding during the unpunished periods remained unchanged from previous saline control day, and time-out responding was significantly increased in the sham-operated controls. L.C. lesions did not affect the suppression of punished responding or the lack of effect on unpunished responding. However, although an increase in responding during Time-Out was observed in the L.C. lesioned animals, this was not statistically significant (Tables 6.3; 6.4).

3.7 The effect of phenylephrine.

Phenylephrine 0.5 mg/kg significantly decreased punished responding as well as unpunished behaviour in both L.C. lesioned and sham-operated animals. Time-Out responding remained unaffected in both groups of rats. There were no differences in responding during all 4 period with L.C. lesioned animals (Tables 6.3; 6.4).

3.8 The effect of quipazine.

Quipazine 1.0 mg/kg did not affect unpunished behaviour or time-out responding in either group of rats. L.C. lesioned and sham-operated animals both decreased punished responding to a similar extent with this dose of quipazine (Tables 6.3; 6.4).

3.9 The effect of ACTH.

Only punished responding was significantly altered with ACTH 0.075 mg/kg. On drug day both L.C. lesioned animals and sham-operated controls intensely suppressed punished behaviour. Unpunished responding as well as Time-Out were unaffected in both groups of animals (Tables 6.3; 6.4).

Discussion.

In the X maze exploratory activity was generally lower than that with animals used previously (see Chapter 2). Various factors may have contributed to this reduction in exploratory activity. For instance, animals used in this study were larger than those used in Chapter 2 (see methods) therefore unsatisfactory maze dimensions (with respect to accommodating larger animals) may account for the reduced exploratory activity observed in both groups of rats. During these experiments it appeared as if animals were uncomfortable in turning around within any enclosed or open arm because of their larger size. Moreover, exploratory activity also decreases with age (see Barnett, 1958) hence it would be expected that both L.C. lesioned and sham-operated controls would explore considerably less than control animals used previously (Chapter 2).

Food restriction and isolation may also account for the general reduction in exploratory activity observed in these animals. File and Day (1972) reported that increased food deprivation from 2 - 6 hours results in a significant linear increase in exploration although Halliday (1968) showed that food deprivation only increased exploratory activity until the animal finds that there is no food in the situation; thereafter, exploratory activity decreases with deprivation. In another study, rats kept on a restricted diet (maintained on 7g of food per day for 6 days) showed no increase in amount of exploratory behaviour over rats allowed free access to food (Montgomery, 1953).

In the present study animals had been maintained on a restricted diet for 18 days before the maze experiments were performed. They were fed at 17.00 hours each day hence food deprivation as well as a restricted diet maintenance could be in part responsible for the reduction in exploratory activity. Furthermore animals were singly caged (unlike previous experiments (Chapter 2) where they were caged in groups of 6's) and this may also have affected their behaviour in the maze.

The difference in exploratory activity in these animals (compared to controls in other maze experiments - Chapter 2) may also be a reflection in the emotionality of the rats used since differences have been found in behavioural measures of anxiety in rats of the same

strain obtained from different sources (File and Vellucci, 1979). Rats used in this study were of the same strain but were from Glaxo (see Introduction) which may also account for the reduced exploratory activity in sham-operated controls.

L.C. lesioned animals showed no differences to sham-operated controls in the open to total ratio in the maze, suggesting that the L.C. does not modulate fear-motivated behaviour in this model. Results are consistent with those of Crow et al (1978) who demonstrated that 6-OHDA lesions lateral to the L.C., which decrease cortical and cerebellar NA, have no effect on a social interaction test of anxiety. However, in the "open-field" test, L.C. lesions reduced exploratory activity, (Kostowski, 1980) an effect that was not observed in the maze, since the total number of entries were not significantly changed. It is unlikely that the failure to detect any change in exploratory activity was due to the maze dimensions (see above) since both groups would be equally affected, although the fact that a different measure of exploratory activity was used in this study may account for the discrepancy from the work of Kostowski (1980).

Initial magazine training commenced 2 days after operation for both groups of animals so as to ensure that L.C. lesioned animals were conditioned before the development of the lesion. Therefore if change did occur in their baseline response rate 7 - 10 days after operation this could be attributed to the impairment in learning or memory caused by the lesion. Training for operant conflict in L.C. lesioned animals and sham-operated controls showed slight differences. The former appeared to be very slow in responding, explored less in the Skinner box and took a longer time to "shape up". It has been demonstrated that the destruction of the L.C. leads to an impairment in the acquisition of both one- and two-way avoidance (Ogren et al, 1980) which may parallel the delay in learning in this study, although at the time of initial training lesions would not have developed. 6-OHDA has been shown to increase aggression in animals (Sorensen and Gordon, 1975) hence the effect of 6-OHDA administration may explain, in part, the delay in learning. However, further work requires to be done on learning effects after lesions before any firm conclusions can be made.

The effect of diazepam, ACTH and the α-adrenoceptor and 5-HT receptor ligands on conflict behaviour in sham-operated controls demonstrated that the effects of these agents were reproducible; effects similar to those reported in Chapter 5 were obtained.

It is apparent from the results in this study that the L.C. does not mediate the anticonflict effects of diazepam or anxiogenic-like action of ACTH since the effects of these agents on punished responding were unaffected by L.C. lesions. Furthermore, the decrease in punished responding during the first period with diazepam was also observed in lesioned animals, suggesting that the depressant activity is not modulated by the L.C. system.

The effects of clonidine and yohimbine were also unaffected by L.C. lesions. Clonidine suppressed unpunished responding and released punishment-induced suppression. Although the effect of L.C. lesions on clonidine or yohimbine in conflict behaviour have not been previously examined, results are consistent with those of Davis et al (1977) who demonstrated that clonidine also depressed acoustic startle reflex in rats with bilateral electrolytic lesions of the L.C. The effect of clonidine on unpunished responding may be due to its sedative effects (see Chapter 4) and the failure to modulate unpunished responding with clonidine in L.C. lesioned animals is also consistent with results obtained by Nassif et al (1983) who demonstrated that the sedative effect of clonidine in 6-OHDA L.C. lesioned animals was the same as in sham-operated controls.

The present results suggest that the effect of yohimbine and clonidine on conflict behaviour is not due to their effects on presynaptic α_2-adrenoceptors located in the terminals or cell bodies of the L.C. system. Two explanations can be offered for this effect. First, given the widespread distribution of α_2-adrenoceptors in the brain (Young and Kuhar, 1981), their effects would be due to an action on α_2-adrenoceptors located pre-synaptically on the terminals or cell bodies of the lateral and dorsal tegmental cell groups (see Moore, 1980) which of course, are spared after the L.C. lesion. The second possibility is that the effect on conflict behaviour might result from an action on postsynaptic α_2-adrenoceptors, although if this was so, then effects of yohimbine and clonidine would be expected to be

potentiated (see Nassif et al, 1983).

The effects of α_1-adrenoceptors and 5-HT receptor ligands and ACTH showed a similar picture. L.C. lesions did not alter their actions on conflict behaviour suggesting that an intact L.C. system is not required in mediating the anxiogenic-like and anticonflict effects of the agents.

Results in the present study have suggested that the L.C. is not involved in the modulation of conflict behaviour produced by the α-adrenoceptor and 5-HT receptor ligands. The involvement of the ventral bundle or the raphe nuclei in mediating these effects have not been examined with these agents and further work examining the effects of these agents on conflict behaviour in ventral bundle lesioned animals would help clarify whether noradrenergic systems play a role in mediating the effects of these agents on aversive motivated behaviours.

Brain Region	Controls n = 6	6-OHDA Treated n = 6
	Noradrenaline ng/g	
Hippocampus + Cortex	285.5 ± 8.1	55.1 ± 8.2***
Cerebellum	457.7 ± 7.9	99.2 ± 8.5***
Hypothalamus	1434.3 ± 21.2	1520.4 ± 17.6
	Dopamine (ng/g)	
Hippocampus + Cortex	151.4 ± 29.8	127.3 ± 8.9
Striatum	3009 ± 22.3	2895 ± 50.3
Hypothalamus	231.1 ± 18.6	221.4 ± 9.5

TABLE 6.1 Regional concentrations of noradrenaline and dopamine in LC lesioned and control animals. *** 2p <0.001 (Student's 't' test)

	Number of entries ± s.e.m.		Open =
	Open	Total	Total Ratio
Sham-operated n = 8	0.25 ± 0.1	2.5 ± 0.6	0.056 ± 0.03
L.C.lesioned n = 6	0.20 ± 0.2	2.5 ± 0.9	0.05 ± 0.01

TABLE 6.2 The effect of L.C.lesions on maze exploration Animals were tested 18 days after lesions were performed.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m. Sham operated	L.C. lesioned
(a) Punished responding (VI - 30 secs)			
ACTH	0.075	1.8 ± 0.4 (6.3 ± 3.5)*	0.8 ± 4.4 (6.8 ± 0.9)*
Diazepam	2.5	42.5 ± 9.3 (3.5 ± 0.9)*	31.0 ± 5.1 (3.5 ± 1.2)*
Clonidine	0.025	13.5 ± 4.4 (5.4 ± 2.2)*	8.5 ± 2.3 (2.8 ± 0.7)*
Yohimbine	2.5	1.0 ± 0.9 (5.9 ± 1.2)*	1.3 ± 0.7 (6.3 ± 1.0)*
Phenylephrine	0.5	1.1 ± 0.27 (6.9 ± 1.0)*	0.3 ± 0.4 (6.6 ± 0.5)*
Prazosin	0.05	7.4 ± 0.4 (2.6 ± 1.4)*	8.3 ± 1.9 (3.5 ± 1.7)*
Quipazine	1.0	0.9 ± 0.6 (6.6 ± 1.3)	0.3 ± 0.2 (6.5 ± 0.8)*
Ketanserin	0.1	7.1 ± 2.3 (3.8 ± 1.1)*	9.6 ± 1.6 (2.9 ± 0.6)*
(b) Time-Out			
ACTH	0.075	11.4 ± 3.1 (19.4 ± 3.1)	7.8 ± 4.3 (5.2 ± 3.1)
Diazepam	2.5	46.5 ± 5.9 (21.5 ± 2.1)*	37.6 ± 8.1 (27.3 ± 6.1)*
Clonidine	0.025	8.9 ± 1.9 (22.0 ± 5.1)*	7.6 ± 2.5 (21.6 ± 4.1)*
Yohimbine	2.5	24.5 ± 15.6 (11.1 ± 6.4)*	15.0 ± 3.7 (11.7 ± 3.7)
Phenylephrine	0.5	13.8 ± 4.6 (17.8 ± 2.6)	8.2 ± 1.3 (4.8 ± 1.8)
Prazosin	0.05	11.6 ± 1.9 (11.4 ± 3.7)	13.2 ± 3.7 (9.7 ± 3.3)
Quipazine	1.0	14.0 ± 1.5 (13.3 ± 4.4)	8.6 ± 2.6 (10.3 ± 8.5)
Ketanserin	0.1	13.3 ± 5.4 (12.8 ± 3.6)	8.7 ± 1.4 (9.6 ± 1.5)

Table 6.3 The effect of L.C. lesion on (a) punished and (b) time-out responding in a VI - 30 sec schedule with α-adrenoceptor, 5-HT receptor ligands, diazepam and ACTH
Previous salaine control day values are shown in brackets. Drugs were administered 30 mins before placement in the box.

* $p<0.05$ (Wilcoxon) compared to previous saline control day.

+ $p<0.05$ (Mann-Whitney 'U' test) compared to sham-operated controls.

DRUG	mg/kg	Mean no. of lever presses ± s.e.m. Sham-operated	L.C. lesioned
(a) First unpunished period (VI - 30 secs)			
ACTH	0.075	59.3 ± 3.3 (59.3 ± 4.5)	49.8 ± 6.3 (54.5 ± 5.2)
Diazepam	2.5	21.5 ± 2.1 (49.6 ± 5.2)*	24.7 ± 5.8 (44.8 ± 9.2)*
Clonidine	0.025	30.8 ± 6.0 (50.6 ± 5.2)*	19.3 ± 4.6 (41.3 ± 8.7)*
Yohimbine	2.5	65.5 ± 7.2 (64.2 ± 8.4)	58.7 ± 1.0 (59.1 ± 7.1)
Phenylephrine	0.5	38.1 ± 3.2 (59.8 ± 5.9)*	25.3 ± 3.1 (57.3 ± 5.6)*
Prazosin	0.05	50.3 ± 3.9 (65.0 ± 4.3)	53.8 ± 6.8 (54.2 ± 8.9)
Quipazine	1.0	58.9 ±4.5 (52.4 ± 5.4)	71.1 ± 2.9 (61.0 ± 6.7)
Ketanserin	0.1	57.8 ± 6.5 (54.3 ± 3.9)	58.1 ± 6.3 (52.3 ± 7.8)
(b) Third unpunished period (VI - 30 secs)			
ACTH	0.075	63.0 ± 3.3 (64.5 ± 6.3)	53.2 ± 6.6 (48.5 ± 1.2)
Diazepam	2.5	61.9 ± 15.3 (48.3 ± 6.4)	55.5 ± 8.3 (47.0 ± 7.3)
Clonidine	0.025	21.9 ± 7.5 (51.1 ± 4.8)*	21.3 ± 7.7 (43.7 ± 9.8)*
Yohimbine	2.5	62.0 ± 6.9 (55.6 ± 3.4)	68.7 ± 9.9 (49.5 ± 1.0)
Phenylephrine	0.5	34.3 ± 3.6 (54.6 ± 3.8)*	35.3 ± 4.1 (64.8 ± 4.3)*
Prazosin	0.05	49.4 ± 7.2 (52.5 ± 4.9)	47.3 ± 1.1 (49.7 ± 1.3)
Quipazine	1.0	58.5 ± 4.6 (63.4 ± 6.6)	52.5 ± 9.2 (58.7 ± 2.3)
Ketanserin	0.1	58.9 ± 4.9 (58.5 ± 6.3)	58.2 ± 7.5 (56.3 ± 5.6)

Table 6.4 The effect of L.C. lesions on (a) first and (b) third unpunished periods in a VI - 30 sec schedule, with α-adrenoceptor, 5-HT receptor ligands, diazepam and ACTH.

Previous saline control day values are shown in brackets.

Drugs were administered 30 mins before placement in the box.

* $p < 0.05$ (Wilcoxon) - compared to previous saline control day.

+ $p < 0.05$ (Mann-Whitney 'U' test) compared to sham-operated controls.

CHAPTER 7

REGIONAL CHANGES IN BRAIN 5-HT AND 5-HIAA CONCENTRATIONS AFTER ANXIOLYTIC AND PUTATIVE ANXIOGENIC ADMINISTRATION.

Introduction

Considerable attention has been focussed on the role of 5-HT in mediating the anxiolytic effects of benzodiazepines. There have been many previous reports that benzodiazepines reduce central 5-HT turnover (Stein et al, 1975; see Haefely et al, 1981). Indeed, it has been suggested that their anxiolytic effects may be due to a reduction in the activity of a serotonergic punishment system (Wise et al, 1972). File and Vellucci (1978) have further suggested that since ACTH caused a large elevation in brain concentration of 5-HIAA, one of the critical events in the induction of anxiety may be the stimulation of 5-HT pathways by ACTH, and that anxiolytics could act by counteracting this effect. These findings suggest that serotonergic systems may be involved in drug effects on anxiety. However the effect of these in discrete brain regions have so far received relatively little attention (Haefely et al 1981; File and Vellucci, 1978) and other drugs such as the "anxiogenic" agent picrotoxin (File and Lister, 1983) does not appear to have been extensively studied. Interpretation of the effects is further complicated by the fact that they have not been compared directly with each other under standard conditions.

As an initial approach, it was desirable to undertake a systematic examination of the effects of anxiolytics and putative anxiogenic agents on the concentrations of 5-HT and 5-HIAA in six brain regions, in order to investigate whether they share effects on regional serotonergic function consistent with a central role for serotonergic systems in anxiety.

The design of the present experiments was dictated partly by the decision to use rats after they had been evaluated in the maze (Chapter 2). By performing both experiments in the same animals, it was hoped that any congruence between fear-motivated behaviour and biochemical effects would become apparent. For this reason a more direct measure of 5-HT turnover for example, that of determining 5-HIAA levels after probenecid, could not be used, since prior administration of probenecid may have affected the absorption or elimination of certain drugs, and therefore would alter the time at which behavioural effects would be observed, depending on the extent to which absorption had been retarded by the presence of probenecid.

The rats used here were killed 3 hrs post-injection. The choice of 3 hrs as the time of determination was arrived at from a consideration of the different drugs which were investigated. The effect of diazepam on brain 5-HT and 5-HIAA has been shown to be apparent at this time (Jenner et al, 1975). Furthermore, the effect of α-adrenoceptor ligands on brain 5-HT and 5-HIAA were also to be determined (Chapter 8), and both clonidine and yohimbine have been shown to have an effect on brain indoleamines at this time (Reinhard and Roth, 1982; Papeschi et al, 1971).

In the present experiments, the concentrations of 5-HT and 5-HIAA have been studied after various doses of picrotoxin, amylobarbitone, acute and chronic diazepam, and ACTH. However, the effect of ACTH was also examined 10 and 30 mins post-injection for comparison with the findings of File and Vellucci (1978) and also because at 10 mins post-injection ACTH would be unlikely to have triggered corticosterone production (Hodges and Sadow, 1967), hence the effect (if any) would not be due to corticosterone release. The effect of these drugs were investigated in six brain regions which include pons-medulla, midbrain, hypothalamus, striatum, hippocampus and cortex (details of dissection are described in "Methods").

For every experiment, controls were performed since levels of 5-HT and 5-HIAA to be detected were very low (nanogram (ng) range) and slight changes in the technique would affect the values obtained. Also, although the recovery was shown to be fairly consistent for each assay (see "Methods") a small difference in recovery would cause a relatively large variation in the regional concentrations of 5-HT and 5-HIAA.

For display in figures, results are expressed as

$$\frac{[\text{Mean concentration of 5-HT or 5-HIAA}]\ \text{drug treated}}{[\text{Mean concentration of 5-HT or 5-HIAA}]\ \text{control}} \times 100\%$$

since this gives a clear representation of changes in brain indolamines. However, actual values for each groups (mean ± s.e.m.) are shown in Tables 7.1 - 7.7.

1. The effect of prior exposure to the maze.

It was essential to establish whether 5-HT and 5-HIAA would be affected if rats had been exposed to a novel environment (i.e. maze study, Chapter 2), since if so, the effect of drugs on brain 5-HT and 5-HIAA could also be influenced by their previous experience.

Table 7.1 shows that there was no significant difference in 5-HT or 5-HIAA in any brain region between rats which had been exposed to the maze 3 hrs previously compared with rats which had only been handled at this time.

2. The effect of acute diazepam.

Diazepam 0.5 - 10.0 mg/kg generally increased 5-HT in all regions except the pons-medulla where 1 - 5 mg/kg caused a significant fall in 5-HT. In the midbrain, hypothalamus and striatum the rise in 5-HT appeared to be biphasic, with the two middle doses 2.0 and 5.0 mg/kg producing a lesser effect. In the hippocampus, 0.5 mg/kg showed a slight (insignificant) decrease, and 5.0 mg/kg a significant decrease in 5-HT. A similar effect with the 5.0 mg/kg dose in the cortex was also observed. 5-HIAA was generally reduced for all doses in the midbrain, striatum, and hippocampus. In the hypothalamus, all doses except 10 mg/kg reduced 5-HIAA, and in the cortex 5.0 mg/kg caused a significant rise in 5-HIAA. In the pons-medulla 5-HIAA was reduced in all doses except 5.0 and 10.0 mg/kg where significant increases in this metabolite were observed (Tables 7.1 - 7.3).

3. The effect of chronic diazepam.

Chronic 1.0 mg/kg diazepam (given daily for 6 days, and killed 3 hrs after last injection) caused a significant decrease in hippocampal 5-HT. 5-HIAA was significantly decreased in the pons-medulla only - an effect similar to that obtained with the acute dose of diazepam (see Table 7.1). Chronic 5.0mg/kg generally decreased 5-HT in the hypothalamus, cortex and midbrain (significantly only in the latter), but caused a significant fall in 5-HT in the pons-medulla, striatum and hippocampus. 5-HIAA decreased significantly in the midbrain, hypothalamus and striatum, whereas an increase was obtained in the hippocampus. Cortical 5-HT and 5-HIAA levels at this chronic dose were not significantly changed. At 10 mg/kg, 5-HT was generally

increased in all regions except the pons-medulla. 5-HIAA was significantly increased in the pons-medulla with no significant changes in this metabolite in any other region at this dose, (Tables 7.1 - 7.3).

4. The effect of amylobarbitone.

Amylobarbitone 15 and 30 mg/kg elevated 5-HT in the cortex and the 30 mg/kg dose also increased 5-HT in the pons-medulla with a slight but significant increase in the hypothalamus. There was no change in 5-HT or 5-HIAA levels in the midbrain or hippocampus, but both doses of amylobarbitone caused a marked decrease in striatal 5-HT. 5-HIAA was significantly reduced in the hypothalamus at both doses, and the midbrain showed a marked decrease in 5-HIAA at the 30 mg/kg dose. (Tables 7.2 - 7.7).

5. The effect of ACTH.

ACTH (0.075 mg/kg) decreased both 5-HT and 5-HIAA in the midbrain at 10 mins. (These animals had of course not been exposed to the maze.) 5-HT was also reduced at this time in the cortex and 5-HIAA reduced in the hypothalamus. Increases in 5-HIAA were also observed in the pons-medulla and striatum. There were no changes in the 5-HT levels in the hypothalamus, pons-medulla, striatum of hippocampus. 5-HIAA in the hippocampus and cortex also remained unchanged at this time.

At 30 mins, 5-HT was reduced and 5-HIAA elevated in the hypothalamus and hippocampus, while both were reduced in the striatum and midbrain (only the 5-HIAA reduction being significant for the latter). Both 5-HT and 5-HIAA remained unchanged in the cortex and pons-medulla although in the latter, a small (insignificant) decrease in 5-HIAA was observed (Table 7.2).

At 3 hrs, the effect of two doses of ACTH on 5-HT and 5-HIAA was investigated. Both 0.05 and 0.075 mg/kg ACTH showed similar effects in the hypothalamus as obtained at 30 mins with 0.075 mg/kg ACTH - i.e., a significant decrease in 5-HT and an elevation in 5-HIAA respectively. At 3 hrs, the effects obtained with 0.075 mg/kg at 30 mins were still visible in the striatum for the same dose. At the lower dose in 3 hrs, only the decreases in 5-HIAA in striatum and midbrain were observed, but changes in 5-HT were not recorded. The

effect of ACTH 0.075 mg/kg on 5-HT in the midbrain at 3 hrs was reversed - a significant increase in this indole was observed, although both doses of ACTH showed decreases in 5-HIAA levels - the effect being similar to that obtained at 30 mins. Furthermore, a significant rise in hippocampal 5-HT at 0.075 mg/kg and a decrease in cortical 5-HIAA at both doses was also observed. There were no changes in 5-HT or 5-HIAA in the pons-medulla for either doses or in the hippocampus for the lower dose. (Tables 7.2 - 7.7).

6. The effect of picrotoxin.

Picrotoxin 2 and 4 mg/kg produced a large elevation of 5-HT in the striatum. (Table 7.4). Picrotoxin 4 mg/kg also caused a large increase in 5-HT in the midbrain. 5-HIAA was decreased in the striatum and pons-medulla; 4 mg/kg however, caused a slight but significant increase in 5-HIAA in the midbrain. (Tables 7.2 - 7.4).

Discussion

Previous investigations using diazepam have shown that, from 1 to at least 3 hrs after doses of 10 - 30 mg/kg, there is a rise in whole-brain 5-HT and 5-HIAA (Fernstrom, 1974; Bourgoin et al, 1975; Chung Hwang and van Woert, 1979) which is reflected in the hippocampus, hypothalamus and midbrain (Rastogi et al, 1977), although a non-significant fall was recorded in the pons-medulla (ibid.). The incease in 5-HT is as expected, since turnover is apparently reduced in whole-brain (Chung Hwang and van Woert, 1979), cortex (Biswas and Carlsson, 1978), midbrain (Rastogi et al, 1977) and brainstem (Setoguchi et al, 1978) which may be due to a decrease in release of 5-HT, although conflicting results have been obtained where more than one brain region has been combined for analysis (Biswas and Carlsson, 1978; Lidbrink et al, 1974). The rise in 5-HIAA however, is thought to be at least partly due to retarded egress via acid transport (Chase et al, 1970).

In contrast to previous work, the present experiments failed to detect any general rise in 5-HIAA after diazepam, although 5-HT consistently rose in all except pons-medulla. When effects on 5-HIAA were examined by dose and by region, it could be seen that slight, although not always significant, falls in 5-HIAA occurred in all brain regions at doses below 5 mg/kg. At 5 and 10 mg/kg however, although the predominant effect was still of a fall, 5-HIAA rose in pons-medulla, hypothalamus and cortex after one or both doses. At the lower doses used here, (0.5 - 5.0 mg/kg) the effects of diazepam on acid transport may have been too weak to cause net 5-HT accumulation, the overall tendency for 5-HIAA to be reduced is also more in line with previous reports that turnover of 5-HT is reduced by diazepam (see above). However, a rise in regional 5-HIAA concentrations has previously been reported for doses as low as the highest dose used here, i.e. 10 mg/kg (Rastogi et al, 1977).

A speculative explanation for this may lie in the rigid stockholding and handling protocol used in these experiments (since animals were previously used for the maze study - Chapter 2). Diazepam, in a dose sufficient to impair acid transport, would only cause net 5-HIAA accumulation when 5-HT turnover was sufficiently high

to saturate the transport system. Furthermore, a variety of stressful stimuli have been shown to increase the concentration of 5-HIAA in the brain without altering 5-HT concentrations (Bliss et al, 1972), and to increase 5-HT turnover (Morgan et al, 1975). It is therefore possible that in the animals used here, 5-HT turnover was unusually low following the prolonged absence of "stress".

The opposite effects on 5-HIAA of acid transport inhibition and of reduced 5-HT turnover could well explain the failure to demonstrate a dose-effect relationship with diazepam. However, this was also true for the rise in 5-HT. Indeed there were signs that the 5-HT dose-response relationship was biphasic, having an "inverted" U shape in terminal field regions. In the midbrain, however, where the cell body groups B1 to B6 are found (see Breese, 1975), there was a consistent linear dose-response relationship of shallow slope. The complex effect in terminal field regions may indicate more than one mode of action for diazepam.

The effects on 5-HT in the pons-medulla for 1 - 5 mg/kg diazepam were opposite from those in the rest of the brain. A similar but slight fall has been recorded previously for this area (Rastogi et al, 1977) and may possibly indicate reduced 5-HT synthesis in this region.

5-HT and 5-HIAA concentrations after chronic diazepam in this study showed varied effects which not only depended on the dose, but also on the brain region concerned. With chronic chlordiazepoxide (5 mg/kg for 5 days) significant increases in 5-HT have been reported in the hypothalamus, midbrain and cortex (File and Vellucci, 1978) and Stein et al (1973) have shown that 5-HT turnover is substantially reduced after six doses of oxazepam. The fact that different benzodiazepines were used in these studies could account for the discrepancies in the data obtained. Furthermore, File and Vellucci (1978) determined the 5-HT and 5-HIAA concentrations 30 mins after the last injection (as opposed to 3 hrs in this study), and Stein et al (1973) determined 5-HT turnover only in two regions - the midbrain-hindbrain, and the diencephalon-forebrain regions respectively.

Amylobarbitone has not previously been studied although pentobarbitone reduced whole-brain 5-HT turnover without affecting

steady state levels (Corrodi et al, 1967) while phenobarbitone reduced 5-HT levels only in doses in excess of 100 mg/kg (Lidbrink et al, 1974). In the present experiments, there were profound regional differences in the effects of amylobarbitone and more direct measures of turnover would be needed to establish whether this had indeed been reduced in the cortex and pons-medulla (increased 5-HT) as well as in the hypothalamus and midbrain (5-HIAA decline).

Picrotoxin has been shown to reverse the reduction in 5-HT turnover induced by chronic diazepam and microinjection of picrotoxin in the dorsal raphe of rats enhanced 5-HT turnover in the cerebral cortex (Collinge et al, 1982). Furthermore, since GABA depresses unit firing at least, in the dorsal raphe (Gallager, 1978) signs of an increase in serotonergic activity might have been predicted. Despite the fact that both doses used produced significant "anxiogenic" activity (Chapter 2) there was no sign of any major increase in serotonergic function in any brain region. In fact, in pons-medulla and especially in the striatum, serotonergic neuronal activity appeared to have been reduced as evidenced by the rise in 5-HT and decline in 5-HIAA.

Interpretation of the effects of ACTH is complicated by the potential effects of released corticosteroids, the effects of which on serotonergic functions are extremely complex (e.g. Green and Curzon, 1968; Millard et al, 1972; Kovacs et al, 1975); however, an abrupt rise in 5-HIAA with unchanged or reduced 5-HT as seen by File and Vellucci (1978), after ACTH is not among the effects reported for corticosteroids. Moreover, this latter effect was seen by File and Vellucci (1978) 10 mins after ACTH administration, when it is unlikely that corticosteroids had increased significantly (Hodges and Sadow, 1967). In the present experiments, such a rise in 5-HIAA was not seen until 30 mins after injection, when it appeared in the hypothalamus and hippocampus (although not in the midbrain, where 5-HIAA was actually reduced).

The failure to obtain an immediate rise in 5-HIAA might speculatively be attributed to an unresponsiveness of serotonergic systems following the prolonged absence of stress. This could also account for the considerable reduction in 5-HT seen after ACTH in

regions where 5-HIAA was increased. Since stress increases brain tryptophan (Kennett and Joseph, 1981) "stress" reduction might be expected to reduce it and synthesis of 5-HT may not have been able to rise rapidly enough to meet demand following ACTH. Hence further experiments are required to investigate the effect of ACTH on "stressed" and "unstressed" animals (perhaps by using restraint stress) in order to establish whether the failure to obtain an immediate rise in 5-HIAA as reported by File and Vellucci (1978) was due to a "stress-related" effect.

In general, the anxiolytics, amylobarbitone and diazepam produced signs of reduced serotonergic activity in the cortex and hippocampus, although their effects in other areas were inconsistent. Among the anxiogenic agents, there was no one brain area where both picrotoxin and ACTH produced the same effect, suggesting that although 5-HT may well be involved in some way in their anxiogenic actions, the drugs may differ in their site and mode of action on 5-HT systems. However, results do tend to reinforce the hypothesis that serotonergic pathways are integrally involved in the modulation of anxiety. On this basis it was decided to examine the effects of α-adrenoceptor and 5-HT receptor ligands having an anxiogenic- or anxiolytic- like activity, on serotonergic function, and to see whether these effects can be paralleled with those of the anxiolytics and putative anxiogenic agents.

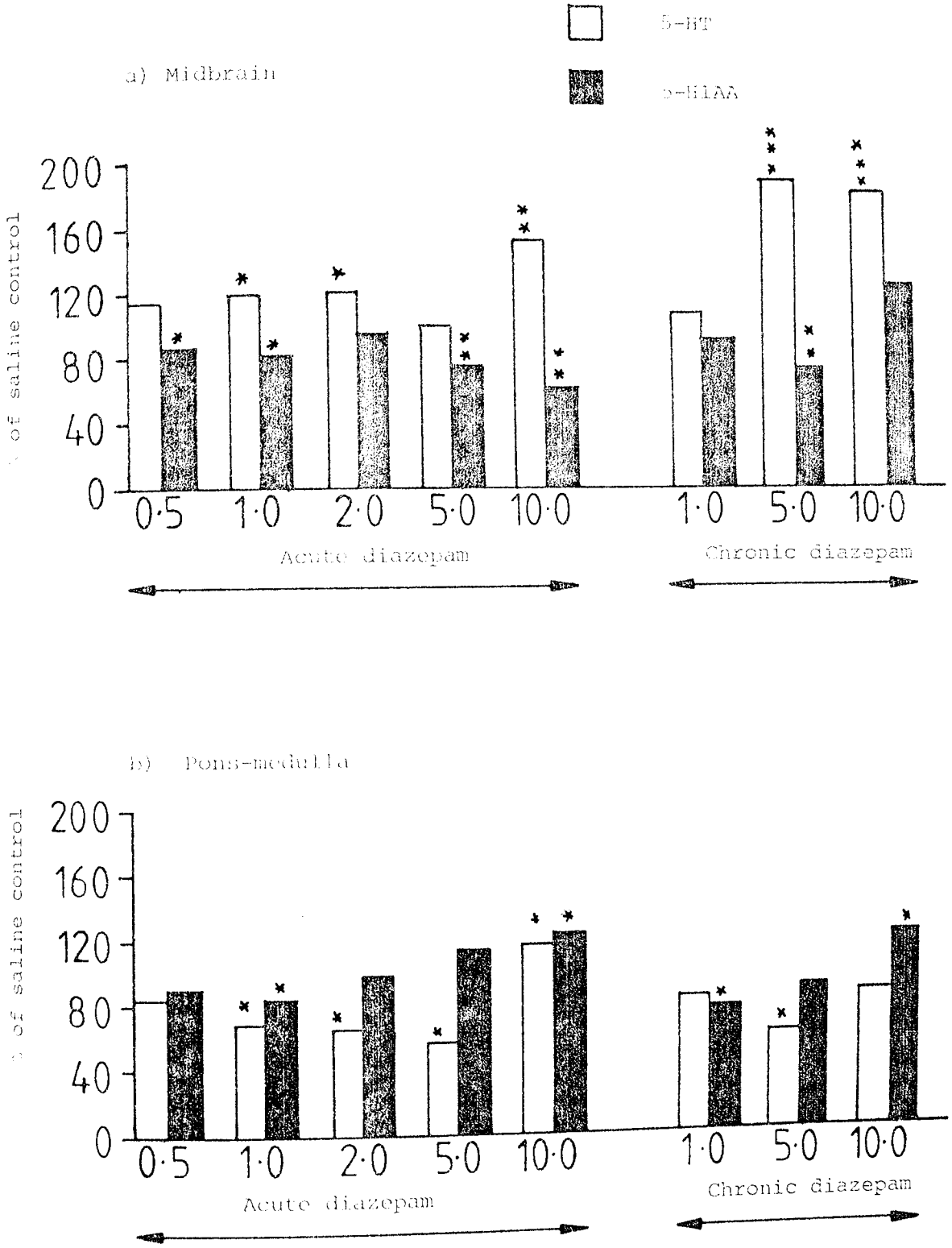


Fig. 7.1 The effect of acute and chronic diazepam on 5-HT and 5-HIAA in a) Midbrain and b) Pons-medulla.

Results are expressed as % of control. Animals were killed 3hrs. Post injection.
*2p <0.05 **2p <0.01 ***2p <0.001 (Students t test).

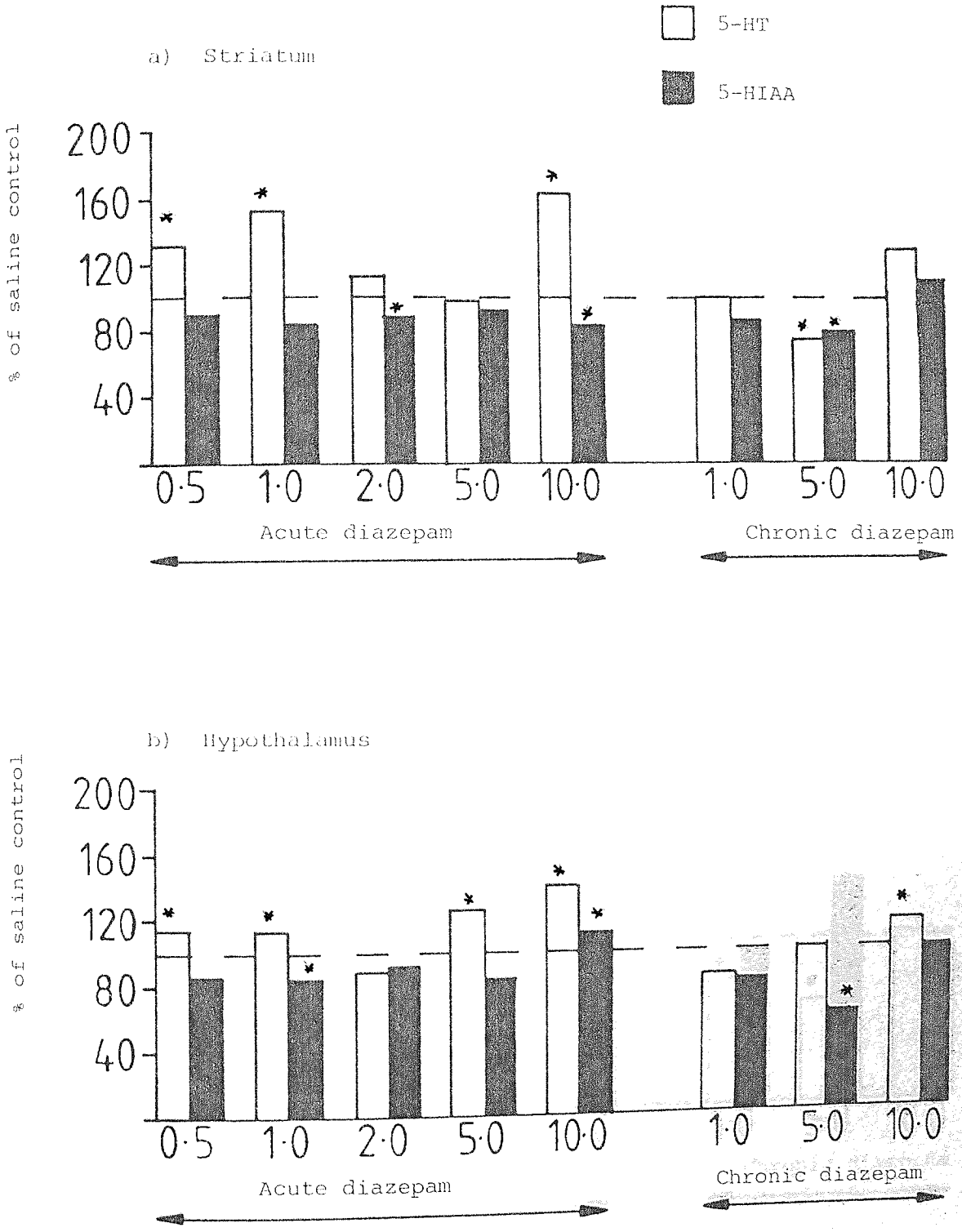


Fig. 7.2. The effect of acute and chronic diazepam on 5-HT and 5-HIAA in a) Striatum and b) Hypothalamus.

Results are expressed as % of control. Animals were killed 3hrs post-injection.
*2p <0.05 **2p <0.01 (Students t test).

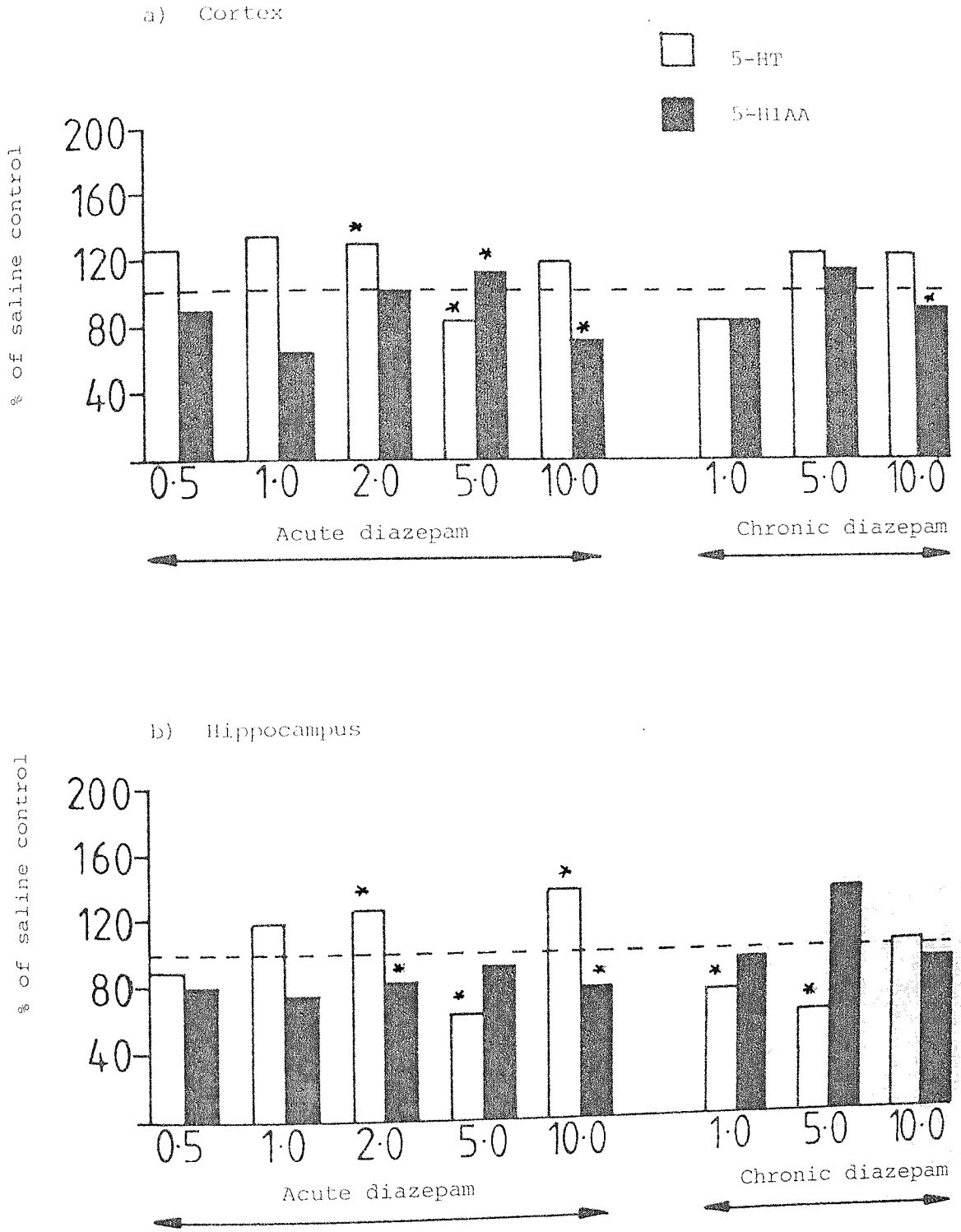


Fig. 7.3 The effect of acute and chronic diazepam on 5-HT and 5-HIAA in a) Cortex and b) Hippocampus.

Results are expressed as % of control. Animals were killed 3hrs post-injection.
*2p <0.05 **2p < 0.01 (Students t test).

BRAIN REGION	5-HT (ng/g)	5-HIAA (ng/g)
PONS/MEDULLA	790 ± 26 (759 ± 36)	1225 ± 64 (1201 ± 63)
MIDBRAIN	749 ± 42 (691 ± 28)	1614 ± 45 (1668 ± 54)
HYPOTHALAMUS	1292 ± 84 (1445 ± 75)	1636 ± 38 (1686 ± 75)
STRIATUM	872 ± 48 (873 ± 32)	1138 ± 38 (1168 ± 43)
HIPPOCAMPUS	863 ± 54 (851 ± 36)	1132 ± 66 (1196 ± 26)
CORTEX	348 ± 16 (320 ± 14)	357 ± 42 (340 ± 10)

TABLE 7.1 Effect of previous exposure to "maze" on the 5-HT and 5-HIAA concentrations in various regions of the rat brain.

5HT and 5HIAA values of animals only handled (not exposed to the maze) are in brackets.

*2p<0.05 **2p<0.01

DRUG/Dose (mg/ g)	5-HT ($\mu g g^{-1}$)	5-HIAA ($\mu g g^{-1}$)
Diazepam (0.5)	637 ± 41 (734 ± 37)	1095 ± 55 (1175 ± 48)
(1.0)	500 ± 18 (734 ± 37)*	995 ± 54 (1175 ± 48)*
(2.0)	425 ± 34 (655 ± 36)*	1222 ± 26 (1295 ± 45)
(5.0)	447 ± 81 (768 ± 52)*	1461 ± 37 (1265 ± 53)
(10.0)	926 ± 66 (799 ± 61)*	1591 ± 82 (1272 ± 53)*
Amylobarbitone (15.0)	851 ± 68 (772 ± 61)	1080 ± 45 (1121 ± 42)
(30.0)	1155 ± 49 (772 ± 61)**	1095 ± 75 (1121 ± 42)
Chronic diazepam		
Diazepam (1.0)	583 ± 26 (680 ± 41)	932 ± 79 (1178 ± 79)*
(5.0)	501 ± 80 (774 ± 34)*	1185 ± 80 (1285 ± 24)
(10.0)	669 ± 65 (729 ± 40)	1630 ± 115 (1246 ± 105)*
ACTH (0.05)	795 ± 21 (808 ± 33)	1298 ± 21 (1343 ± 30)
(0.075)	733± 69 (808 ± 33)	1290 ± 30 (1393 ± 30)
Picrotoxin (2.0)	611 ± 29 (653 ± 52)	725 ± 45 (1171 ± 42)**
(4.0)	626 ± 25 (653 ± 52)	780 ± 84 (1171 ± 42)**
ACTH (0.075) (10 mins later)†	812 ± 44 (725 ± 30)	1229 ± 44 (1104 ± 38)*
(30 mins later)††	884 ± 20 (808 ± 33)	1154 ± 63 (1343 ± 30)

TABLE 7.2 The effect of anxiolytic and putative anxiogenic agents on 5-HT and 5-HIAA in the pons medulla.

Results are expressed as mean ± s.c.m. Vehicle control values are shown in brackets. Animals were killed 3 hrs after injection except $^{+}$ACTH- killed 10 mins later, and $^{++}$ACTH killed 30 mins later.

* $2p<0.05$ **$2p<0.01$ ***$2p<0.001$ (Student's t test)

DRUG/DOSE (mg/kg)	5-HT (ngg^{-1})	5-HIAA (ngg^{-1})
Diazepam (0.5)	874 ± 46 (729 ± 73)	1859 ± 38 (2009 ± 68)*
(1.0)	905 ± 42 (769 ± 73)*	1642 ± 40 (2009 ± 68)*
(2.0)	936 ± 41 (763 ± 30)*	1875 ± 34 (2005 ± 40)
(5.0)	866 ± 33 (851 ± 20)	1573 ± 57 (2058 ± 38)**
(10.0)	1255 ± 50 (852 ± 56)**	1418 ± 64 (1972 ± 63)**
Amylobarbitone (15.0)	786 ± 63 (760 ± 41)	1625 ± 120 (1282 ± 66)
(30.0)	743 ± 33 (760 ± 40)	1400 ± 73 (1782 ± 66)**
Chronic diazepam		
Diazepam (1.0)	928 ± 64 (792 ± 65)	2142 ± 69 (2079 ± 107)
(5.0)	1572 ± 57 (813 ± 37)***	1596 ± 31 (2052 ± 57)**
(10.0)	1511 ± 42 (792 ± 34)***	2190 ± 56 (2090 ± 160)
ACTH (0.005)	759 ± 35 (729 ± 35)	1799 ± 57 (2017 ± 119)*
(0.075)	800 ± 25 (729 ± 35)*	1769 ± 68 (2017 ± 119)*
Picrotoxin (2.0)	958 ± 95 (836 ± 69)	1941 ± 76 (1878 ± 46)
(4.0)	1379 ± 84 (836 ± 69)**	2056 ± 41 (1878 ±66)*
ACTH (0.075) (10 mins)†	492 ± 26 (716 ± 29)***	1129 ± 63 (1989 ± 68)***
(30 mins)††	1534 ± 32 (729 ± 35)*	1859 ± 92 (2017 ± 119)*

Table 7.3. The effect of anxiolytics and putative anxiogenic agents on 5-HT and 5-HIAA in the midbrain.

Results are expressed as mean ± s.e.m. Vehicle control values are shown in brackets. Animals were killed 3 hours after injection except †ACTH- killed 10 mins later, and ††ACTH killed 30 mins later

* 2p <0.05 **2p <0.01 ***2p <0.001 (Student's t test)

DRUG/DOSE (mg/kg)	5-HT (μgg^{-1})	5-HIAA (μgg^{-1})
Diazepam (0.5)	883 ± 45 (668 ± 36)*	1270 ± 76)1413 ± 17)
(1.0)	1011 ± 55 (668 ± 36)*	1181 ± 24 (1413 ± 17)
(2.0)	949 ± 62 (845 ± 16)	1186 ± 56 (1309 ± 66)*
(5.0)	878 ± 52 (901 ± 37)	1323 ± 55 (1434 ± 29)
(10.0)	1228 ± 71 (757 ± 17)*	1227 ± 43 (1424 ± 71)*
Amylobarbitone (15.0)	557 ± 53 (905 ± 70)**	1132 ± 98 (1178 ± 56)
(30.0)	344 ± 69 (905 ± 70)***	1204 ± 64 (1178 ± 56)
Chronic diazepam		
Diazepam (1.0)	1030± 63 (935 ± 93)	1252 ± 37 (1407 ± 56)
(5.0)	518 ± 23 (694 ± 62)*	1002 ± 33 (1292 ± 68)*
(10.0)	760 ± 64 (657 ± 20)	1421 ± 138 (1281 ± 79)
ACTH (0.05)	653 ± 22 (707 ± 19)	1103 ± 60 (1481 ± 61)*
(0.075)	606 ± 36 (707 ± 19)*	1155 ± 95 (1481 ± 61)*
Picrotoxin (2.0)	1221 ± 84 (835 ± 96)**	979 ± 61 (1171± 43)**
(4.0)	1491 ± 47 (835 ± 96)**	855 ± 68 (1121 ± 43)***
ACTH (0.075) (10 mins)$^{+}$	859 ± 105 (973 ± 40)	1321 ± 97 (1184 ± 32)*
(30 mins)$^{++}$	600 ± 35 (707 ± 19)*	1236 ± 92 (1481 ± 61)

TABLE 7.4. The effect of anxiolytics and putative anxiogenic agents on 5-HT and 5-HIAA in the striatum.

Results are expressed as mean ± s.e.m. Vehicle control values are shown in brackets. Animals were killed 3 hours after injection except $^{+}$ACTH - killed 10 mins later, and $^{++}$ACTH killed 30 mins later.

*2p<0.05 **2p<0.01 ***2p,0.001 (Student's t test)

DRUG/DOSE (mg/kg)		5-HT (ngg^{-1})	5-HIAA (ngg^{-1})
Diazepam	(0.5)	1564 ± 25 (1339 ± 38)*	1938 ± 54 (2149 ± 84)
	(1.0)	1595 ± 62 (1339 ± 38)*	1894 ± 57 (2149 ± 54)*
	(2.0)	1164 ± 58 (1266 ± 79)	1526 ± 40 (1648 ± 52)
	(5.0)	1562 ± 32 (1386 ± 110)*	1667 ± 52 (1884 ± 53)
	(10.0)	1910 ± 44 (1362 ± 82)*	2454 ± 40 (2124 ± 95)*
Amylobarbitone	(15.0)	1563 ± 80 (1399 ± 56)	1306 ± 64 (1813 ± 84)**
	(30.0)	1580 ± 65 (1399 ± 56)*	1162 ± 89 (1813 ± 84)***
Chronic diazepam			
Diazepam	(1.0)	1203 ± 53 (1333 ± 61)	1578 ± 126 (1870 ± 115)
	(5.0)	1434 ± 16 (1334 ± 109)	1184 ± 63 (1813 ± 45)*
	(10.0)	1630 ± 30 (1394 ± 37)*	1137 ± 97 (1745 ± 72)
ACTH	(0.05)	1312 ± 47 (1415 ± 25)*	2381 ± 35 (2188 ± 61)*
	(0.075)	1278 ± 47 (1415 ± 25)*	2541 ± 72 (2188 ± 61)*
Picrotoxin	(2.0)	1243 ± 78 (1168 ± 72)	2038 ± 49 (1991 ± 57)
	(4.0)	1175 ± 84 (1168 ± 73)	2133 ± 28 (1991 ± 57)
ACTH	(0.075) (10 mins)+	1443 ± 46 (1343 ± 22)	1376 ± 63 (1589 ± 65)*
	(30 mins)++	1085 ± 53 (1415 ± 25)	2595 ± 94 (2188 ± 61)*

Table 7.5 The effect of anxiolytics and putative anxiogenic agents on 5-HT and 5-HIAA in the hypothalamus.

Results are expressed as mean ± s.e.m. Vehicle control values are shown in brackets. Animals were killed 3 hours after injection except +ACTH - killed 30 mins later, and ++ACTH killed 30 mins later.

* $2p<0.05$ ** $2p<0.01$ *** $2p,0.001$ (Student's t test)

DRUG/DOSE (mg/kg)		5-HT ($ng g^{-1}$)	5-HIAA ($ng g^{-1}$)
Diazepam	(0.5)	800 ± 14 (870 ± 32)	1100 ± 58 (1360 ± 170)
	(1.0)	1035 ± 115 (870 ± 32)	1005 ± 140 (1369 ± 170)
	(2.0)	1011 ± 84 (802 ± 35)*	1054 ± 61 (1226 ± 81)*
	(5.0)	537 ± 33 (882 ± 69)*	1358 ± 76 (1389 ± 48)
	(10.0)	929 ± 24 (703 ± 27)*	1044 ± 28 (1336 ± 22)*
Amylobartitone	(15.0)	931 ± 42 (929 ± 62)	1022± 54 (1140 ± 36)
	(30.0)	902 ± 89 (919 ± 62)	1131 ± 37 (1140 ± 36)
Chronic diazepam			
Diazepam	(1.0)	589 ± 67 (762 ± 101)*	1271 ± 107 (1331 ± 90)
	(5.0)	652 ± 81 (756 ± 21)*	1651 ± 81 (1247 ± 48)
	(10.0)	863 ± 54 (770 ± 45)	1195 ± 83 (1258 ± 100)
ACTH	(0.05)	824 ± 36 (734 ± 25)	1357 ± 59 (1305 ± 45)
	(0.075)	892 ± 32 (734 ± 25)*	1140 ± 45 (1305 ± 45)
Picrotoxin	(1.0)	923 ± 95 (886 ± 63)	1097 ± 118 (1226 ± 115)
	(2.0)	1013 ± 24 (886 ± 63)	1204 ± 44 (1226 ± 115)
ACTH	(0.075) (10 mins)$^{+}$	791 ± 21 (717 ± 34)	1190 ± 65 (1284 ± 46)
	(30 mins)$^{++}$	606 ± 32 (734 ± 25)*	1628 ± 24 (1305 ± 45)*

Table 7.6 The effect of anxiolytics and putative anxiogenic agents on 5-HT and 5-HIAA in the hippocampus

Results are expressed as mean ± s.e.m. Vehicle control values are shown in brackets. Animals killed 3 hours after injection except $^{+}$ ACTH - killed 10 mins later, and $^{++}$ACTH killed 30 mins later.

*2p<0.05 **2p<0.01 ***2p<0.001 (Student's t test)

DRUG/DOSE (mg/kg)		5-HT (ngg^{-1})	5-HIAA (ngg^{-1})
Diazepam	(0.5)	314 ± 15 (255 ± 14)	276 ± 21 (313 ± 27)
	(1.0)	340 ± 18 (255 ± 14)	219 ± 28 (313 ± 27)
	(2.0)	495 ± 21 (390 ± 12)*	391 ± 15 (388 ± 21)
	(5.0)	321 ± 13 (384 ± 24)*	495 ± 15 (419 ± 18)*
	(10.0)	392 ± 24 (319 ± 27)	224 ± 12 (323 ± 22)*
Amylobarbitone	(15.0)	421 ± 17 (322 ± 11)**	376 ± 3.0 (349 ± 36)
	(30.0)	477 ± 26 (322 ± 11)**	361 ± 9.3 (349 ± 36)
Chronic diazepam			
Diazepam	(1.0)	259 ± 10 (294 ± 32)	257± 14 (291 ± 20)
	(5.0)	501 ± 45 (392 ± 74)	319 ± 61 (270 ± 63)
	(10.0)	456 ± 28 (349 ± 8.0)	202 ± 29 (263 ± 28)*
ACTH	(0.05)	395 ± 16 (303 ± 13)	201 ± 20 (300 ± 13)*
	(0.075	263 ± 25 (303 ± 13)	234 ± 20 (300 ± 13)*
Picrotoxin	(2.0)	399 ± 31 (409 ± 37)	401 ± 27 (420 ± 30)
	(4.0)	393 ± 37 (409 ± 37)	340 ± 30 (420 ± 30)
ACTH	(0.075) (10 mins)$^{+}$	246 ± 12 (342 ± 9.4)	352 ± 12 (343 ± 63)
	(30 mins)$^{++}$	354 ± 34 (303 ± 13)	286 ± 36 (300 ± 13)

Table 7.7 The effect of anxiolytics and putative anxiogenic agents on 5-HT and 5-HIAA in the cortex.

Results are expressed as mean ± s.e.m. Vehicle control values are shown in brackets. Animals killed 3 hours after injection except $ACTH^{+}$ - killed 10 mins later, and $^{++}ACTH$ killed 30 mins later.

*2p<0.05 **2p<0.01 ***2p ,0.001 (Student's t test)

CHAPTER 8

REGIONAL CHANGES IN BRAIN 5-HT AND 5-HIAA LEVELS AFTER α-ADRENOCEPTOR AGONISTS AND ANTAGONISTS

Introduction

The involvement of 5-HT in the action of benzodiazepines and ACTH as reported by Stein et al (1973) and File and Vellucci (1978) has been discussed in Chapter 7, and changes in the regional concentrations of 5-HT and 5-HIAA have also been observed with other anxiolytics and putative anxiogenic agents (Chapter 7). It was therefore of interest to examine the effects of α-adrenoceptor agonists and antagonists on brain indoleamines in order to ascertain whether there was a consistent regional pattern in the effects of these agents (especially at doses which showed anxiolytic- or anxiogenic- like activity) as compared to those observed with the anxiolytics and putative anxiogenics (Chapter 7).

Following pilot experiments which demonstrated that prior exposure to the maze (Chapter 2) did not affect regional 5-HT and 5-HIAA concentrations (Chapter 7), the effects of α-adrenoceptor ligands on brain indoleamines were determined 3 hrs post-injection in animals that had been previously exposed to the maze.

The time of determination was chosen to be 3 hrs since both clonidine and yohimbine have been shown to exhibit significant changes in brain 5-HT and 5-HIAA concentrations at this time (Reinhard and Roth 1982; Papeschi et al, 1971). Furthermore, since the effects of diazepam, amylobarbitone and picrotoxin were also investigated at 3 hrs post-injection, this would enable an adequate comparison to be made.

The effect of drugs on 5-HT and 5-HIAA in 6 brain regions have been examined. For display in figures with some agents, results are expressed as

$$\frac{[\text{Mean 5-HT or 5-HIAA concentration}]_{\text{drug}}}{[\text{Mean 5-HT or 5-HIAA concentration}]_{\text{control}}} \times 100\%$$

However, actual values for each group (mean $\pm$ s.e.m.) are shown in the Tables 8.1 - 8.15.

1. The Effect of clonidine on 5-HT and 5-HIAA levels 3 hrs post-injection.

In the pons-medulla, clonidine 0.01 and 0.05 mg/kg significantly increased 5-HT, whereas the higher doses (0.075 - 0.3 mg/kg) decreased it. 5-HIAA however, was only significantly increased at 0.3 - 1.0 mg/kg (Table 8.1). In the midbrain, 5-HT generally decreased with 0.005 - 0.05 mg/kg clonidine, whereas 0.075 mg and 1.0 mg/kg significantly increased it. 5-HIAA was decreased in this region at 0.005 - 0.01 mg/kg but increased significantly with 0.05 - 0.075 and 1.0 mg/kg respectively (Table 8.2).

Hypothalamic 5-HT decreased significantly with 0.005 and 0.025 mg/kg but 0.01 mg and 0.05 - 0.1 mg/kg generally increased it (Table 8.3). 5-HIAA was only significantly increased at 0.025 - 0.05 mg and at 1.0 mg/kg clonidine. Striatal 5-HT significantly decreased at 0.005 mg/kg, but there was a general increase in 5-HT at 0.01 - 0.1 mg/kg. In the striatum there was a general fall in 5-HIAA except at 0.01 mg/kg where 5-HIAA remained unchanged and 0.1 mg/kg where it was slightly increased (Table 8.4).

In the hippocampus 5-HT was increased with 0.005 - 0.01 mg/kg but decreased with 0.025 - 0.05 mg/kg. A significant increase in 5-HT was also obtained with 0.075 and 1.0 mg/kg in the hippocampus. Hippocampal 5-HIAA generally decreased between 0.005 -0.025 mg/kg whereas 0.05 - 0.3 mg/kg increased it; however, at the highest dose (1.0 mg/kg), hippocampal 5-HIAA decreased significantly (Table 8.5).

Cortical 5-HT was increased significantly at 0.01 mg and 0.3 - 1.0 mg/kg respectively. 5-HIAA appeared to have decreased at lower doses (0.005 - 0.01 mg/kg) and higher doses (0.3 - 1.0 mg/kg) whereas between 0.025 - 0.1 mg/kg clonidine increased it, with peak effect being observed at 0.075 mg/kg clonidine (Table 8.6).

2. The effect of azepexole on 5-HT and 5-HIAA levels 40 mins and 3 hrs post-injection.

In the pons-medulla, azepexole 1.0 - 2.0 mg/kg increased 5-HT at 3 hrs although at 40 mins, 2.0 mg/kg decreased it, and 1.0 mg/kg had no effect. 5-HIAA remained unchanged at 3 hrs, but at 40 mins, both doses increased this metabolite in the pons-medulla (significantly at

2.0 mg/kg - Table 8.7). Neither 5-HT nor 5-HIAA were altered at 40 mins with azepexole in the midbrain. At 3 hrs, however, both 5-HT and 5-HIAA were decreased (Table 8.2). Hypothalamic 5-HT remained unchanged at 40 mins, although 1.0 mg/kg azepexole significantly decreased 5-HT at 3 hrs. Only 2.0 mg/kg azpexole at 40 mins increased 5-HIAA significantly in the hypothalamus (Tables 8.2; 8.8). Striatal 5-HT decreased significantly with azepexole 1.0 - 2.0 mg/kg at 40 mins but remained unchanged 3 hrs later. There were no significant changes in 5-HIAA in the striatum except with 2.0 mg/kg at 3 hrs, where a significant increase was observed (Tables 8.4; 8.8).

Azepexole 1.0 mg/kg at 40 mins did not alter 5-HT or 5-HIAA levels in the hippocampus or cortex, although with 2.0 mg/kg hippocampal 5-HT decreased, and 5-HIAA increased (Table 8.9). At 3 hrs, hippocampal 5-HT also decreased with 1.0 mg/kg azepexole, and cortical 5-HIAA increased with 2.0mg/kg azepexole (Table 8.6).

3. The effect of guanabenz on 5-HT and 5-HIAA levels 40 mins and 3 hrs post-injection.

Guanabenz (0.1 - 1.0 mg/kg) did not significantly alter 5-HT levels in the pons-medulla, except with 1.0 mg/kg at 3 hrs where an increase in this indole was obtained. At 0.1 mg/kg, 5-HIAA in the pons-medulla increased significantly at 40 mins - this increase was still visible at 3 hrs. 1.0 mg/kg guanabenz had no effect on 5-HIAA levels at 40 mins although a significant fall was observed 3 hrs post-injection (Tables 8.1; 8.7).

In the midbrain, 0.1 mg/kg decreased 5-HT significantly 40 mins post-injection, but at 3 hrs the decrease was non-significant, whilst 1.0 mg/kg guanabenz increased 5-HT at 3 hrs only (Tables 8.2; 8.7).

Both doses of guanabenz significantly increased 5-HIAA in the midbrain at 40 mins, and these increases were also observed at 3 hrs (Tables 8.2; 8.7). At 40 mins, neither 5-HT nor 5-HIAA were significantly altered with guanabenz 0.1 - 1.0 mg/kg in the hypothalamus. At 3 hrs however, both doses significantly increased hypothalamic 5-HIAA but only 1.0 mg/kg increased 5-HT significantly (Tables 8.3 - 8.8).

In the striatum, 0.1 mg/kg guanabenz did not alter 5-HT levels although at both times (40 mins and 3 hrs post-injection) 5-HIAA was

significantly increased at this dose. Striatal 5-HT significantly decreased with guanabenx 1.0 mg/kg at 40 mins and 3 hrs, but 5-HIAA remained unchanged.

Hippocampal 5-HT significantly decreased and 5-HIAA increased with 0.1 mg/kg guanabenz at 40 mins, and was still visible 3 hrs later. Neither 5-HT nor 5-HIAA were changed with guanabenz 1.0 mg/kg at 40 mins although a significant increase was observed at 3 hrs (Tables 8.5; 8.9).

Cortical 5-HT and 5-HIAA remained unchanged with 0.1 mg/kg guanabenz at 40 mins although 1.0 mg/kg increased both 5-HT and 5-HIAA in the cortex. At 3 hrs, both doses increased 5-HT significantly in the cortex although 5-HIAA remained unchanged at this time. (Tables 8.6; 8.9).

4. Effect of clonidine and RS 21361 on 5-HT and 5-HIAA 40 mins post-injection.

Clonidine

In the pons-medulla, clonidine (0.01 - 0.075 mg/kg) did not alter 5-HT levels, but 5-HIAA was significantly decreased with 0.05 and 0.075 mg/kg at this time. However, the decrease in 5-HIAA was not observed 3 hours later in this region (Tables 8.1; 8.7).

In the midbrain, clonidine 0.05 and 0.075 mg/kg significantly decreased 5-HT levels, whereas 5-HIAA was only increased at 0.01 mg/kg. The effects on 5-HT in the midbrain were still visible at 3 hrs with 0.05 mg/kg but with 0.075 mg/kg at 3 hrs an increase was observed (Tables 8.2; 8.7).

Hypothalamic 5-HT was increased, and 5-HIAA decreased at this time, with 0.05 and 0.0875 mg/kg; these effects were not observed 3 hrs later. Striatal 5-HT was only increased with 0.05 mg/kg and 5-HIAA with 0.01 mg/kg - none of these effects were seen 3 hrs later. In the hippocampus at 40 mins clonidine 0.05 mg/kg increased 5-HT but 5-HIAA remained unchanged at all doses. Cortical 5-HT decreased and 5-HIAA increased with 0.05 and 0.075 mg/kg. The increase in cortical 5-HIAA was still visible at 3 hrs (Tables 8.3 - 8.9).

RS 21361

Only one dose of RS 21361 was looked at 40 mins after injection. Results show that in the midbrain RS 213671 10mg/kg increased 5-HT and 5-HIAA was increased in the hypothalamus. 5-HT and 5-HIAA levels in the other brain regions remained unaltered. (Tables 8.6 - 8.9).

5. The effect of α_2-Adrenoceptor Antagonists on 5-HT and 5-HIAA levels 3 hrs post-injection.

a. Piperoxane

Piperoxane 5.0 mg/kg elevated 5-HT in the pons-medulla, and reduced 5-HIAA significantly in the midbrain, pons-medulla and hippocampus (Figs. 8.1 - 8.3). At 10.0 mg/kg piperoxane, 5-HT was elevated significantly in all regions except the pons-medulla. 5-HIAA was generally reduced in all areas except the hypothalamus, but significant decreases were observed only in the midbrain, striatum and cortex (Figs. 8.1 - 8.3).

b. RS 21361

RS 21361 5 mg/kg generally increased 5-HT in all brain regions looked at (Figs. 8.1 - 8.3) and decreased 5-HIAA with significant effects only in the hypothalamus and hippocampus (Figs. 8.2; 8.3).

c. Idazoxan

Idozoxan 0.125 and 0.25 mg/kg did not affect 5-HT or 5-HIAA levels in the pons-medulla (Fig.8.1). In the midbrain 5-HT was not affected, but significant decreases in 5-HIAA in this region were observed with both doses. Hypothalamic 5-HT and 5-HIAA were both decreased with idazoxan 0.125 - 0.25 mg/kg. Neither doses of idazoxan altered 5-HT levels in the striatum, hippocampus or cortex, but 5-HIAA levels in all these regions were significantly decreased (Figs. 8.2; 8.3).

d. Yohimbine

Yohimbine (1.25 - 5.0 mg/kg) consistently elevated 5-HT in all areas of the brain looked at although the effect was not dose-dependent. 5-HIAA was reduced in all areas except the striatum and pons-medulla where the reduction was non-significant (Figs. 8.1 - 8.3).

6. The effect of α_1-adrenoceptor agonists on 5-HT and 5-HIAA levels 3 hrs post-injection.

a. Phenylephrine

In the pons-medulla, only 0.25 mg/kg phenylephrine caused a significant rise in 5-HT. 5-HIAA remained unchanged at all doses of phenylephrine used in this region. In the midbrain, 0.25 mg/kg and 2.5 mg/kg caused a significant increase in 5-HT, while 1.0 mg/kg decreased 5-HT levels in the midbrain. Only 2.5 mg/kg phenylephrine increased 5-HIAA significantly in this region (Tables 8.10; 8.11).

At 0.25 mg/kg both 5-HT and 5-HIAA markedly decreased in the hypothalamus, although at a slightly higher dose (1.0 mg/kg) a significant increase was observed with 5-HT and 5-HIAA. At 2.5 mg/kg, the increase in 5-HIAA obtained with 1.0 mg/kg was still visible, but 5-HT was markedly decreased at this dose (Table 8.12).

All three doses decreased striatal 5-HT, although only 0.25 mg/kg decreased 5-HIAA significantly in the striatum. Hippocampal 5-HT and 5-HIAA were decreased with 0.25 mg/kg phenylephrine; the decrease in 5-HT was also seen at 2.5 mg/kg, while the 5-HIAA effect was visible at 1.0 mg/kg phenylephrine. Cortical 5-HT generally decreased with phenylephrine but no significant effects were seen in cortical 5-HIAA (Tables 8.13 - 8.15).

b. St 587

St 587 caused a general decrease in 5-HT in the pons-medulla region (significant only at 0.1 mg/kg), although the highest dose (2.0 mg/kg) increased it slightly. 5-HIAA in this region generally decreased with significant effects being observed at 1.0 and 2.0 mg/kg respectively. In the midbrain, both 5-HT and 5-HIAA remained unchanged, whereas in the hypothalamus, 0.1 mg and 1.0 mg/kg decreased 5-HT but 5-HIAA remained unchanged at all doses in this region (Tables 8.10 - 8.12).

St 587 did not affect striatal 5-HT significantly, but increased 5-HIAA at the lowest and the highest doses. At 0.1 mg and 0.5 mg/kg St 587, neither hippocampal, nor cortical 5-HT or 5-HIAA were altered. The 1.0 mg/kg dose decreased significantly, hippocampal 5-HT and hippocampal and cortical 5-HIAA. Both 5-HT and 5-HIAA were significantly decreased in the cortex at 2.0 mg/kg St 587 (Tables 8.13

- 8.15).

7. The Effect of α_1-Adrenoceptor Antagonists on 5-HT and 5-HIAA levels 3 hrs post-injection.

a. Prazosin

Prazosin (0.025 - 0.5 mg/kg) generally decreased 5-HT levels in the pons-medulla and 1.0 mg/kg slightly increased it. Only 1.0 mg/kg prazosin increased 5-HIAA significantly in this region. In the midbrain, 0.025 - 0.5 mg/kg significantly decreased 5-HT except the 0.25 mg/kg dose which did not affect 5-HT or 5-HIAA. At 1.0 mg/kg, both 5-HT and 5-HIAA in the midbrain were increased, whereas 0.05 and 0.1 mg/kg decreased 5-HIAA, although 0.025 mg/kg prazosin increased 5-HIAA significantly in the midbrain. In the hypothalamus only 0.05 mg and 0.5 mg/kg prazosin significantly increased 5-HT; 5-HIAA in this region was significantly decreased at 0.1 - 0.25 mg/kg, but increased at 1.0 mg/kg prazosin (Tables 8.10 - 8.12).

Striatal 5-HT generally decreased with all doses of prazosin used, whereas the 5-HIAA levels in this region increased significantly at 0.05 mg/kg prazosin. Hippocampal 5-HT significantly decreased with 0.1 mg and 1.0 mg/kg prazosin, whereas 5-HIAA decreased 0.025 mg/kg but increased significantly with 0.1 mg and 1.0 mg/kg prazosin. Doses of 0.05 mg, 0.25 mg and 0.5 mg/kg had no effect on either hippocampal 5-HT or 5-HIAA. Cortical 5-HT decreased with all doses of prazosin used, whereas 5-HIAA remained unchanged at 0.025 - 0.05 mg/kg, increased between 0.1 - 0.5 mg/kg, and decreased significantly at 1.0 mg/kg (Tables 8.13 - 8.15).

b. Thymoxamine

Thymoxamine (0.1 - 1.0 mg/kg) caused a significant increase in 5-HT in the pons-medulla. 5-HIAA however decreased with 0.1 mg/kg but increased with 0.5 - 1.0 mg/kg. In the midbrain, only 0.1 mg/kg thymoxamine significantly decreased 5-HT. 0.5 - 1.0 mg/kg increased 5-HIAA in the midbrain. 5-HT in the hypothalamus decreased significantly at 1.0 mg/kg; 5-HIAA was also decreased in the hypothalamus at 0.5 - 1.0 mg/kg thymoxamine, but there was no effect on either 5-HT or 5-HIAA at 0.1 mg/kg (Tables 8.10 - 8.12).

Thymoxamine 1.0 mg/kg caused a significant fall in striatal 5-HT, but 5-HIAA was increased at 0.1 mg/kg only. In the hippocampus,

0.5 - 1.0 mg/kg thymoxamine decreased 5-HT levels, but 5-HIAA remained unaffected at all doses. In the cortex, only 1.0 mg/kg thymoxamine had an effect on 5-HT and 5-HIAA - both were decreased significantly at this dose; the lower doses had no effect (Tables 8.13 - 8.15).

Discussion

In previous experiments, the predominant effects of clonidine (0.05 to 10 mg/kg) was a fall in 5-HIAA which has been reported at times from 45 min to 3 hrs after administration; the effect of 5-HT appears to be more variable with no effect or a rise, having been reported for a variety of doses, times and brain regions (Maj et al, 1973; Rochette and Bralet, 1975; Scheel-Kruger and Hasselager, 1974). Turnover was found to be increased in the brainstem and hypothalamus 45 mins after 0.05 mg/kg (Rochette and Bralet, 1975) but synthesis was reduced in the cortex 3 hrs after 0.3 mg/kg (Reinhard and Roth, 1982) and a detailed study of clonidine on cortical synthesis and metabolism of 5-HT revealed that 5-HIAA was reduced only by doses of 0.3 mg/kg and above, whereas 5-HIAA was unaffected (Reinhard and Roth, 1982).

In this study the effect of clonidine on brain 5-HT and 5-HIAA levels appeared to vary according to the dose and brain region concerned and no one brain region gave similar effects on brain 5-HT or 5-HIAA with increasing dose. Furthermore, although at some doses the effects of clonidine 40 min post-injection were still maintained or exaggerated at 3 hrs, with other doses and certain brain regions significant effects observed at 40 mins were either reversed or remained unchanged (compared to controls) at 3 hrs. This variability in results with clonidine could be due to its effect on α_1-adrenoceptors, since results obtained from the study by Bradshaw et al (1982) suggest that clonidine acts as a partial agonist at central α_1-adrenoceptors. Also from this study it is apparent that α_1-adrenoceptor agonists also have an effect on serotonergic activity which may in part explain the inconsistent effects of clonidine at high doses. However, the anxiolytic dose of clonidine (0.01 mg/kg as seen in Chapter 2) produced marked effects. 5-HT was elevated in all regions except the midbrain (where it declined), and falls in 5-HIAA were also recorded (this effect being insignificant in the cortex and midbrain) indicating a general reduction in 5-HT turnover.

The effects of clonidine in the present study on brain 5-HT and 5-HIAA shows much greater sensitivity than reported by Maj et al (1973) or Reinhard and Roth (1982), although the reason for this is not clear.

However, the overall pattern of changes in brain 5-HT and 5-HIAA with the anxiolytic dose of clonidine strongly resembled that seen with diazepam in the terminal field areas of cortex, hippocampus, striatum and hypothalamus (Chapter 7).

The effects of azepexole and guanabenz have not been previously examined. Results in the present study show that the effects of these agents at 40 min and 3 hrs are not similar to those obtained with clonidine. There appears to be no significant trend or an increase or decrease in 5-HT or 5-HIAA. Moreover the anxiolytic doses of these α_2-adrenoceptor agonists (as seen in Chapter 2) have not shown any resemblance to the effects obtained with the anxiolytics (Chapter 7).

The failure to detect any significant changes resembling clonidine or the anxiolytics with azepexole and guanabenz could be due to the time at which 5-HT and 5-HIAA determinations were performed since although clonidine has been shown to produce maximal effects at 3 hrs the effect of 5-HT and 5-HIAA at a range of times after dosing with azepexole and guanabenz have not been determined. Further experiments are required to investigate the dynamics of the changes which occur at a range of times after dosing with these two agents.

The effect of yohimbine on brain indoleamines has also been investigated. An increase in whole-brain 5-HT and a decrease in 5-HIAA concentrations following yohimbine have been reported (Papeschi et al, 1971; Sanghvi and Gershon, 1974). However, Reinhard and Roth (1982) have found yohimbine without effect of cortical indoleamines, although it prevented the action of clonidine. In this study, yohimbine produced consistent, but non dose-dependent elevations in 5-HT in all brain regions. 5-HIAA also declined in all regions, although this was not significant in pons-medulla or striatum. The effect of yohimbine therefore appeared to be very general.

The effect of piperoxane and RS 21361 appeared to be similar to those of yohimbine, although they were less marked, and in the cortex, pons-medulla and striatum with some doses of piperoxane, varied insignificant changes were observed. The reason for some of these inconsistent results may be due to the time at which these determinations were performed, since it is possible that with these α_2-adrenoceptor antagonists, changes would have been apparent at a time

other than 3 hrs.

The effect of idazoxan on brain indoleamines was less clear. Whereas the hippocampus and cortex showed effects similar to those of yohimbine, 5-HT decreased in the hypothalamus and both indoleamines remained unchanged in the pons-medulla. The inconsistent effects of idazoxan on brain 5-HT and 5-HIAA could be due to the lack of selectivity of this agent. Although Doxey et al (1983) have demonstrated an α_2-adrenoceptor antagonistic effect with idazoxan, the cardiovascular effects of idazoxan suggest that it may have a partial agonist activity at post-junctional α_1-adrenoceptors (Dalrymple et al, 1982), and Paciorek and Shepperson (1983) have demonstrated a α_1-adrenoceptor agonistic activity at similar doses to those at which it exhibits α_2-antagonistic properties. Furthermore, Goldstein et al (1983) showed that at lower doses, idozoxan had clonidine-like α_2-agonistic activity when its effects were examined on L.C. unit activity.

The effects of α_1-adrenoceptor agonists and antagonists have been investigated, and results indicate that phenylephrine decreased 5-HIAA in whole-brain, whereas phenoxybenzamine and phentolamine increased it in the forebrain, although neither of these were statistically significant (Plaznik et al, 1983).

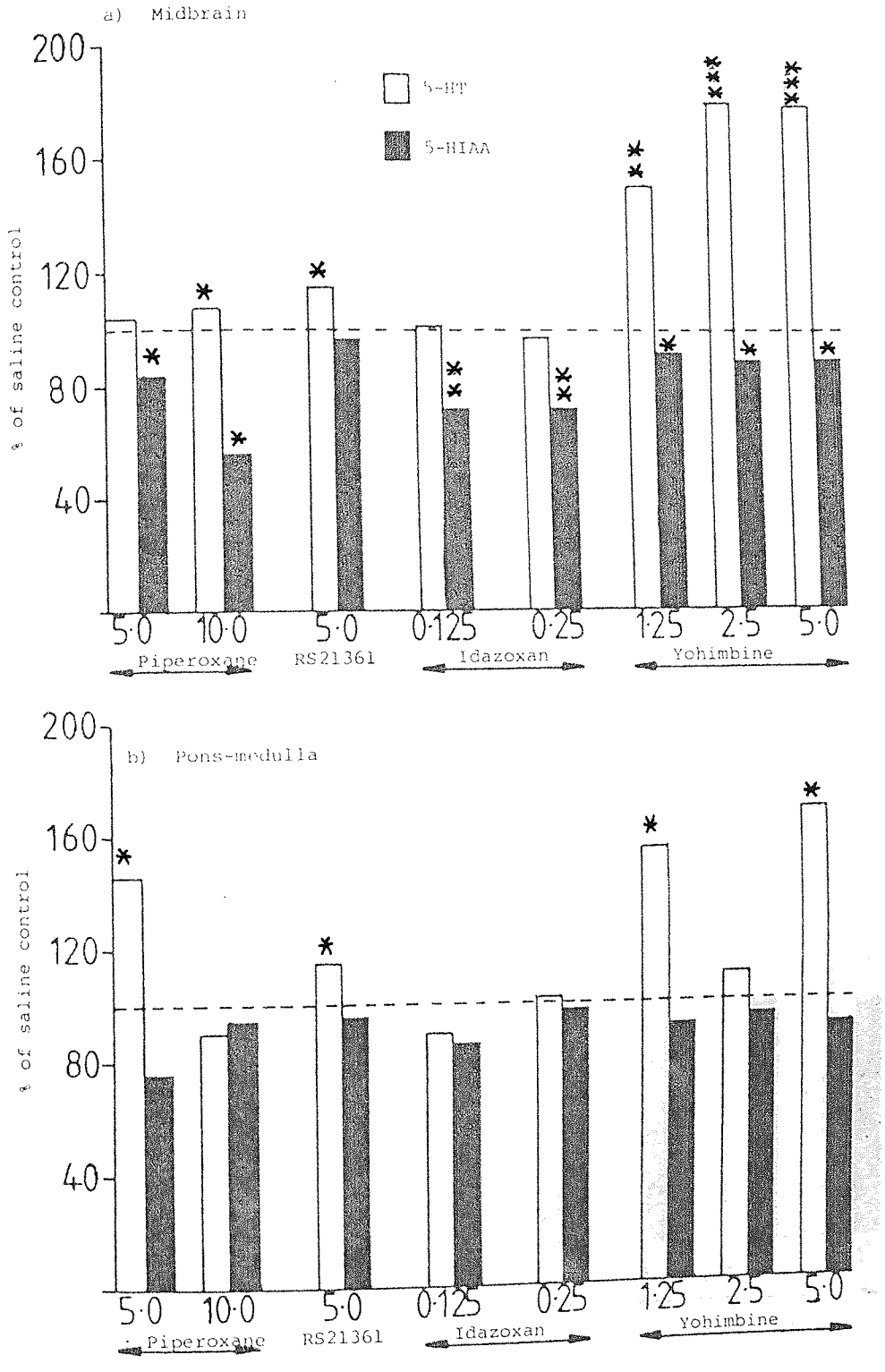
In this study the effect of α_1-adrenoceptor agonists were inconsistent, since although both phenylephrine and St587 induced significant changes in brain 5-HT and 5-HIAA, these varied according to the dose and the brain region concerned. Furthermore, no one region showed a consistent effect on 5-HT or 5-HIAA at the anxiogenic doses (as seen in Chapter 2). The effects of the antagonists were similar. The anxiolytic dose of prazosin increased 5-HT in the hypothalamus, and 5-HIAA in the striatum, whereas thymoxamine which had no significant anxiolytic-like effect (Chapter 2) produced marked effects in the pons-medulla - 5-HT was elevated at all doses and 5-HIAA increased at the two higher doses.

The interpretation of the effects of α-adrenoceptor agonists and antagonists was difficult since no consistent effect (even with the anxiolytic- or anxiogenic- doses) was observed. Furthermore, results in this study could not be compared with those of Plaznik et al (1983)

because drugs were injected in to the median raphe nucleus (as opposed to ip injections in this study). Also, different α_1-adrenoceptor antagonists were used, and the time at which 5-HIAA determinations were performed was also different.

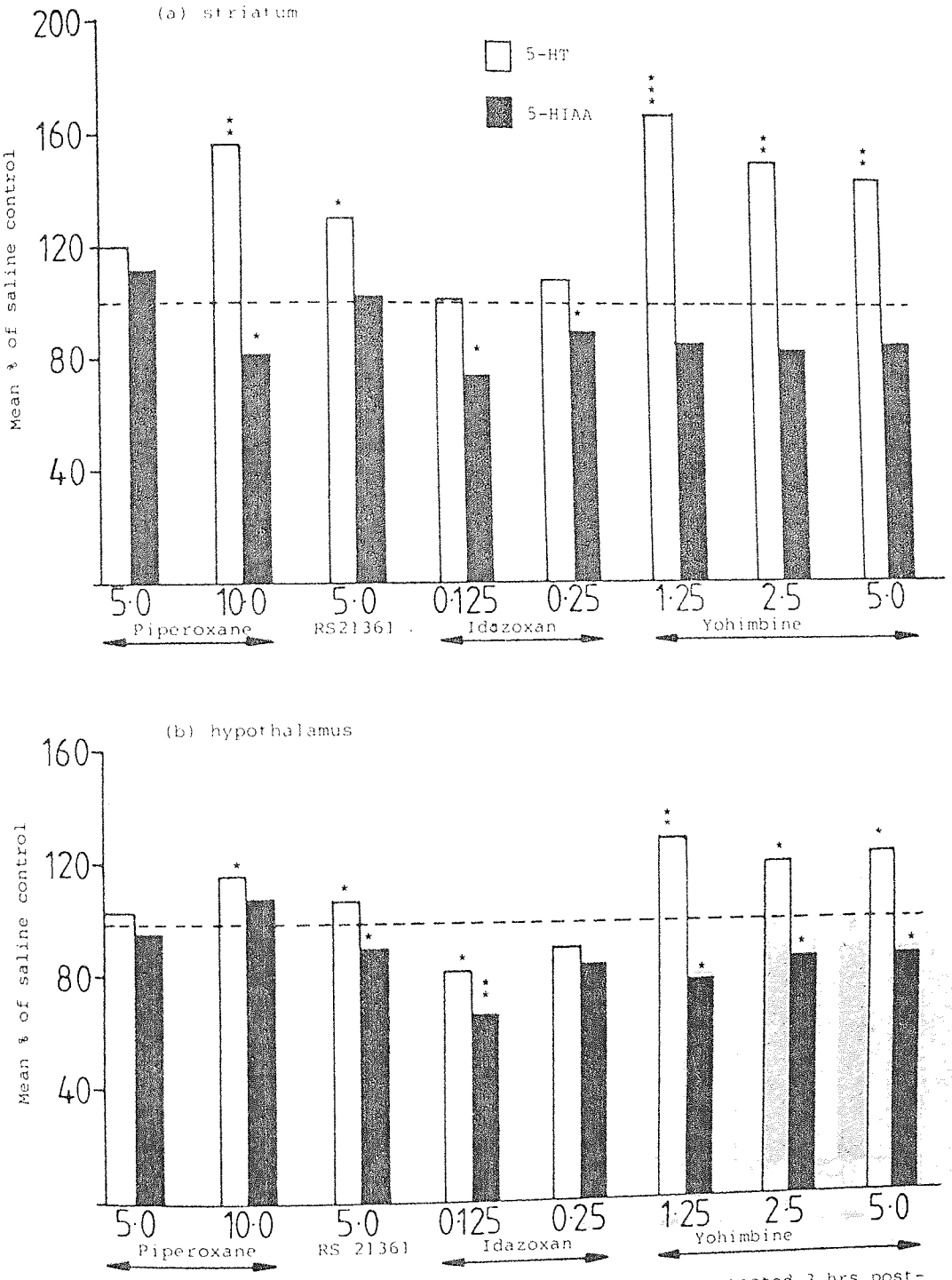
Although all the drugs examined did show effects on regional concentrations of 5-HT and 5-HIAA, anxiolytic- and anxiogenic- doses of the α-adrenoceptor ligands did not consistently have opposite effects. For instance, the anxiogenic doses of yohimbine and piperoxane appeared to increase 5-HT and decrease 5-HIAA, and similar effects were observed with the anxiolytic dose of clonidine, whereas none of the α-adrenoceptor ligands showed consistent changes in these indoleamines. To some extent, this may have been due to the time at which 5-HT and 5-HIAA determinations were performed, since maximal effects may become apparent at different times with different agents. From the results obtained in this study it appears that the effect of α-adrenoceptor agonists and antagonists are idiosyncratic to individual drugs and that the effect of these agents on anxiety cannot therefore be explained by regional changes in 5-HT and 5-HIAA concentrations.

Fig. 8.1. The effect of α_2 adrenoceptor antagonists on 5-HT and 5-HIAA in a) Midbrain and b) Pons-medulla.



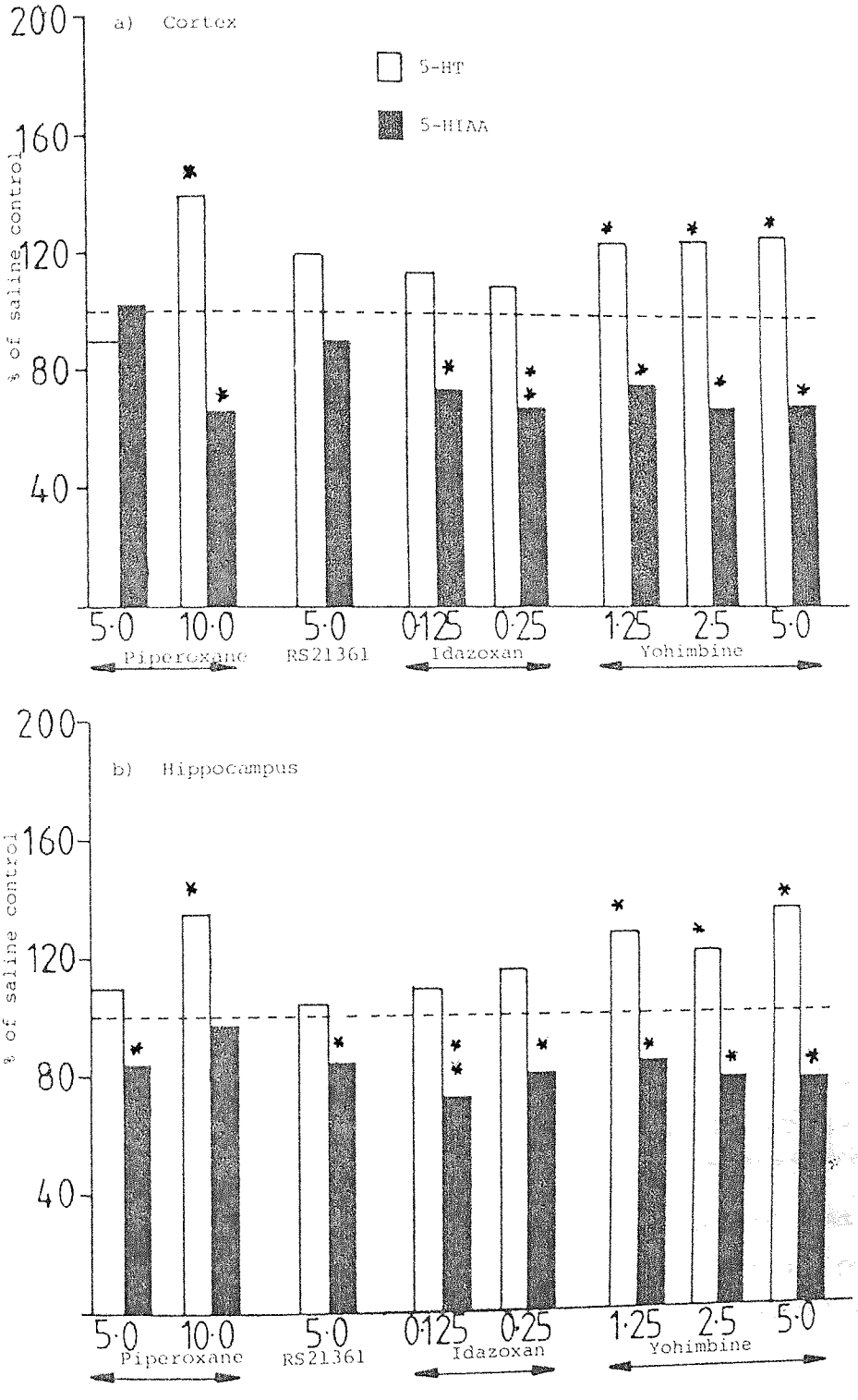
Results are expressed as % control.
*2p <0.05 **2p <0.01 ***2p <0.001 (Students t test)

Fig 8.2 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the (a) striatum and (b) hypothalamus.



Results are expressed as % of control. Animals were rested 3 hrs post-injection. * 2p <0.05; ** 2p <0.01; *** 2p <0.001 (Student's t test).

Fig. 8.3 The effect of α_2 adrenoceptor antagonists on 5-HT and 5-HIAA in a) Cortex and b) Hippocampus.



Results are expressed as % control.
*2p <0.05 **2p <0.01 ***2p <0.001 (Students t test)

DRUG (mg/kg)	5-HT (ng/g)	5-HIAA (ng/g)
Azepexole (1.0)	938 ± 44 (638 ± 34)*	1033 ± 50 (1163 ± 42)
(2.0)	955 ± 41 (638 ± 34)*	1075 ± 50 (1167 ± 42)
Clonidine (0.005)	619 ± 75 (702 ± 65)	1273 ± 31 (1250 ± 27)
(0.01)	1054 ± 138 (638 ± 31)**	1054 ± 45 (1162 ± 42)
(0.025)	777 ± 62 (761 ± 37)	1396 ± 55 (1275 ± 48)
(0.05)	899 ± 9 (761 ± 37)*	1034 ± 11 (1275 ± 48)
(0.075)	561 ± 50 (761 ± 37)*	1469 ± 64 (1275 ± 48)
(0.1)	651 ± 24 (734 ± 24)*	1219 ± 95 (1228 ± 33)
(0.3)	884 ± 36 (1035 ± 60)*	1336 ± 18 (1194 ± 17)*
(1.0)	995 ± 60 (1035 ± 60)	1342 ± 31 (1194 ± 17)*
Guanabenz (0.1)	804 ± 10 (806 ± 36)	1441± 30 (1205 ± 25)*
(1.0)	1050 ± 50 (795 ± 34)*	910 ± 38 (1163 ± 35)*
Piperoxane (5.0)	973 ± 34 (661 ± 37)*	875 ± 44 (1155 ± 48)
(10.0)	669 ± 24 (734 ± 28)	1159 ± 50 (1235 ± 33)
RS 21361 (5.0)	1045 ± 15 (927 ± 11)*	1069 ± 38 (1127 ± 15)
RX781094 (0.125)	729 ± 55 (791 ± 24)	1178 ± 47 (1333 ± 58)
(0.25)	808 ± 65 (791 ± 24)	1270 ± 99 (1333 ± 58)
Yohimbine (1.25)	1050 ± 21 (687 ± 33)*	1175 ± 74 (1270 ± 93)
(2.5)	760 ± 20 (687 ± 33)	1210 ± 70 (1270 ± 93)
(5.0)	1162 ± 48 (687 ± 33)*	1175 ± 50 (1270 ± 93)

Table 8.1 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the pons-medulla 3 hours post injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p<0.05; **2p<0.01; ***2p,0.001 (Student's 't' test)

DRUG (mg/kg)	5-HT (ng/g)	5-HIAA (ng/g)
Azepexole (1.0)	541 ± 69 (848 ± 105)*	1862 ± 27 (2120 ± 74)*
(2.0)	759 ± 79 (848 ± 105)	1874 ± 77 (2120 ± 74)*
Clonidine (0.0005)	539 ± 91 (693 ± 73)	1568 ± 107 (1875 ± 95)*
(0.01)	492 ± 86 (848 ± 110)**	1470 ± 81 (2120 ± 74)*
(0.025)	702 ± 50 (729 ± 73)	2240 ± 36 (2184 ± 69)
(0.05)	588 ± 33 (729 ± 73)*	2884 ± 29 (2184 ± 69)*
(0.075)	888 ± 63 (729 ± 73)*	2540 ± 65 (2184 ± 69)*
(0.1)	652 ± 14 (752 ± 38)	2184 ± 69 (2202 ± 120)
(0.3)	937 ± 74 (921 ± 18)	2229 ± 56 (2180 ± 63)
(1.0)	1209 ± 57 (921 ± 18)*	2350 ± 22 (2180 ± 63)*
Guanabenz (0.1)	579 ± 28 (756 ± 12)	2343 ± 24 (2015 ± 21)*
(1.0)	994 ± 63 (798 ± 69)*	2503 ± 37 (2034 ± 117)*
Piperoxane (5.0)	757 ± 59 (729 ± 73)	1499 ± 70 (1809 ± 70)*
(10.0)	803 ± 69 (675 ± 38)*	845 ± 37 (1543 ± 120)*
RS-21361 (5.0)	865 ± 20 (764 ± 30)*	2100 ± 30 (2147 ± 57)
Idazoxan (0.125)	951 ± 42 (929 ± 30)	1326 ± 39 (1835 ± 62)**
(0.25)	907 ± 69 (929 ± 30)	1350 ± 85 (1835 ± 62)**
Yohimbine (1.25)	1207 ± 100 (787 ± 47)**	2009 ± 44 (2184 ± 74)*
(2.5)	1434 ± 47 (787 ± 47)***	1959 ± 70 (2184 ± 74)*
(5.0)	1439 ± 32 (787 ± 47)***	1965 ± 35 (2184 ± 74)*

Table 8.2 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the midbrain, 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p <0.05; **2p<0.01; ***1 <0.001 (Student's 't' test)

DRUG (mg/kg)	5-HT (ng/g)	5-HIAA (ng/g)
Azepexole (1.0)	810 ± 73 (1405 ± 74)**	1809 ± 143 (1864 ± 145)
(2.0)	1188 ± 97 (1405 ± 74)	1849 ± 109 (1864 ± 145)
Clonidine (0.005)	884 ± 109 (1371 ± 70)**	2004 ± 66 (2038 ± 82)
(0.01)	1730 ± 117 (1405 ± 79)*	1741 ± 150 (1864 ± 145)
(0.025)	1062 ± 22 (1276 ± 39)*	2254 ± 24 (2103 ± 84)*
(0.05)	1457 ± 40 (1376 ± 39)	2263 ± 24 (2103 ± 84)*
(0.075)	1441 ± 35 (1376± 39)	2104 ± 84 (2103 ± 84)
(0.1)	1955 ± 152 (1330 ± 38)*	2153 ± 30 (2101 ± 33)
(0.3)	1355 ± 197 (1479 ± 82)	1815 ±197 (1712 ± 78)
(1.0)	1395 ± 150 (1479 ± 82)	2000 ± 82 (1712 ± 78)*
Guanabenz (0.1)	1329 ± 60 (1376 ± 31)	2298 ± 44 (2163 ± 34)*
(1.0)	1652 ± 28 (1378 ± 24)	2313 ± 130 (2153 ± 138)*
Piperoxane (5.0)	1390 ± 50 (1339 ± 38)	1943 ± 49 (2049 ± 84)
(10.0)	1501 ± 65 (1298 ± 38)*	2187 ± 103 (2024 ± 70)
RS 21361 (5.0)	1613 ± 58 (1490 ± 38)*	1566 ± 63 (1720 ± 87)*
Idazoxan (0.125)	921 ± 109 (1124 ± 28)*	1177 ± 82 (1781 ± 78)**
(0.25)	1025 ± 49 (1124± 28)	1500 ± 135 (1781 ± 78)
Yohimbine (1.25)	1761 ± 51 (1382 ± 35)*	1664 ± 120 (2173 ± 160)*
(2.5)	1638 ± 17 (1382 ± 35)*	1845 ± 107 (2173 ± 160)*
(5.0)	1687 ± 65 (1382 ± 35)*	1890 ± 65 (2173 ± 160)*

Table 8.3 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the hypothalamus 3 hours post injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p<0.05; **2p <0.01; ***2p<0.001 (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Azepexole	(1.0)	929 ± 80	(861 ± 49)	1530 ± 129	(1336 ± 105)
	(2.0)	811 ± 37	(861 ± 49)	1991 ± 129	(1336 ± 195)*
Clonidine	(0.005)	412 ± 69	(786 ± 77)**	1168 ± 98	(1441 ± 63)*
	(0.01)	1176 ± 25	(861 ± 49)*	1336 ± 89	(1336 ± 105)
	(0.025)	851 ± 59	(668 ± 36)*	1285 ± 35	(1404 ± 35)*
	(0.05)	771 ± 16	(668 ± 36)	1382 ± 45	(1404 ± 35)
	(0.075)	1185 ± 34	(668 ± 36)***	1354 ± 45	(1404 ± 35)
	(0.1)	752 ± 36	(703 ± 35)	1324 ± 36	(1313 ± 25)
	(0.3)	861 ± 38	(887 ± 73)	1195 ± 25	(1409 ± 14)*
	(1.0)	939 ± 30	(887 ± 73)	1272 ± 88	(1409 ± 14)*
Guanabenz	(0.1)	695 ± 21	(656 ± 11)	1714 ± 73	(1428 ± 67)*
	(1.0)	495 ± 11	(756 ± 20)*	1300 ± 48	(1271 ± 70)
Piperoxane	(5.0)	679 ± 61	(568 ± 36)	1473 ± 93	(1313 ± 77)
	(10.0)	1064 ± 82	(681 ± 19)**	1245 ± 131	(1525 ± 40)*
RS-21361	(5.0)	868 ± 39	(676 ± 42)*	1255 ± 49	(1246 ± 30)
Idazoxan	(0.125)	867 ± 34	(858 ± 42)	975 ± 36	(1301 ± 49)*
	(0.25)	914 ± 66	(858 ± 42)	1166 ± 52	(1301 ± 49)*
Yohimbine	(1.25)	1266 ± 53	(755 ± 65)**	1095 ± 140	(1257 ± 34)
	(2.5)	1158 ± 69	(755 ± 65)**	1084 ± 24	(1257 ± 34)
	(5.0)	1107 ± 35	(755 ± 65)**	1087 ± 37	(1257 ± 34)

Table 8.4 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the shriatum, 3 hours post injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.
*2p CO.05; **2p<0.01; *** 2p CO.001 (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Azepexole	(1.0)	361 ± 37	(726 ± 41)**	1391 ± 70	(1274 ± 94)
	(2.0)	733 ± 84	(726 ± 41)	1187 ± 105	(1274 ± 94)
Clonidine	(0.005)	742 ± 90	(669 ± 34)	1173 ± 29	(1273 ± 80)
	(0.01)	902 ± 81	(726 ± 41)	1102 ± 54	(1270 ± 94)
	(0.025)	482 ± 20	(870 ± 32)*	1303 ± 27	(1360 ± 17)
	(0.05)	601 ± 41	(870 ± 32)*	1507 ± 17	(1360 ± 17)*
	(0.075)	1049 ± 39	(870 ± 32)	1708 ± 89	(1369 ± 17)*
	(0.1)	816 ± 45	(894 ± 32)	1315 ± 135	(1298 ± 26)
	(0.3)	762 ± 46	(782 ± 34)	1182 ± 26	(1077 ± 28)*
	(1.0)	1008 ± 41	(782 ± 34)*	871 ± 31	(1977 ± 28)*
Guanabenz	(0.1)	488 ± 16	(669 ± 12)*	1613 ± 22	(1261 ± 37)*
	(1.0)	943 ± 41	(674 ± 17)*	1395 ± 17	(1318 ± 100)
Piperoxane	(5.0)	964 ± 12	(870 ± 32)	1095 ± 11	(1305 ± 18)*
	(10.0)	819 ± 38	(619 ± 34)*	1253 ± 77	(1297 ± 26)
RS-21361	(5.0)	916 ± 27	(882 ± 10)	1034 ± 100	(1224 ± 57)*
Idazoxan	(0.125	907 ± 36	(818 ± 41)	963 ± 40	(1362 ± 79)**
	(0.25)	943 ± 35	(818 ± 41)	1062 ± 44	(1362 ± 79)*
Yohimbine	(1.25)	1008 ± 31	(786 ± 30)*	1074 ± 50	(1284 ± 51)*
	(2.5)	954 ± 16	(786 ± 30)*	1004 ± 57	(1284 ± 51)*
	(5.0)	1062 ± 53	(786 ± 30)*	985 ± 36	(1284 ± 51)*

Table 8.5 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the hyppocampus 3 hours post injections. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p<0.05; **2p<0.01; ***2p<0.001 (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Azepexole	(1.0)	474 ± 74	(423 ± 24)	459 ± 31	(389 ± 29)
	(2.0)	365 ± 26	(423 ± 24)	593 ± 22	(389 ± 29)*
Clonidine	(0.005)	299 ± 25	(306 ± 14)	269 ± 10	(334 ± 17)*
	(0.01)	576 ± 16	(423 ± 23)*	302 ± 19	(389 ± 29)*
	(0.025)	246 ± 16	(255 ± 14)	315 ± 36	(313 ± 27)
	(0.05)	296 ± 28	(255 ± 14)	439 ± 8.0	(313 ± 27)*
	(0.075)	286 ± 47	(255 ± 14)	557 ± 28	(313 ± 27***
	(0.1)	201 ± 15	(226 ± 11)	304 ± 30	(297 ± 37)
	(0.3)	416 ± 16	(363 ± 18)*	280 ± 32	(351 ± 21)
	(1.0)	462 ± 22	(363 ± 18)*	303 ± 14	(351 ± 21)
Guanabenz	(0.1)	414 ± 18	(316 ± 10)*	433 ± 21	(389 ± 23)
	(1.0)	422 ± 23	(292 ± 6.0)*	279 ± 17	(280 ± 15)
Piperoxane	(5.0)	232 ± 9.0	(255 ± 14)	290 ± 10	(285 ± 14)
	(10.0)	314 ± 22	(226 ± 15)*	186 ± 32	(277 ± 36)*
RS-21361	(5.0)	398 ± 15	(334 ± 13)	291 ± 26	(327 ± 15)
Idazoxan	(0.125)	456 ± 21	(401 ± 18)	266 ± 40	(359 ± 15)*
	(0.25)	441 ± 22	(401 ± 18)	243 ± 29	(359 ± 15)**
Yohimbine	(1.25)	393 ± 10	(314 ± 11)*	302 ± 35	(392 ± 29)*
	(2.5)	395 ± 26	(314 ± 11)*	268 ± 24	(392 ± 29)*
	(5.0)	401 ± 9.0	(314 ± 11)*	276 ± 31	(392 ± 29)*

Table 8.6 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrates in the cortex 3 hours post injection.
Results are expressed as mean ± s.e.m.
* 2p <0.05; ** 2p <0.01; *** 2p <0.001 (Student's 't' test)

(a) Pons/medulla

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Azepexole	(1.0)	812 ± 22	(833± 18)	1549 ± 48	(1343 ± 37)
	(2.0)	766 ± 34	(845 ± 32)*	1426 ± 30	(1187 ± 26)*
Clonidine	(0.01)	793 ± 26	(792 ± 25)	1146 ± 14	(1153 ± 20)
	(0.05)	906 ± 74	(775 ± 21)	411 ± 46	(1255 ± 45)***
	(0.075)	806 ± 43	(775 ± 21)	834 ± 84	(1255 ± 45)**
Guanabenz	(0.1)	801 ± 33	(806 ± 36)	1408 ± 26	(1205 ± 25)*
	(1.0)	792 ± 100	(795 ± 34)	1208 ± 61	(1163 ± 35)
RS-21361	(10.0)	918 ± 25	(908 ± 33)	1340 ± 15	(1334 ± 21)
(b) Midbrain					
Azepexole	(1.0)	683 ± 26	(805 ± 12)	2264 ± 99	(2065 ± 32)
	(2.0)	753 ± 37	(799 ± 13)	2058 ± 91	(2124 ± 117)
Clonidine	(0.01)	767 ± 18	(728 ± 17)	2323 ± 49	(2119 ± 30)*
	(0.05)	493 ± 13	(802 ± 39)**	2280 ± 31	(2184 ± 30)
	(0.075)	371 ± 46	(802 ± 39)**	2250 ± 46	(2184 ± 30)
Guanabenz	(0.1)	655 ± 18	(756 ± 11)*	2259 ± 120	(2015 ± 21)*
	(1.0)	834 ± 72	(798 ± 69)	2416 ± 62	(2034 ± 117)*
RS-21361	(10.0)	845 ± 35	(730 ± 35)*	2018 ± 56	(2011 ± 36)

Table 8.7 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations on (a) pons-medulla and (b) midbrain 40 mins post-injection.

Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* $2p < 0.05$; ** $2p < 0.01$; *** $2p < 0.001$ (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
(a) hypotholamus					
Azepexole	(1.0)	1092 ± 61	(1296 ± 24)	2275 ± 65	(2172 ± 23)
	(2.0)	1179 ± 47	(1276 ± 22)	2239 ± 34	(2041 ± 48)*
Clonidine	(0.01)	1287 ± 26	(1304 ± 13)	2281 ± 36	(2290 ± 66)
	(0.05)	1516 ± 32	(1376 ± 27)*	1993 ± 71	(2103 ± 31)*
	(0.075)	1505 ± 16	(1376 ± 27)*	1965 ± 54	(2103 ± 31)*
Guanabenz	(0.1)	1401 ± 28	(1376 ± 31)	2143 ± 32	(2163 ± 34)
	(1.0)	1445 ± 25	(1378 ± 25)	2166 ± 110	(2153 ± 138)
RS 21361	(10.0)	1416 ± 25	(1441 ± 35)	2240 ± 35	(2110 ± 35)*
(b) striatum					
Azepexole	(1.0)	462 ± 29	(688 ± 33)**	1648 ± 52	(1407 ± 83)
	(2.0)	597 ± 38	(709 ± 15)*	1490 ± 36	(1473 ± 34)
Clonidine	(0.01)	672 ± 23	(650 ± 10)	1779 ± 48	(1612 ± 13)*
	(0.05)	1117 ± 128	(742 ± 25)**	1255 ± 33	(1326 ± 23)
	(0.075)	806 ± 33	(742 ± 25)	1157 ± 67	(1326 ± 23)
Guanabenz	(0.1)	648 ± 16	(656 ± 11)	1685 ± 85	(1428 ± 67)*
	(1.0)	543 ± 11	(756 ± 20)*	1228 ± 46	(1271 ± 70)
RS 21361	(10.0)	673 ± 21	(707 ± 19)	1474 ± 43	(1481 ± 33)

Table 8.8 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in (a) hypothalamus and (b) striatum 40 mins post-injection.

Results are expressed as mean ± s.e.m. Control values are expressed in brackets.

* $2p < 0.05$; ** $2p < 0.01$; *** $2p < 0.001$ (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
(a) hippocampus					
Azepexole	(1.0)	659 ± 31	(687 ± 30)	1354± 25	(1369 ± 14)
	(2.0)	543 ± 43	(891 ± 43)*	1630 ± 33	(1410 ± 48)*
Clonidine	(0.01)	824 ± 11	(826 ± 8)	1383 ± 19	(1356 ± 15)
	(0.05)	1044 ± 52	(763 ± 26)	1020 ± 33	(1305 ± 44)
	(0.075)	632 ± 39	(763 ± 26)	1402 ± 75	(1305 ± 44)
Guanabenz	(0.1)	522 ± 18	(619 ± 12)*	1575 ± 69	(1361 ± 47)*
	(1.0)	695 ± 13	(674 ± 17)	1396 ± 50	(1318 ± 100)
RS 21361	(10.0)	764 ± 23	(634 ± 40)	1310 ± 35	(1298 ± 25)
(b) cortex					
Azepexole	(1.0)	267 ± 18	(275 ± 13)	364 ± 6	(310 ± 19)
	(2.0)	221 ± 39	(257 ± 23)	309 ± 20	(320 ± 15)
Clonidine	(0.01)	288 ± 6	(302 ± 5)	362 ± 10	(323 ± 8)
	(0.05	239 ± 12	(358 ± 39)*	355 ± 27	(267 ± 12)*
	(0.075	223 ± 26	(358 ± 39)*	334 ± 50	(267 ± 12)
Guanabenz	(0.1)	330 ± 14	(316 ± 10)	367 ± 25	(389 ± 23)
	(1.0)	393 ± 26	(292 ± 6)*	373 ± 17	(280 ± 17)*
RS 21361	(10.0)	310 ± 5	(300 ± 13)	341 ± 17	(321 ± 23)

Table 8.9 The effect of α_2-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in (a) hippocampus and (b) cortex 40 mins post-injection.

Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* 2p <0.05; ** 2p <0.01; *** 2p <0.001 (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Phenephrine	(0.25)	699 ± 39	(642 ± 21)	1272 ± 30	(1237 ± 32)
	(1.0)	632 ± 73	(775 ± 48)	1194 ± 31	(1255 ± 45)
	(2.5)	990 ± 31	(699 ± 53)*	1301 ± 69	(1243 ± 36)
St 587	(0.1)	716 ± 36	(837 ± 39)*	1175 ± 60	(1231 ± 102)
	(0.5)	646 ± 61	(728 ± 35)	1370 ± 105	(1352 ± 93)
	(1.0)	641 ± 40	(728 ± 35)	988 ± 93	(1352 ± 93)*
	(2.0)	863 ± 77	(704 ± 50)	856 ± 14	(1397 ± 50)
Prazosin	(0.025)	612 ± 46	(837 ± 39)*	1264 ± 70	(1231 ± 102)
	(0.05)	807 ± 58	(799 ± 34)	1391 ± 117	(1272 ± 100)
	(0.1)	482 ± 30	(768 ± 52)*	1239 ± 71	(1265 ± 53)
	(0.25)	643 ± 27	(642 ± 39)	1306 ± 28	(1237 ± 32)
	(0.5)	664 ± 43	(775 ± 48)	1305 ± 40	(1255 ± 45)
	(1.0)	776 ± 74	(699 ± 53)	1488 ± 63	(1243 ± 36)*
Thymoxamine	(0.1)	1282 ± 75	(704 ± 59)***	921 ± 56	(1397 ± 50)**
	(0.5)	881 ± 43	(696 ± 83)*	1381 ± 67	(1132 ± 53)*
	(1.0)	1014 ± 97	(696 ± 83)**	1609 ± 97	(1132 ± 53)**

Table 8.10 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the pons-medulla 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* $2p < 0.05$: ** $2p < 0.01$; *** $2p < 0.001$ (Students 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Phenylephrine					
	(0.25)	1177 ± 50	(822 ± 91)	2129 ± 55	(2159 ± 43)
	(1.0)	656 ± 22	(882 ± 23)	2228 ± 99	(2125 ± 61)
	(2.5)	989 ± 27	(849 ± 16)*	2443 ± 149	(2052 ± 130)*
St 587	(0.1)	703 ± 107	(754 ± 57)	2173 ± 60	(1988 ± 97)
	(0.5)	801 ± 75	(927 ± 66)	2121 ± 114	(2065 ± 104)
	(1.0)	861 ± 84	(927 ± 66)	2029 ± 84	(2065 ± 104)
	(2.0)	596 ± 52	(700 ± 38)	1691 ± 86	(1861 ± 88)
Prazosin	(0.025)	440 ± 35	(754 ± 57)*	2322 ± 82	(1988 ± 97)*
	(0.05)	620 ± 68	(852 ± 56)*	1775 ± 65	(1972 ± 63)*
	(0.1)	605 ± 60	(851 ± 20)*	1203 ± 58	(2058 ± 38)*
	(0.25)	1013 ± 106	(822 ± 91)	2169 ± 102	(2159 ± 43)
	(0.5)	692 ± 65	(882 ± 23)	2454 ± 35	(2125 ± 61)*
	(1.0)	952 ± 20	(849 ± 16)*	3029 ± 38	(2052 ± 23)*
Thymoxamine	(0.1)	574 ± 47	(700 ± 38)*	1631 ± 110	(1861 ± 88)
	(0.5)	921 ± 60	(813 ± 33)	2212 ± 102	(1905 ± 68)**
	(1.0)	811 ± 52	(813 ± 33)	2201 ± 51	(1905 ± 68)**

Table 8.11 The effect of α_1-adrenoceptor ligands as 5-HT and 5-HIAA concentrations in the midbrain 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* $2p < 0.05$; ** $2p < 0.01$: *** $2p < 0.001$ (Student's 't' test).

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Phenylephrine					
	(0.25)	1288 ± 55	(1478 ± 79)*	1501 ± 117	(1904 ± 108)*
	(2.0)	1857 ± 40	(1395 ± 54)*	2033 ± 59	(1803 ± 31)*
	(2.5)	859 ± 55	(1457 ± 88)*	2226 ± 153	(1734 ± 88)*
St 587	(0.1)	1069 ± 70	(1401 ± 124)*	1697 ± 101	(1816 ± 73)
	(0.5)	1978 ± 154	(1209 ± 105)	1553 ± 143	(1726 ± 156)
	(1.0)	792 ± 64	(1209 ± 105)*	1619 ± 54	(1726 ± 156)
	(2.0)	1380 ± 55	(1215 ± 33)	1965 ± 54	(1945 ± 46)
Prazasin	(0.025)	1081 ± 53	(1401 ± 124)*	1726 ± 114	(1816 ± 73)
	(0.05)	1773 ± 89	(1362 ± 82)*	2338 ± 57	(2124 ± 92)
	(0.1)	1479 ± 41	(1386 ±111)	1563 ± 36	(1884 ± 53)*
	(0.25)	1416 ± 69	(1478 ± 79)	1410 ± 87	(1904 ± 108)*
	(0.5)	1557 ± 40	(1395 ± 54)	1822 ± 35	(1803 ± 31)
	(1.0)	1512 ± 51	(1457 ± 88)	2012 ± 83	(1734 ±88)*
Thymoxamine	(0.1)	1269 ± 44	(1215 ± 33)	1941 ± 53	(1942 ± 55)
	(0.5)	1358 ± 37	(1367 ± 48)	1494 ± 62	(1674 ± 57)*
	(1.0)	1184 ± 67	(1367 ± 48)*	1452 ± 59	(1674 ± 57)*

Table 8.12 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the hypothalamus 3 hours post injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.
*2p <0.05: ** 2p <0.01: *** 2p <0.001 (Student's 't' test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Phenylephrine					
	(0.25)	611 ± 78	(849 ± 35)*	1069 ± 58	(1379 ± 44)*
	(1.0)	475 ± 48	(780 ± 53)*	1409 ± 106	(1339 ± 106)
	(2.5)	549 ± 27	(716 ± 19)*	1240 ± 40	(1266 ± 73)
St 587	(0.1)	722 ± 89	(802 ± 72)	1791 ± 89	(1501 ± 65)*
	(0.5)	712± 56	(763 ± 36)	1419 ± 54	(1363 ± 54)
	(1.0)	732 ± 35	(763 ± 36)	1499 ± 73	(1363 ± 54)
	(2.0)	860 ± 49	(844 ± 53)	2014 ± 99	(1459 ± 39)*
Prazosin	(0.025)	709 ± 80)	(802 ± 73)	1539 ± 106	(1502 ± 65)
	(0.05)	728 ± 72	(757 ± 17)	1923 ± 97	(1423 ± 34)*
	(0.1)	584 ± 24	(901 ± 37)*	1409 ± 57	(1434 ± 29)
	(0.25)	796 ± 77	(849 ± 35)	1210 ± 150	(1379 ± 44)
	(0.5)	534 ± 44	(78- ± 53*	1282 ± 78	(1339 ± 26)
	(1.0)	529 ± 20	(714 ± 19)*	1365 ± 71	(1266 ± 73)
Thymoxamine	(0.1)	665 ± 36	(844 ± 53)	1817 ± 98	(1459 ± 39)*
	(0.5)	839 ± 67	(882 ± 53)	1226 ± 76	(1316 ± 62)
	(1.0)	699 ± 34	(882 ± 53)**	1329 ± 45	(1316 ± 62)

Table 8.13 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the striatum 3 hours post injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* $2p < 0.05$: ** $2p < 0.01$; *** $2p < 0.001$ (Student's 't' test).

DRUG (mg/kg)		5-HT (ng/g)		5 HIAA (ng/g)	
Phenylephrine					
	(0.25)	486 ± 56	(662 ± 35)*	1006 ± 39	(1297 ± 54)*
	(1.0)	896 ± 65	(763 ± 26)	1020 ± 33	(1305 ± 44)*
	(2.5)	569 ± 39	(885 ± 34)*	1481 ± 35	(1347 ± 55)
St 587	(0.1)	770 ± 49	(777 ± 59)	1179 ± 68	(1239 ± 118)
	(0.5)	783 ± 44	(772 ± 34)	1341 ± 36	(1368 ± 80)
	(1.0)	617 ± 35	(772 ± 34)*	1174 ³ 42	(1368 ± 80)*
	(2.0)	939 ± 60	(802 ± 59)	1352 ± 95	(1211 ± 67)
Prazosin	(0.025)	893 ± 48	(777 ± 59)	1008 ± 50	(1239 ± 118)*
	(0.05)	669 ± 39	(703 ± 42)	1374 ± 52	(1336 ± 71)
	(0.1)	548 ± 40	(882 ± 69)*	1617 ± 96	(1309 ± 48)*
	(0.25)	685 ± 42	(662 ± 35)	1004 ± 102	(1297 ± 84)
	(0.5)	842 ± 52	(763 ± 26)	1281 ± 34	(1305 ± 44)
	(1.0)	461 ± 28	(885 ± 35)*	1549 ± 65	(1347 ± 55)*
Thymoxamine	(0.1)	709 ± 42	(802 ± 50)	1252 ± 95	(1211 ± 67)
	(0.5)	683 ± 31	(918 ± 57)**	1252 ± 76	(1214 ± 58)
	(1.0)	526 ± 58	(918 ± 57)**	1310 ± 48	(1214 ± 58)

Table 8.14 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the hippocampus 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.
* $2p < 0.05$; ** $2p < 0.01$; *** $2p < 0.001$ (Student's 't' test).

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
Phenylephrine					
	(0.25)	232 ± 14	(292 ± 14)*	222 ± 19	(199 ± 9.0)
	(1.0)	354 ± 39	(374 ± 39.4)	345 ± 20	(351 ± 21)
	(2.5)	345 ± 37	(342 ± 20)	350 ± 22	(381 ± 29(
St587	(0.1)	286 ± 26	(292 ± 24)	223 ± 13	(258 ± 12)
	(0.5)	360 ± 31	(412 ± 22)	438 ± 15	(440 ± 13)
	(1.0)	381 ± 32	(412 ± 22)	307 ± 13	(440 ± 13)*
	(2.0)	260 ± 19	(317 ± 17)*	180 ± 13	(388 ± 27)**
Prazosin	(0.025)	278 ± 12	(292 ± 24)	210 ± 10	(258 ± 12)
	(0.05)	286 ± 17	(319 ± 27)	328 ± 26	(323 ± 22)
	(0.1)	273 ± 20	(384 ± 24)	536 ± 20	(419 ± 18)*
	(0.25)	260 ± 17	(292 ± 14)	251 ±13	(198 ± 9.0)*
	(0.5)	311 ± 34	(374 ± 39)	395 ± 35	(351 ± 21)
	(1.0)	201 ± 15	(347 ± 29)*	312 ± 7.0	(381± 29)*
Thymoxamine	(0.1)	275 ± 16	(317 ± 17)	359 ± 17	(388 ± 27)
	(0.5)	365 ± 33	(409 ± 24)	236 ± 14	(265 ± 9.0)
	(1.0)	344 ± 8.0	(409 ± 24)*	175 ± 15	(265 ± 9.0)*

Table 8.15 The effect of α_1-adrenoceptor ligands on 5-HT and 5-HIAA concentrations in the cortex, 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p <0.05: ** 2p <0.01; *** 2p <0.001 (Student's 't' test).

CHAPTER 9

REGIONAL CHANGES IN BRAIN 5-HT AND 5-HIAA LEVELS AFTER THE ADMINISTRATION OF 5-HT RECEPTOR LIGANDS

Introduction

The aim of this study was to attempt to establish whether the behavioural effects observed with 5-HT receptor ligands in the maze exploration study (Chapter 2) as well as conflict behaviour (Chapters 3 and 5) could be correlated with their effects on regional brain concentrations of 5-HT and 5-HIAA.

The pharmacology of many drugs affecting serotonergic neurotransmission within the CNS is not yet completely understood. For example, it has been suggested that quipazine acts at dopamine receptors (Grabowska et al, 1974b) and has also been shown to act as a weak monoamine oxidase inhibitor both in vitro and in vivo as well as 5-HT receptors (Green et al, 1976); on the other hand, a range of "classical" 5-HT receptor antagonists (which include methysergide, metergoline and pizotifen) have been demonstrated to possess partial agonistic activity (Colpaert et al, 1982). Furthermore, at present there is no generally satisfactory subclassification of 5-HT receptors to account for differing properties among groups of agonists and antagonists (for classification of 5-HT receptors, see General Introduction).

In view of these considerations, studying the effects of such agents on changes in regional 5-HT and 5-HIAA concentrations would not only indicate the extent to which behavioural and biochemical effects can be correlated, but would also attempt to establish whether these agents have a general effect on serotonergic function or whether these effects vary according to the brain region as well as the dose concerned. Furthermore, although the effects of quipazine on whole-brain (Green et al, 1976) and regional (Hamon et al, 1976) 5-HT and 5-HIAA levels have been previously examined, the effects of ketanserin and pirenperone have not. Experiments were therefore carried out in order to determine the effects of ketanserin, pirenperone and quipazine on 5-HT and 5-HIAA levels in six brain regions, 3 hrs post-injection. For display in figures, results are expressed as

$$\frac{[\text{Mean 5-HT or 5-HIAA concentration}]\ \text{drug}}{[\text{Mean 5-HT or 5-HIAA concentration}]\ \text{control}} \times 100\%$$

However, actual values for each group (mean $\pm$ s.e.m.) are shown in Tables 9.1 - 9.3.

1. The effect of quipazine.

In the pons-medulla, 5-HT was consistently elevated with quipazine 0.5 - 2.0 mg/kg (significant only at 0.5 mg/kg). 5-HIAA in this region was generally decreased, significant effects observed only with the highest dose used (Fig.9.1). In the midbrain, 5-HT was significantly increased at 1.0 and 2.0 mg/kg and there was a significant fall in 5-HIAA for all three doses (Fig.9.1). Hypothalamic and striatal 5-HT and 5-HIAA remained unchanged (generally), at all doses except 2.0 mg/kg where a significant increase in hypothalamic 5-HT was observed (Fig.9.2).

Quipazine had no effect on hippocampal 5-HT, although 5-HIAA was significantly decreased at 1.0 mg/kg. Only 2.0 mg/kg quipazine significantly increased cortical 5-HT, but there was a general fall in cortical 5-HIAA with quipazine (significant changes observed with 1.0 mg and 2.0 mg/kg respectively) (Fig.9.3).

2. The effect of ketanserin.

Ketanserin (0.025 - 0.2 mg/kg) caused a significant fall in 5-HT, and 5-HIAA was also decreased (significant only at 0.2 mg/kg) in the pons-medulla. In the midbrain, 5-HT was consistently decreased (significant changes observed at 0.1 mg and 0.2 mg/kg), but no significant changes occured in 5-HIAA levels (Fig.9.1). In the hypothalamus and striatum 5-HT was decreased, although only 0.025 mg/kg decreased it significantly. Hypothalamic and striatal 5-HIAA was significantly decreased at 0.1 mg and 0.2 mg/kg ketanserin, but 0.05 mg/kg increased it in the striatum (Fig.9.2). In the hippocampus, 5-HT was decreased significantly at all doses and there was also a general decrease in 5-HIAA, although none of these were statistically significant. Ketanserin 0.025 - 0.05 mg/kg decreased both 5-HT and 5-HIAA in the cortex; however, 0.1 - 0.2 mg/kg did not affect either of these (Fig.9.3).

3. The effect of pirenperone.

In the pons-medulla and midbrain, 5-HT decreased significantly only in the midbrain at 0.2 mg/kg pirenperone. 5-HIAA however, increased in the pons-medulla at 0.1 mg/kg pirenperone, but decreased in the midbrain with 0.1 - 0.2 mg/kg (Fig.9.1). Pirenperone (0.05 - 0.2 mg/kg) caused a significant fall in 5-HT in the hypothalamus and

striatum. Hypothalamic 5-HIAA decreased significantly at 0.1 - 0.2 mg/kg whereas striatal 5-HIAA decreased significantly at 0.1 mg/kg only (Fig.9.2).

Hippocampal and cortical 5-HT was generally decreased. Significant effects were only observed at 0.1 - 0.2 mg/kg in the cortex. There was also a general fall in 5-HIAA, significant effects being observed at 0.1 mg/kg in the cortex and the hippocampus (Fig.9.3).

Discussion

The effect of quipazine on 5-HT and 5-HIAA concentrations indicated that in all areas except the hypothalamus and striatum (where effects varied according to the dose), there was a general increase in 5-HT and a decrease in 5-HIAA. Green et al (1976) have reported that quipazine induced a small increase in brain 5-HT - an effect that was only apparent up to 90 mins post-injection. In another study, quipazine was shown to decrease 5-HIAA in the brain-stem and forebrain, the effect being maximal 2 hrs after injection, whereas increases in 5-HT were only seen in the brain-stem 1 hr after administration (Hamon et al, 1976). Furthermore, Grabowska et al, (1974a) demonstrated that 1 - 3 hrs post-quipazine injection 5-HIAA was significantly decreased, but 5-HT remained unaffected at all times, and the effect of quipazine on 5-HIAA was antagonised by pretreating animals with methysergide.

Although generally results obtained here are consistent with those of other studies, differences in detecting changes in these indoleamines may largely be due to the differences in time scale of the experiments, and to a lesser extent the dose range examined. In this study, biochemical determinations were based on the effects of quipazine reported by Grabowska et al (1974a), since one of the doses of quipazine used was 2.5 mg/kg (which could within reason, be paralleled to the 0.5 - 2.0 mg/kg dose range used in this study), as opposed to 10 mg/kg (Hamon et al, 1976) and 25 mg/kg (Green et al, 1976). Moreover, since the effects of α-adrenoceptor agonists and antagonists, anxiolytics and putative anxiogenics were also looked at 3 hrs post injection, it was felt that this time of determination would be useful to compare the effects of quipazine with those reported in Chapters 7 and 8.

In view of the results obtained with quipazine, and those of the α_2-adrenoceptor antagonists yohimbine, piperoxane and RS 21361, (Chapter 8), it appears that they have similar effects on changes in brain indoleamines. Both types of drugs cause anxiogenic-like effects in the behavioural tests examined

(Chapters 2 and 3) therefore suggesting that 5-HT may well be involved in some way in the anxiogenic actions of these drugs, and that both type of agents may have a similar mode of action on 5-HT systems to modulate anxiety, although the action of α_1-adrenoceptor antagonists on 5-HT systems may be a secondary effect. However, it is still unclear why diazepam and the α_2-adrenoceptor antagonists and quipazine, while having opposite behavioural effects, should have similar effects on regional concentrations of 5-HT and 5-HIAA.

In this study, ketanserin and pirenperone 3 hrs post-injection showed some similar effects. There was a general fall in indoleamines in the striatum, hypothalamus, hippocampus and cortex, suggesting a decrease in 5-HT release. 5-HT was generally decreased in the midbrain with both drugs, but only higher doses of pirenperone caused a significant reduction in 5-HIAA. However, although in the pons-medulla ketanserin decreased both 5-HT and 5-HIAA, a significant elevation in 5-HIAA was observed with one dose of pirenperone.

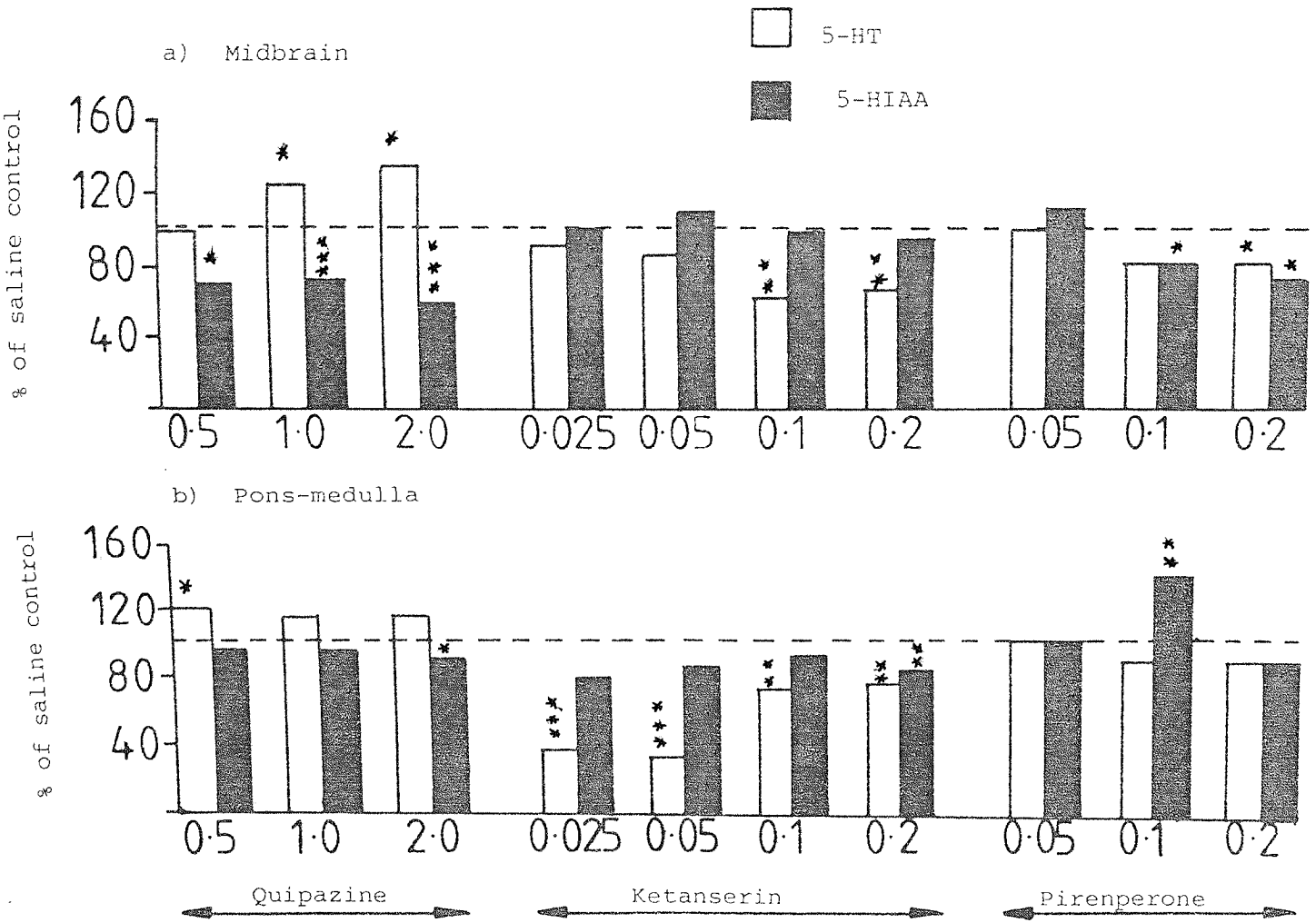
Ketanserin and pirenperone have not been previously examined, although methiothepin was shown to accelerate both the synthesis and utilisation of brain 5-HT 2 hrs post-injection (Bourgoin et al, 1977), whereas metergoline decreased the rate of synthesis and utilisation of 5-HT (Bourgoin et al, 1978). LSD had a similar effect to that of metergoline (Bourgoin et al, 1977), and although pretreatment with metergoline abolished the increase in the rate of 5-HT synthesis and utilisation induced by methiothepin, LSD had no such effect on the action of methiothepin (Bourgoin et al, 1978).

Owing to the fact that metergoline and methiothepin have been demonstrated to possess partial agonistic activity (Colpaert et al, 1982) effects of these agents on brain 5-HT and 5-HIAA cannot be compared to those of pirenperone and ketanserin, since the latter drugs have been shown to be more selective (Colpaert and Janssen, 1983; Leysen et al, 1981). However it is possible that the effect if ketanserin on 5-HT and 5-HIAA may partly be due to its α-adrenoceptor blocking activity, since ketanserin has

been shown to block α_1-adrenoceptors (Kalkman et al, 1983) although from the results obtained in Chapter 8 with the α_1-adrenoceptor antagonists, it seems unlikely that the consistent effect on 5-HT and 5-HIAA produced by ketanserin in a number of brain regions was due to its α_1-blocking activity. This is because the effect of α_1-adrenoceptor antagonists on regional 5-HT and 5-HIAA concentrations was fairly inconsistent.

In general, the opposite effects of quipazine and ketanserin and pirenperone on brain 5-HT concentrations 3 hrs post-injection suggest that the effects of these agents in modulating fear-motivated behaviour is mediated via serotonergic systems. However there is another consideration. The biochemical effects of ketanserin and pirenperone at a range of times after dosing have not been performed, hence the possibility that some peak effects may have occured at a time other than 3 hrs post-injection cannot be excluded. Further experiments are required to examine the dynamics of the changes which occur. However these experiments were beyond the scope of this study since essentially the requirement that dictated the decision that biochemistry should be performed on multiple doses rather than at multiple times was that of the dose-response relationships which were of considerable importance especially in evaluating the maze as a model of fear-motivated behaviour.

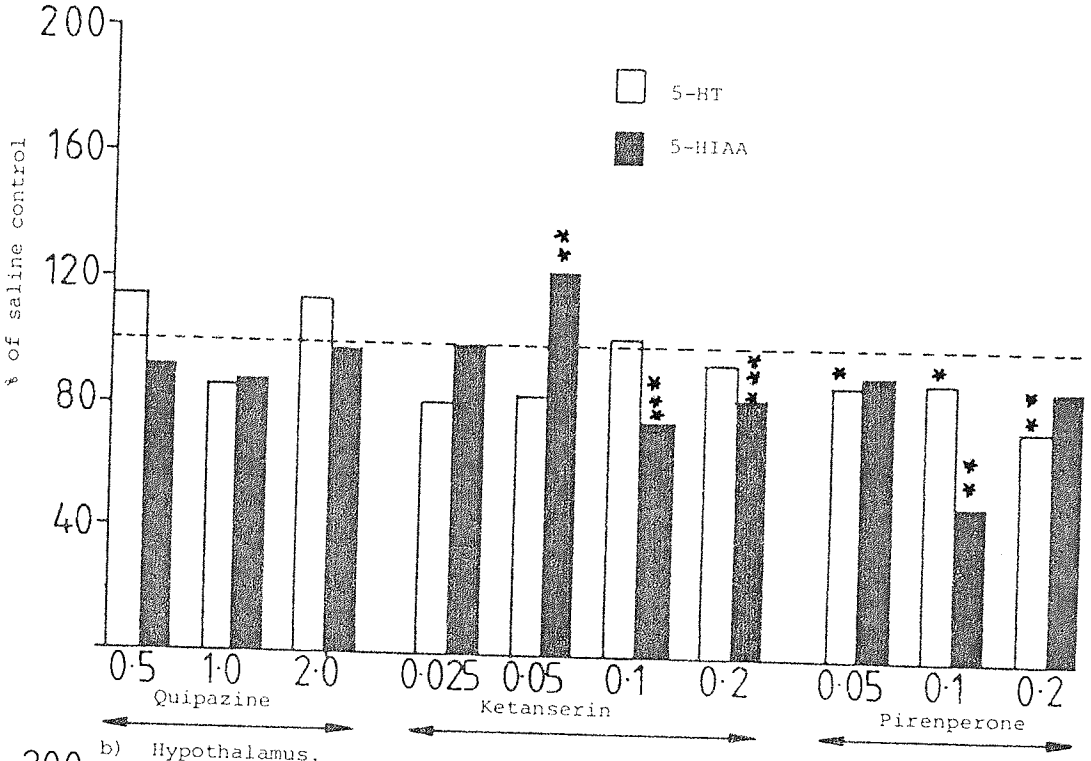
Fig. 9.1 *The effect of 5-HT receptor ligand and changes in 5-HT and 5-HIAA concentrations in a) Midbrain and b) Pons-medulla 3hrs post-injection.*



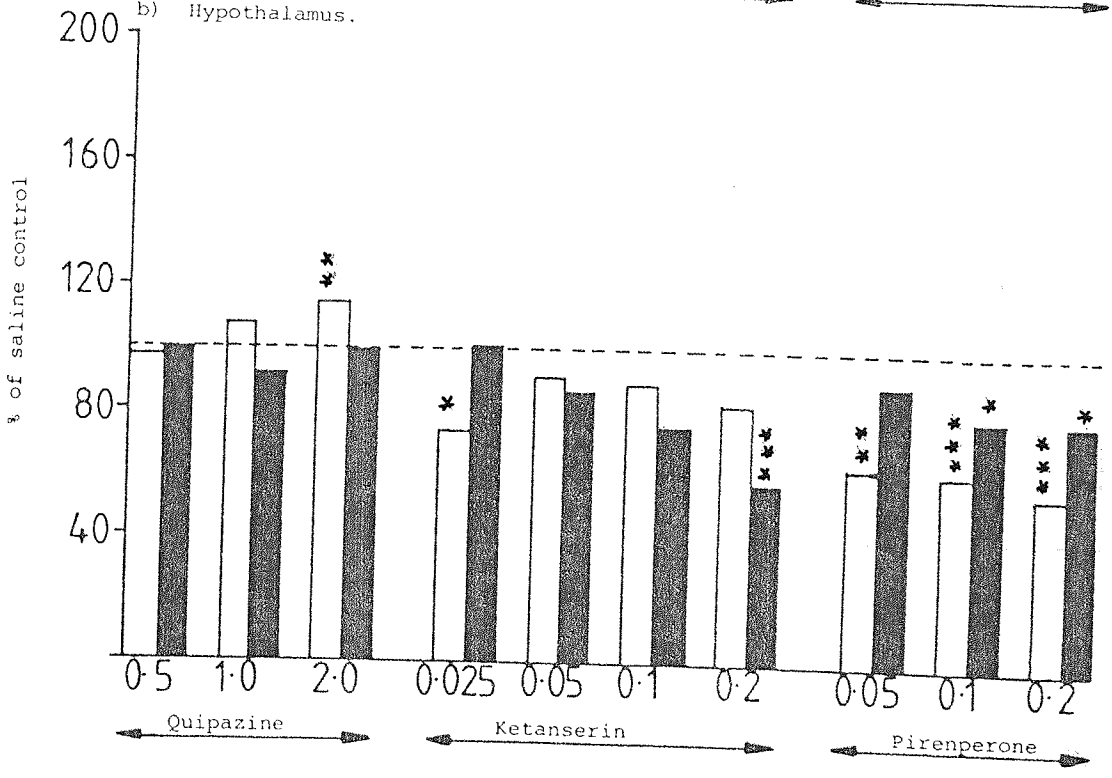
Results are expressed as % of control
*2p < 0.05 **2p < 0.01 ***2p < 0.001 (Students t test)

Fig. 9.2 The effect of 5-HT receptor ligands on changes in 5-HT and 5-HIAA concentrations in a) Striatum and b) Hypothalamus.

a) Striatum



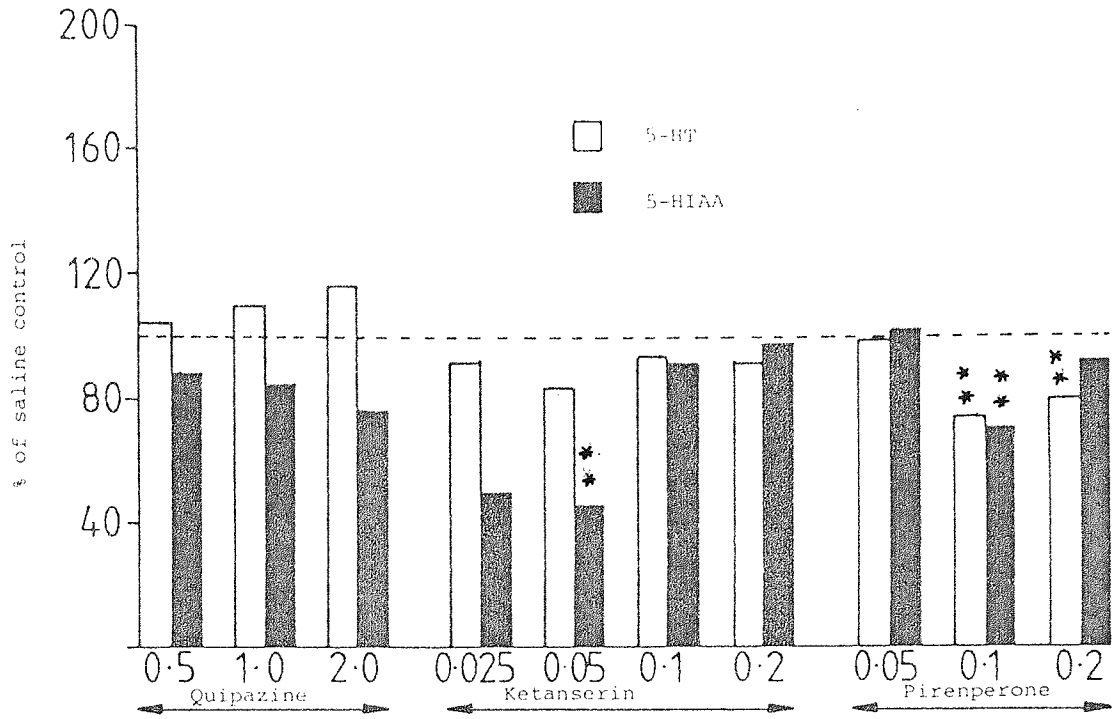
b) Hypothalamus.



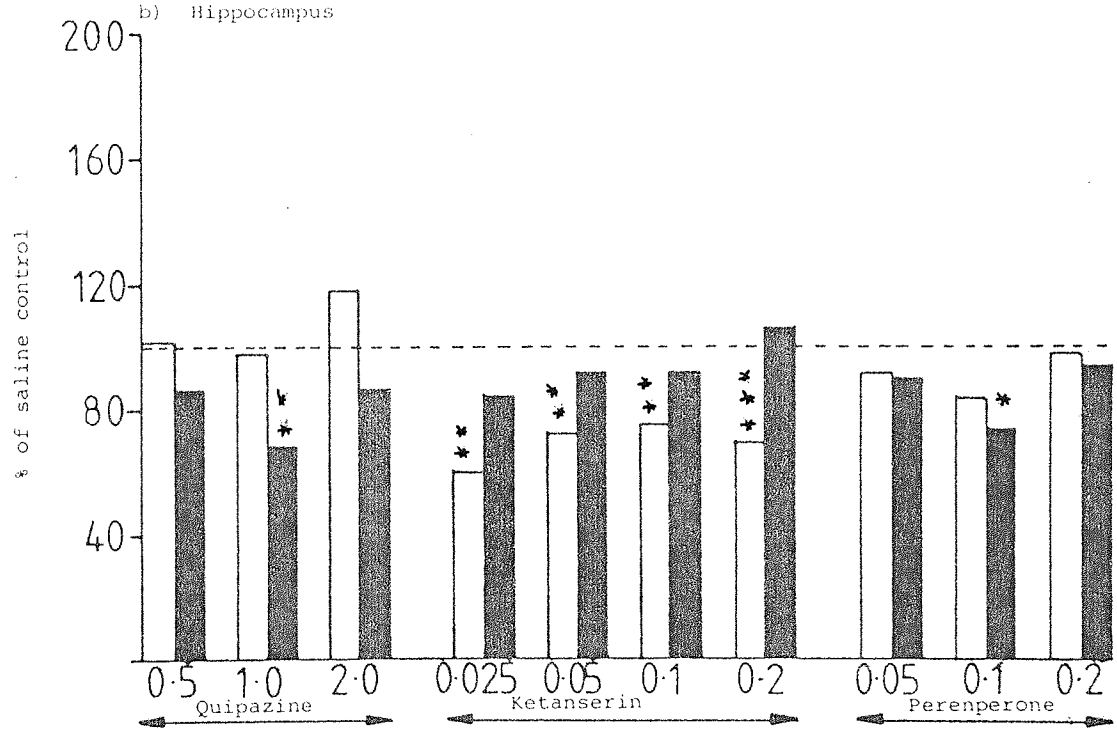
Results are expressed as % of control.
*2p < 0.05 **2p <0.01 ***2p <0.001 (Students t test).

Fig. 9.3. The effect of 5-HT receptor ligands on changes in 5-HT and 5-HIAA concentrations in a) Cortex and b) Hippocampus 3hrs post-injection.

a) Cortex



b) Hippocampus



Results are expressed as % of control.
*2p <0.05 **2p <0.01 ***2p <0.001 (Students t test)

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
(a) pons-medulla					
Quipazine	(0.5)	754 ± 27	(617 ± 25)*	1195 ± 75	(1235 ± 56)
	(1.0)	814 ± 47	(707 ± 64)	1181 ± 70	(1250 ± 27)
	(2.0)	829 ± 43	(702 ± 64)	1119 ± 40	(1259 ± 27)*
Ketanserin	(0.025)	262 ± 38	(782 ± 76)***	911± 64	(1118 ± 57)
	(0.05)	254 ± 35	(782 ± 76)***	962 ± 59	(1118 ± 57)
	(0.1)	524 ± 42	(709 ± 24)**	1340 ± 91	(1448 ± 33)
	(0.2)	534 ± 45	(709 ± 24)**	1206 ± 35	(1448 ± 33)**
	(0.05)	776 ± 16	(759 ± 36)	1226 ± 74	(1201 ± 53)
Pirenperone	(0.1)	549 ± 44	(617 ± 25)	1711 ± 46	(1135 ± 56)**
	(0.2)	674 ± 30	(759 ± 36)	1071 ± 31	(1201 ± 53)
(b) mid-brain					
Quipazine	(0.5)	882 ± 41	(952 ±43)	1251 ± 49	(1759 ± 59)*
	(1.0)	867 ± 55	(693 ± 73)*	1357 ± 18	(1875 ± 95)***
	(2.0)	912 ± 105	(693 ± 73)*	1138 ± 48	(1875 ± 45)***
Ketanserin	(0.025)	842 ± 112	(909 ± 112)	1888 ± 87	(1872 ± 70)
	(0.05)	796 ± 105	(909 ± 112)	2057 ± 106	(1872 ± 70)
	(0.1)	521 ± 69	(805 ± 57)**	1618 ± 46	(1656 ± 33)
	(0.2)	556 ± 60	(805 ± 57)**	1572 ± 41	(1656 ± 33)
Pirenperone	(0.05)	693 ± 30	(691 ± 28)	1960 ± 59	(1668 ± 54)
	(0.1)	808 ± 33	(952 ± 43)	1502 ± 95	(1759 ± 59)*
	(0.2)	581 ± 25	(691 ± 28)*	1263± 54	(1668 ± 54)*

Table 9.1 The effect of 5-HT receptor ligands on 5-HT and 6-HIAA in the (a) pons-medulla and (b) midbrain 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p <0.05; ** 2p <0.01; ***2p<0.001(Student's 't' test).

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
(a) hypothalamus					
Quipazine	(0.5)	1282 ± 69	(1311 ± 36)	1960 ± 81	(1957 ± 115)
	(1.0)	1490 ± 88	(1371 ± 70)	1929 ± 77	(2038 ± 81)
	(2.0)	1692 ± 38	(1371 ± 70)**	2066 ± 43	(2038 ± 81)
Ketanserin	(0.025)	879 ± 77	(1162 ± 89)*	1861 ± 131	(1820 ± 64)
	(0.05)	1068 ± 102	(1167 ± 89)	1680 ± 180	(1815 ± 101)
	(0.1)	1214 ± 44	(1336± 67)	1358 ± 170	(1815 ± 101)
	(0.2)	1104 ± 61	(1332 ± 67)	1052 ± 32	(1815 ±101)***
Pirenperone	(0.05)	908 ± 80	(1445 ± 75)**	1529 ± 45	(1682 ± 75)
	(0.1)	811 ± 71	(1311 ± 36)***	1553 ± 150	(1957± 115)*
	(0.2)	830 ± 23	(1445 ± 75)***	1351 ± 57	(1682 ± 75)*
(b) striatum					
Quipazine	(0.5)	947 ± 79	(823 ± 64)	1295 ± 96	(1393 ± 56)
	(1.0)	681 ± 43	(786 ± 77)	1259 ± 96	(1441 ± 63)
	(2.0)	891 ± 112	(786 ± 77)	1410 ± 38	(1441 ± 63)
Ketanserin	(0.025)	671 ± 79	(834 ± 49)	1111 ± 131	(1109 ± 68)
	(0.05)	706 ± 82	(834 ± 49)	1380 ± 56	(1109 ± 68)**
	(0.1)	619 ± 46	(601 ± 30)	1079 ± 26	(1411 ± 51)***
	(0.2)	560 ± 47	(601 ± 30)	1180 ± 16	(1411 ± 51)***
Pirenperone	(0.05)	762 ± 23	(873 ± 32)*	1064 ± 61	(1168 ± 43)
	(0.1)	730 ± 75	(823 ± 64)*	693 ± 65	(1393 ± 56)**
	(0.2)	657 ± 30	(873 ± 32)**	1048 ± 15	(1168 ± 43)

Table 9.2 The effect of 5-HT receptor ligands on 5-HT and 5-HIAA in (a) hypothalamus and (b) striatum; 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

*2p <0.05; ** 2p <0.01; *** 2p <0.001 (Student's 't' test).

DRUG (mg/kg)		5-HT (ng/g)		5-HIAA (ng/g)	
(a) hippocampus					
Quipazine	(0.5)	589 ± 54	(584 ± 35)	956 ± 45	(1116 ± 115)
	(1.0)	648 ± 41	(669 ± 34)	845 ± 35	(1237 ± 80)**
	(2.0)	791 ± 65	(669 ± 34)	1074 ± 47	(1237 ± 80)
Ketanserin	(0.025)	475 ± 65	(799 ± 73)**	962 ± 70	(1142 ± 66)
	(0.05)	580 ± 30	(799 ± 73)**	1053 ± 91	(1142 ± 66)
	(0.1)	737 ± 29	(979 ± 33)**	1063 ± 42	(1147 ± 33)
	(0.2)	681 ± 18	(979 ± 33)***	1214 ± 39	(1147 ± 33)
Pirenperone	(0.05)	784 ± 23	(851 ± 36)	1076 ± 29	(1196 ± 26)
	(0.1)	495 ± 30	(584 ± 35)	824 ± 68	(1116 ± 115)*
	(0.2)	832 ± 31	(851 ± 36)	1119 ± 45	(1196 ± 26)
(b) cortex					
Quipazine	(0.5)	316 ± 5.0	(303 ± 14)	278 ± 29	(316 ± 13)
	(1.0)	338 ± 20	(306 ± 14)	284 ± 35	(334 ± 17)
	(2.0)	354 ± 12	(306 ± 14)	250 ± 12	(334 ± 17)
Ketanserin	(0.025)	336 ± 11	(364 ± 11)	183 ± 20	(375 ± 12)
	(0.05)	301 ± 17	(364 ± 11)**	174 ± 25	(375 ± 12)
	(0.1)	328 ± 23	(346 ± 12)	333 ± 5.7	(353 ± 7.0)
	(0.2)	319 ± 12	(346 ± 12	342 ± 6.3	(353 ± 7.0)
	(0.05)	317 ± 20	(320 ± 14)	348 ± 9.5	(340 ± 10)
Pirenperone	(0.1)	222 ± 12	(306 ± 13)**	223 ± 12	(316 ± 14)*
	(0.2)	225 ± 12	(320 ± 14)**	310 ± 12	(340 ± 14)

Table 9.3 The effect of 5-HT receptor ligands on 5-HT and 5-HIAA on (a) hippocampus and (b) cortex 3 hours post-injection. Results are expressed as mean ± s.e.m. Control values are shown in brackets.

* 2p <0.05; **2p <0.01; *** 2p <0.001 (Student's 't' test).

General Discussion

The purpose of this study has been to investigate the proposed roles of noradrenergic and serotonergic neurones in aversively-motivated behaviour (AMB) by establishing the effect of α-adrenoceptor and 5-HT receptor agonists and antagonists in behavioural models that could be regarded as sensitive to "fear" and its modulation. Behavioural and biochemical interactions between the α-adrenoceptor and 5-HT receptor ligands had also been examined, and an attempt had been made to interpret these actions in the light of the effects obtained with known anxiolytic and putative anxiogenic agents.

The effects of α-adrenoceptor and 5-HT receptor ligands were examined in two behavioural models - an elevated X-maze (which was developed for this study) and operant conflict. The X-maze was initially validated by examining the effects of amylobarbitone, diazepam, ACTH and picrotoxin. Results showed that, as expected, diazepam and amylobarbitone increased the proportion of open arm entries whereas ACTH and picrotoxin reduced it. Furthermore, similar behavioural effects were observed in the Geller-Seifter conflict test - a widely accepted test for anxiety (see Cook and Davidson, 1973), suggesting that the elevated X-maze model of AMB gives a reliable measure of the effect of drugs on such behaviour. However, expected inactive drugs such as neuroleptics antidepressants and amphetamine (see Cook and Davidson, 1973) have not yet been investigated in this model; it would therefore be desirable to establish that such inactive drugs have no effect in the proportion of open arm entries, which would further confirm the validity of this model.

A. Effect of drugs acting at α-adrenoceptors.

Behavioural effects in the X-maze as well as the Geller-Seifter conflict test yielded similar results. The α_2- adrenoceptor agonists had anxiolytic-like effects, and the antagonists were anxiogenic. Phenylephrine and St 587 which are selective for α_1-adrenoceptors (Drew 1976; Kobinger and Pi chler, 1982) decreased the proportion of open arm entries in the X-maze and suppressed punished responding in operant conflict, whereas prazosin had the opposite effect.

The high selectivity for α_2-adrenoceptors with azepexole and guanabenz have been previously reported (Kobinger and Pichler, 1977;

Jarrott et al, 1979). The partial agonist effect of clonidine as reported by Bradshaw et al (1982) was also confirmed in the X-maze, since the reduction in the proportion of open arm entries with a high dose of clonidine was antagonised by prazosin (Chapter 2). Yohimbine, piperoxane, RS21361 and idazoxan, all of which have been shown to act preferentially at α_2-adrenoceptors (Drew, 1976; Michel et al, 1981; Doxey et al, 1977), had the opposite effect to those of the α_2-adrenoceptor agonists.

The α_1-adrenoceptor agonists phenylephrine and St 587 increased punishment-induced suppression in operant conflict behaviour, and reduced the proportion of open arm entries in the X-maze, whereas the selective α_1-adrenoceptor antagonist prazosin (Doxey et al, 1977) had the reverse effect. Thymoxamine however, did not have an effect in either of the behavioural tests, but this may be explained by its lack of selectivity for α_1-adrenoceptors that has been reported peripherally (Drew, 1976).

The modulation of fear-motivated behaviour with the α-adrenoceptor ligands has been previously shown in other behavioural models. For instance, in the potentiated startle paradigm in rats, phenylephrine, yohimbine and piperoxane increased the startle amplitude, whereas clonidine and phenoxybenzamine decreased it (Davis and Astrachan, 1981; Davis et al, 1979). Clonidine has also been shown to increase punished reponding (Kruse et al, 1981) and prazosin caused a marked increase in punished drinking (Gardner and Piper, 1982). In this study the effect of selective α_1- and α_2- adrenoceptor agonists and antagonists have been examined (see above) and the consistency with which anxiogenic-like and anxiolytic-like effects have been observed strongly suggest that these effects are mediated via α_1 and α_2-adrenoceptors respectively.

In comparing the results obtained in the X-maze with those of Geller-Seifter conflict, it was observed that in the former, smaller doses of drugs were required in order to observe an effect. This was was particularly true of clonidine and prazosin. In the X-maze, clonidine 0.01 - 0.025 mg/kg and prazosin 0.025 mg/kg showed significant anxiolytic effects (Chapter 2). In the Geller-Seifter test anticonflict activity was observed with 0.025 - 0.05 mg/kg and 0.05

mg/kg, clonidine and prazosin respectively. Results not only suggest that the X-maze is a valid test for examining the anxiogenic and anxiolytic profiles of drugs, but also that it is a more sensitive test than operant conflict. It would be of interest to investigate the effects of benzodiazepine antagonists in the X-maze since the effects of these agents in an exploratory model of anxiety in the mouse have suggested that both the species as well as the paradigm employed determine the pharmacological profile of the drugs (Crawley et al, 1984).

One point to emerge from the Geller-Seifter test was that yohimbine, piperoxane and phenylephrine produced a carry-over effect (Chapter 3) suggesting that these agents might be altering the reaction to the aversive stimuli (light signal) and/or "fear" reinforcing effect of receiving a shock. Sensitisation to aversive conditions is characteristic of phobic types of anxiety (see Gray, 1981) and it is possible therefore that these drugs may not produce "anxiogenic-like" activity in an "emotionally neutral" environment but exposure to aversive or potentially or actually noxious environments could trigger phobic behaviour or panic states. It is noteworthy that yohimbine has been shown to cause panic states in man (Holmberg and Gershon, 1961) and that clonidine has been claimed to be particularly active in this type of anxiety (see Redmond, 1982) which may explain in part the reason for the carry-over effects to post drug days at least with yohimbine and piperoxane.

In accordance with the aims of the project, a modification of the conflict schedule used by de Carvalho et al (1983) was used to study rate dependency and the carry-over effects observed in the Geller-Seifter test. The effect of diazepam on drug sophisticated animals showed that release in punished responding was not dependent upon pre-existing response rates. A similar effect was reported with oxazepam (Babbini et al, 1982) who in addition also demonstrated that the responsiveness to the anxiolytic effect of oxazepam is related to shock-intensity given during training and to the animal variability under control conditions. Clonidine on the other hand, showed rate dependence effects. A release in punished responding was observed only when control baseline rates during these periods were less than 6

responses. Such an effect has been reported by Harris et al (1978) in a "fixed-interval" and "fixed ratio" schedule with no punishment. In this schedule both quipazine and clonidine increased low response rates and decreased high response rates. In the present study, a time-out period was incorporated so that changes in responding (induced by drugs) due to a high or low control baseline rate (rate-dependent effect) could be distinguished from a true anxiolytic or anxiogenic effect. However,on control days, with quipazine and clonidine, it was not possible to reduce time-out responding to less than 6 responses (as with the punished period). This therefore raises the question as to whether or not the effects on punished responding with clonidine and quipazine were due to rate-dependency.

The effects of acute and chronic (5 days) administration of RS 21361 and yohimbine were examined in the VI-30 sec schedule of conflict behaviour, in an attempt to investigate the carry-over effects of the α_1-adrenoceptor antagonists. In this schedule, a carry-over effect with either of these drugs was not observed, and this may be due to the fact that the schedule was not sensitive enough to detect any changes. Also, it is possible that whereas in the Geller-Seifter test, animals had not been previously experienced with an "anxiolytic-like" drug, in this schedule (VVV-T - incorporating a VI-30 sec. component for all 3 periods followed by time-out) animals had been clonidine and diazepam-experienced. Neither RS 21361 nor yohimbine reduced unpunished responding in the first segment, and on the basis of the effect of βCCM in this type of schedule (de Carvalho et al, 1983), it appears that the α_1-adrenoceptor antagonists do not have an effect on anticipatory anxiety.

Although behavioural effects in this study with the α-adrenoceptor ligands have been fairly consistent, it should be noted that these agents have marked effects on the sympathetic nervous system. The results obtained have not been identified with certainty to be exerted on the CNS. In man for instance, it is well known that peripheral administration of adrenaline (but not NA) may be accompanied by subjective feelings of anxiety and apprehension (Rothballer, 1959), an effect that may be linked to an awareness of tachycardia by

adrenaline. Elevation of blood pressure per se has also been shown to be capable of inducing EEG arousal (Baust et al, 1963). The possibility that peripheral effects are at least partly responsible for the results obtained cannot therefore be excluded. This aspect merits further investigation. It would be of interest to investigate the effect of tyramine (a precursor of NA which does not penetrate the blood brain barrier) on AMB to confirm whether the effect of drugs altering noradrenergic function, on AMB are mediated centrally or whether they are due to a consequence of the peripheral actions of these agents. Furthermore, it would be of value to study the effects of α-adrenoceptor ligands on AMB in the presence of phentolamine, since phentolamine is a peripheral non-selective α-adrenoceptor antagonist (see Drew, 1976). Comparing the effects of α-adrenoceptor ligands on AMB in the presence of phentolamine and when administered alone will give an indication as to whether or not behavioural effects of these drugs are centrally mediated.

B. Effect of drugs acting at 5-HT receptors.

The effect of quipazine and ketanserin in the X-maze and the Geller-Seifter conflict test were as expected: quipazine decreased the proportion of open arm entries and also suppressed punished responding, whereas ketanserin, an antagonist at 5-HT_2 receptors (Leysen et al, 1981) had the opposite effect. In comparing the effects of these agents in the X-maze and the Geller-Seifter test, it was found that although an anxiogenic-like effect with quipazine was observed at similar doses in both behavioural models, 0.05 mg/kg ketanserin showed anxiolytic-like activity in the X-maze, whereas 0.1 mg/kg showed anticonflict activity in the Geller-Seifter test. This is comparable with the effects of clonidine and prazosin discussed earlier (see above) and also suggests that the X-maze is more sensitive than the Geller-Seifter test. It is unclear why pirenperone, the selective 5-HT_2 antagonist (Colpaert and Janssen, 1983) was without effect in either of the behavioural models.

Despite the lack of effect of pirenperone, findings in this study suggest that 5-HT receptors also modulate AMB, since in the X-maze the reduction in the proportion of open arm entries by quipazine was antagonised by pretreatment with pirenperone (Chapter 2).

Previous studies investigating the role of 5-HT in punished behaviour have shown some discrepancies. For instance, Graeff (1974) using a multiple fixed-interval and fixed-ratio schedule of food reinforcement with punishment in the latter component found that 3 - 10 mg/kg methysergide increased all responses to about half the peak effect exerted by chlordiazepoxide. Winter (1972) observed that 1 - 10 mg/kg methysergide increased punished behaviour whereas 3 - 25 mg/kg cinanserin was inactive. Since methysergide and cinanserin effectively antagonised a decrement in unpunished behaviour as induced by N,N-dimethyltryptamine, it was concluded that 5-HT antagonism was neither necessary nor sufficient to account for the anticonflict activity of these agents. (A detailed review of the effect of serotonergic agents on AMB has been discussed in Introduction, Section 11.2).

The discrepancies reported in the studies investigating the effects of 5-HT agents on punished behaviour could be explained on the basis of different sensitivities of the conflict component in the various procedures. For example, Stein et al (1975) used the Geller-Seifter conflict schedule where punishment was suppresed on a continuous reinforcement (CRFs) procedure. Graeff (1974) investigated the effects of 5-HT antagonists on punishment suppressed behaviour in a fixed-ratio (FR5) schedule component, and similarly, Winter (1972) used a fixed-ratio (FR10) component during the punished period. Also, the fact that none of these studies give an indication of previous drug experience in the animals used to examine the effects of 5-HT antagonists, which may also contribute towards the differences in the effects observed, since this study has also shown the importance of previous drug experience (Chapters 3 and 4). For example, it was found that in diazepam-experienced animals, the carry-over effect produced on the first exposure to yohimbine was not as intense as that observed in drug-naive animals (Chapter 3), and by the second exposure to yohimbine in diazepam-experienced animals the carry-over effect was virtually abolished, further emphasising the importance of previous drug history of the animals.

In this study, unlike the effects of yohimbine, piperoxane and phenylephrine, quipazine did not produce a carry-over effect in either

of the conflict behavioural schedules used. This not only suggests that quipazine has no effect on anticipatory anxiety (see de Carvalho et al, 1983), but also that the anxiogenic-like effect of quipazine is mediated via a different mechanism to that of the α-adrenoceptor ligands.

Although results from this study also suggest the involvement of serotonergic systems in AMB, further work is required investigating the effects of 5-HT_1 receptor agonists in such behaviour (since as yet, selective antagonists of 5-HT_1 receptors have not been developed). For instance, 8-OHDPAT, a 5-HT_{1A} receptor agonist (Cortes et al, 1984) has been shown to increase the acoustic startle response (Svensson and Ahlneius, 1983) suggesting that 5-HT_1 receptors are involved in AMB. The effect of RU 24969, a 5-HT_{1B} receptor agonist (Cortes et al, 1984) as well as 8-OHDPAT in operant conflict and the X-maze would also help to confirm the involvement of 5-HT_1 receptors in AMB.

C. Interactions of anxiolytic and putative anxiogenic agents with NA and 5-HT.

Behavioural interactions with ACTH have shown that its anxiogenic-like effect is not modulated by 5-HT receptor of α-adrenoceptor ligands. In accordance with one of the aims of the project, which was to examine whether anxiogenic- or anxiolytic-profiles of drugs could be correlated with changes in regional brain 5-HT and 5-HIAA biochemical determinations of 5-HT and 5-HIAA were performed after ACTH administration. Results showed that although the brain 5-HT and 5-HIAA were altered, no one brain region consistently altered these indoleamines in a direction opposite to that observed with amylobarbitone or diazepam. It is possible therefore that the changes in 5-HT and 5-HIAA are idiosyncratic to ACTH and that the effects of ACTH are not mediated via noradrenergic or serotonergic systems. However, the modulation of the anxiogenic-like activity of ACTH by α-adrenoceptor and 5-HT receptor ligands have not been previously examined and since only one dose of each drug was investigated, the lack of effect may not be representative of all doses.

Biochemical determinations of 5-HT and 5-HIAA following the putative anxiogenic picrotoxin (File and Lister, 1983) showed inconsistent effects - no one brain region showed a consistent change

in either of the indoleamines. Furthermore, the effects of picrotoxin on regional 5-HT and 5-HIAA were not opposite to those of diazepam and amylobarbitone. The effects of GABA and its agonists on anxiety have been reported. For instance the GABA agonist THIP (4,5,6,7-tetrahydroisoxazolo [5,4-C] pyridin-3-ol) has been shown to have anxiolytic effects in humans (Hoehn-Saric, 1983) and in the rat dorsal raphe, infusions of GABA released punished responding as did the GABA against muscimol when infused into the amygdala (see Iversen, 1984). The latter effect suggests that the 5-HT pathway of raphe origin is involved in mediating the effects of GABA and GABA agonists. However, the effects of these agents on brain 5-HT function as related to their effects on anxiety have not been examined, and the fact that a time-course study of the effect of picrotoxin on 5-HT and 5-HIAA levels was not performed, therefore it remains to be determined whether or not the anxiogenic-like activity of picrotoxin is mediated via serotonergic systems.

Conflict behavioural interaction experiments with diazepam have suggested that neither α-adrenoceptor nor 5-HT receptor ligands modulate its anticonflict activity. Although this was only a preliminary study in that only one dose of each drug was examined, the lack of effect of yohimbine in modulating the anticonflict activity of diazepam is consistent with the finding that although clonidine guanfacine increased food intake in an unfamiliar situation, these agents were unable to enhance the efficacy of diazepam in stimulating food consumption in a novel environment (Thiebot et al, 1984a). Furthermore, Charney et al (1983) demonstrated that although diazepam antagonised the increase in subjective anxiety produced by yohimbine in humans, it did not attenuate yohimbine-induced increases in plasma-MHPG, blood pressure or autonomic symptoms suggesting that α_1-adrenoceptors do not modulate the anxiolytic effects of diazepam. The lack of effect of phenylephrine is also consistent with the findings of Stein et al (1975) where phentolamine failed to alter the release in punished responding caused by diazepam, suggesting also that the anticonflict effects of benzodiazepines are not mediated into α_1-adrenoceptors.

The anticonflict activity of diazepam remained unaffected in the presence of quipazine, and although only one dose of each drug was examined, it appears that the anticonflict activity of diazepam is not modulated by serotonergic systems. Results are in contrast with those of Stein et al (1975) who showed that icv 5-HT was able to antagonise the punishment-releasing effect of oxazepam, although Shephard et al (1982) demonstrated that although 5-methoxydimethyltryptamine reversed the anticonflict effect of pCPA, it did not affect punished responding in the presence of chlordiazepoxide. Furthermore, 5,7-DHT lesions of the dorsal raphe did not affect behavioural inhibition in control rats nor did it modify the ability of diazepam to release punished responding (Thiebot et al, 1984b), and metergoline was reported to be without effect in conflict behaviour in the rat and also did not alter the increase in punished responding caused by diazepam (Commisaris and Rech, 1982). (A detailed review of the involvement of serotonergic systems in the actions of benzodiazepine can be found in the Introduction, Section 11).

Biochemical determinations of 5-HT and 5-HIAA following diazepam and amylobarbitone showed that with diazepam, there appeared to be a general increase in 5-HT and a decrease in 5-HIAA in most regions investigated. However, with amylobarbitone, there were profound regional differences, and therefore, a direct measure of turnover of 5-HT with both these agents would be more useful in explaining the effects observed.

Although biochemical as well as behavioural results in this study suggest that the effect of diazepam is not modulated by serotonergic systems, further work requires to be done, since the range of agonists and antagonists available for such studies has not been great; also the lack of specificity for 5-HT_1 and 5-HT_2 receptors of the drugs used in this study, and their influence on other neurotransmitter systems (Colpaert et al, 1981; Green et al, 1976) may have influenced the effects obtained in this study.

D. Interactions between α-adrenoceptor and 5-HT receptor ligands.

Behavioural interaction experiments in the maze as well as conflict behaviour showed that the α-adrenoceptor ligands failed to modulate the effects of 5-HT receptor ligands on AMB. Conversely, 5-HT

receptor ligands did not have an effect on the action of α-adrenoceptor ligands on AMB. Although behavioural interactions between noradrenergic and serotonergic systems have been implicated in hypoalgesia (Hammond et al, 1980), 5-HT head twitch (Handley and Brown, 1982) and convulsions (Lazarova and Samanin, 1983), the absence of an interaction in AMB may be due to the fact that both noradrenergic and serotonergic systems act independently to modulate such behaviour. This is supported by the observation that methysergide failed to alter the increase in punished responding induced by clonidine in the Geller-Seifter conflict test (Kruse et al, 1981) and cyproheptadine failed to alter the increase in acoustic startle response amplitude produced by phenylephrine (Davis and Astrachan, 1981) although both methysergide and cyproheptadine have been shown to release punished responding in conflict behaviour (see Sepinwall and Cook, 1978).

In accordance with the aims of the project, the effect of α-adrenoceptor and 5-HT receptor ligands on regional changes in brain 5-HT and 5-HIAA were also determined. With the α-adrenoceptor ligands, there appeared to be no consistent changes in any one brain region, for either 5-HT or 5-HIAA, except for the α_2-adrenoceptor antagonists which caused an increase in 5-HT and a decrease in 5-HIAA in most brain regions. However, although changes in these indoleamines were consistent with those observed by Papeschi et al (1971) diazepam also showed similar changes in this study, as well as reported by other workers (for review, see Haefely et al, 1981). The lack of consistent effects of α-adrenoceptor ligands on regional 5-HT and 5-HIAA may further confirm the likelihood that behavioural effects of these agents are unrelated to their actions on serotonergic systems. However it is possible that since biochemical determinations were performed 3 hrs post-injection peak effects with some drugs may have been missed. Further work examining the effects of these agents on regional 5-HT and 5-HIAA at different times may help to clarify whether these changes are idiosyncratic to the drug in question, or can be related to their action on AMB.

Biochemical determinations of regional 5-HT and 5-HIAA concentration with quipazine ketanserin and pirenperone have shown some consistent effects. Quipazine caused a general increase in 5-HT and a

decrease in 5-HIAA 3 hrs post-injection. Owing to the fact that a direct measure of 5-HT turnover or utilisation was not performed in this study, interpretation of the data is difficult. It appears that quipazine reduces 5-HT turnover although results cannot confirm that this is due to stimulation of 5-HT receptors since the increase in 5-HT could also be due to the inhibition of monoamine oxidase (MAO) activity because quipazine has been shown to act as a weak reversible MAOI both in vivo and in vitro (Green et al, 1976).

The decrease in 5-HIAA observed with quipazine has been shown by Grabowska et al (1974a), who in addition demonstrated that this decrease could be antagonised by methysergide; 5-HT remained unaffected after quipazine. Green et al (1976) however, demonstrated that 10 mg/kg quipazine caused a small increase in 5-HT and did not alter brain tryptophan concentrations. The discrepancies in the results obtained here (as compared to those reported elsewhere - see above) may be due to the differences in doses as well as the method of investigation, since Grabowska et al (1974) examined 5-HIAA accumulation after pargyline which is more representative of 5-HT turnover than the method used by Green et al (1976) and in the present study.

Ketanserin and pirenperone appeared to cause a general decrease in 5-HT and 5-HIAA. The decrease in 5-HT may be explained by the blocking of postsynaptic 5-HT receptors since this would cause an increase in release of 5-HT which would appear to decrease 5-HT levels (via a transneuronal feedback mechanism); however, on this basis 5-HIAA would be expected to increase. 5-HIAA was also shown to decrease - an effect that cannot be explained by postsynaptic receptor blockade. It is possible that an increase might have occured at a time other than 3 hrs post-injection, hence determining the effects of ketanserin and pirenperone at a range of times after dosing may have detected such a change. On the other hand, it is possible that both these agents may have accelerated the egress of 5-HIAA out of the brain therefore causing 5-HIAA depletion. Investigating indoleamine concentrations post probenecid would help to confirm whether a decrease in 5-HIAA was induced by these agents, or whether it was a secondary effect.

While interpreting biochemical results obtained in this study, the functional importance of brain 5-HT metabolism in relation to

behavioural effects has to be considered. Following the work of Green and Grahame Smith (1976) it appears that many of the changes seen in 5-HT metabolism may be irrelevant in terms of 5-HT function. They suggested that there may be two pools of central 5-HT, one being "functional" and the other "non-functional". Hence newly formed 5-HT may not necessarily enter the functional pool of 5-HT but rather, may become bound intraneuronally and may undergo oxidative-deamination without ever being "functional". Thus increases in brain 5-HT and 5-HIAA may not reflect alterations of 5-HT function, but may indicate changes in "non-functional" 5-HT. This factor must also be considered in the interpretation of the results in this study.

E. The role of L.C. in AMB.

Previous studies have examined the role of the L.C. in fear and anxiety, and results from such studies have been very conflicting. For instance, fear-like behaviour was elicited in the stumptailed monkey by electrical stimulation of the L.C. or by drugs increasing L.C. activity, while electrolytic lesions caused a deficit in such behaviour (Redmond and Huang, 1979). Experiments on aversively and novelty motivated behaviour in the rat have shown that lesioning of the dorsal bundle by 6-OHDA failed to disrupt the acquisition and performance of a wide variety of aversively motivated behaviours; responses to novelty were disrupted but in such a way as to suggest "if anything, an increased rather than a decreased fear reaction" (see Mason and Fibiger, 1979a). In fear-motivated acquisition learning, lesioned rats were no different from controls, but resistance to extinction was seen in CER tasks (Mason and Fibiger, 1979b). Crow et al (1978) similarly found no effects in such tests or in a social interaction test of anxiety in animals with lesions lateral to the L.C. (using 6-OHDA), constituting further evidence that the L.C. is not involved in anxiety mechanisms. (A detailed review of previous studies investigating the role of the L.C. in AMB has been considered in the Introduction Section 11.1b).

In accordance with the aims of the project, the effect of L.C. lesions in the X-maze was examined and the effect of α-adrenoceptor and 5-HT receptor ligands in L.C. lesioned animals on conflict behaviour

was also investigated, in order to determine whether the L.C. is involved in AMB and whether an intact L.C. system is required to mediate the effects of these agents on such behaviour.

Consistent with the findings of Crow et al (1978) as well as Mason and Fibiger (1979b), L.C. lesions appeared to have no effect on AMB as measured by the exploratory activity in the X-maze (Chapter 2). However, Adams and Geyer (1981) demonstrated that L.C. lesioned animals (with 6-OHDA) reduced the startle amplitude in the potentiated startle paradigm in rats which may be explained by the fact that a different behavioural model was being used. The lack of effect of L.C. lesions in the X-maze also suggests that noradrenergic inputs to the septo-hippocampal system is not involved in the modulation of anxiety, as described by Gray (1981), since lesions of the L.C. also causes destruction of noradrenergic neurones to the septo-hippocampal system (Chapter 6).

Operant conditioning experiments showed that L.C. lesions did not affect the release in punished responding produced by diazepam. Results are consistent with Koob et al (1980) who demonstrated that virtual total destruction of the dorsal noradrenergic innervation of forebrain by 6-OHDA lesion to the central tegmental tract failed to significantly alter the anxiolytic effect of chlordiazepoxide. However, Grant et al (1980) showed that diazepam antagonised the increase in firing rate of the L.C. caused by yohimbine and in rats subjected to immobilisation stress suggesting that diazepam attenuates the neural and behavioural aspects of L.C. activation.

The discrepancy in the results obtained by Grant et al (1980) may be due to the fact that during immobilisation stress, although animals are exposed to an aversive stimulus, a behavioural response to the aversive stimulus is not being measured. This naturally raises the question whether it is valid to attempt to correlate L.C. firing with immobilisation stress, since essentially a behavioural measure of anxiety is not being investigated.

The lack of effect of L.C. lesions in modulating the anxiogenic action of ACTH in conflict behaviour is also consistent with those of ACTH in the social interaction test of anxiety, the response to ACTH in L.C. lesioned animals (with 6-OHDA) was similar to sham-operated

controls (File et al, 1979b). Results in this study support the contention that the effects of ACTH and diazepam on AMB do not require an intact L.C. system.

L.C. lesions did not affect the release in punished responding induced by clonidine, suggesting that the L.C. is not involved in mediating the effects of clonidine, since if the effect of clonidine was mediated via presynaptic α_2-adrenoceptors then a decrease in response would be expected; on the other hand, if it was mediated postsynaptically then L.C. lesions would cause a significant potentiation. Similar results were obtained with prazosin, phenylephrine and yohimbine -whereby L.C. lesioned animals were no different from sham-operated controls, therefore suggesting that the L.C. is not involved in the effects of α-adrenoceptor ligands on AMB. The effects of L.C. lesions on 5-HT receptor ligands were similar - behavioural effects were not modified by the lesions, suggesting that an intact L.C. system is not required to mediate the effects of these agents. However, the effect of L.C. lesions on conflict behaviour with α-adrenoceptor and 5-HT receptor ligands have been performed at only one dose for each drug. By examining the effect of L.C. lesions at a range of doses for these drugs on conflict behaviour, would help to confirm that the lack of involvement of the L.C. in such behavioural effects is maintained at all doses.

In conclusion, the results from this study suggest the involvement of both α-adrenoceptors and 5-HT receptors in AMB. It has also been demonstrated that noradrenergic and serotonergic systems act independently to modulate such behaviour, and that the behavioural effects of known anxiolytic and putative anxiogenic agents do not appear to involve changes in noradrenergic and serotonergic activity. The results reported here go some way towards reconciling two opposing schools of thought, since it appears that although noradrenergic systems are involved in anxiety, the L.C. does not modulate AMB. The function of the L.C. is yet to be elucidated, although from the results reported here, its involvement in other physiological or behavioural effects cannot be assessed.

It is possible that the ventral bundle may be involved in mediating the effects of drugs on AMB, since this noradrenergic system

is intact during L.C. lesioning. This area merits further investigation, since the effects of lesioning this pathway on AMB has not been examined. The involvement of serotonergic systems in AMB has also been established from the results in this study, although further lesioning experiments are required in order to establish which pathways play a role in AMB.

References

Abrams, R. (1978). Serotonin and affective disorders. In Serotonin in health and disease. Vol 3. Ed W B Essman. Spectrum Publications, N.Y.

Adams, L.M., Geyer, M. (1981). Effects of 6-OHDA lesions of the LC on startle in rats. Psychopharmac. 73 394-398.

Ahlquist, R.P. (1948). A study of the adrenotropic receptors. Amer. J. Physiol. 153 586-599.

Anden, N-E., Corrodi, H., Fuxe, K., Hokfelt, B., Hokfelt, T., Rydin, C., Svensson, T.H. (1970). Evidence for a central noradrenaline receptor stimulation by clonidine. Life Sci. 9 513-523.

Anisman, H. (1978). Neurochemical changes and stress.In Psychopharmacology of aversively motivated behaviour. Ed H Anisman & G Bignami. Plenum Press, N.Y.

Anzelark, G.M., Crow, T.J., Greenway, A.P. (1973). Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. Science 181 682-684.

Aprison, M.H., Ferster, C.B. (1961). Neurochemical correlates of behaviour: II. correlation of brain monoamine oxidase activity with behavioural changes after iproniazid and 5-hydroxytryptophan. J. Neurochem. 6 350-357.

Aprison, M.H., Takahashi, R., Tachiki, K. (1968). Serotonergic receptors in clinical depression.In Neuropharmacology and behaviour Ed B Haber & M H Aprison. Plenum Press, N.Y.

Arbilla, S., Langer, S.Z., Maurin, Y. (1982). Inhibition by α_2-adrenoceptor antagonists of calcium-dependent electrically evoked release of [^{3}H]-Gaba from cerebral cortex slices. Brit. J. Pharmacol. 77 446p.

Babbini, M., Giardi, M., Bartoletti, M. (1982). Benzodiazepine effects upon Geller Seifter conflict test in rats: analysis of individual variability. Pharmacol. Biochem. Behav. 17 43-48.

Banki, C.M. (1977). Correlation of anxiety and related symptoms with cerebrospinal fluid 5-hydroxyindole acetic acid in depressed women. J. Neural Transm. 41 135-143.

Baraban,J.M., Aghajanian, G.K. (1981). Noradrenergic innervation of serotonergic neurones in the dorsal raphe: demonstration by electron microscopic autoradiography. Brain Res. 204 1-11.

Barbeau, H., Filion, M., Bedard, P. (1980). Effects of agonists and antagonists of serotonin on spontaneous hindlimb EMG activity in chronic spinal rats. Neuropharmacol. 20 99-107.

Barnett, S.A. (1958). Exploratory behaviour. Brit. J. Psychol. 49 289-310.

Baust W., Niemezyk, H., Vieth, J. (1963). The action of blood pressure on the ascending reticular activating system with special reference to adrenaline induced arousal. Electroenceph. Clin. Neurophysiol. 15 63-72.

Beckett, P.J., Finch, L. (1982). The α_1 and α_2 adrenoceptor involvement in the central cardiovascular action of clonidine in the conscious renal hypertensive cat. Eur. J. Pharmacol. 82 155-160.

Berger, B.D., Wise, C.D., Stein, L. (1971). Norepinephrine: reversal of anorexia in rats with lateral hypothalamic damage. Science 172 281-284.

Biswas, B., Carlsson, A. (1968). On the mode of action of diazepam on brain catecholamine metabolism. Naunyn Schmeid. Arch. Pharmacol. 303 73-78.

Blier, P., De Montigny, C. (1983). Effects of quipazine on pre- and postsynaptic serotonin receptors: single cell studies in the rat CNS. Neuropharmacol. 22 495-500.

Bliss, E.L., Ailon, J., Zwanziger, J. (1968). Metabolism of norepinephrine, serotonin and dopamine in rat brain with stress. J. Pharmacol. Exp. Ther. 164 122-134.

Blundell, J.E. (1979). Serotonin and feeding. In Serotonin in health and disease. Vol 5. Ed W B Essmann. London Spectrum Publications Inc.

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

Bolin, P.Jr., Davanzo, J.P. (1982). The influence of isolation and amino-oxyacetic acid (AOAA) on GABA in muricidal rats. Psychopharmacol. 76 367-370.

Bourgoin, S., Artaud, F., Bolkaert, J., Hery, F., Glowinski, J., Hamon, M. (1978). Paradoxical decrease of brain 5-HT turnover by metergoline, a central 5-HT receptor blocker. Naunyn Schmied. Arch. Pharmacol. 302 313-321.

Bourgoin, S., Enjalbert, A., Adrien, J., Hery, F., Glowinski, J., Hamon, M. (1977). Acute changes in central 5-HT metabolism induced by the blockade of stimulation of serotoninergic receptors during ontogenesis in the rat. J. Pharmacol. Exp. Ther. 202 519-531.

Bourgoin, S., Hery, F., Ternaux, J.P., Hamon, M.(1975). Effects of benzodiazepine on the binding of tryptophan to serum. Consequences on 5-hydroxyindoles concentration in the rat brain. Psychopharmacol. Commun. 1 209-216.

Bradshaw, C.M., Stoker, M.J., Szabadi, E. (1982). The effect of microelectrophoretically applied clonidine on single cerebral cortical neurones in the rat. Evidence for interaction with α_1-adrenoceptors. Naunyn Schmied. Arch. Pharmacol. 320 230-234.

Breese, G.R. (1975). Chemical and immunochemical lesions by specific neurotoxic substances and antisera. In Handbook of Psychopharmacology Vol 1. Ed L L Iversen, S D Iversen, & S H Snyder Plenum Press, N.Y.

Brown, J. (1980). Central α-adrenoceptors and behaviour. PhD thesis. University of Aston in Birmingham.

Bullock, S.A., Kruse, H., Fielding, S. (1978). The effect of clonidine on conflict behaviour in rats: is clonidine an anxiolytic agent? Pharmacologist. 20 223.

Carlsson, A., Corrodi, H., Fuxe, K., Hokfelt, T. (1969). Effects of some antidepressant drugs on the depletion of intraneuronal brain catecholamine stores caused by 4 α dimethylmetatyramine. Eur. J. Pharmacol. 5 367-373.

Cassens, G., Roffman, M., Kuruc, A., Orsulak, P.J., Schildkraut, J.J. (1980). Alterations in brain norepinephrine induced by environmental stimuli previously paired with

inescapable shock. Science. 209 1138-1140.
Cavero, J., Lefevre, F., Roach, A.G. (1977). Differential effects of prazosin on the pre- and postsynaptic α-adrenoceptors in the rat and dog. Brit. J. Pharmacol. 61 469p.
Cerrito, F., Raiteri, M. (1979). Evidence for an autoreceptor-mediated presynaptic control of serotonin release in central nerve endings. Brit. J. Pharmacol. 67 424p.
Charney, D.S., Heninger, G.R., Redmond, D.E.Jr. (1983). Yohimbine induced anxiety and increased noradrenergic functions in humans: Effects of diazepam and clonidine. Life Sci. 33 19-30.
Chase, T.N., Katz, R.I., Kopin, I.J. (1970). Effect of diazepam on fate of intracisternally injected serotonin C14 Neuropharmacol. 9 103-108.
Chi, C.C. (1965). The effect of amobarbital sodium on conditioned fear as measured by the potentiated startle response in rats. Psychopharmacol. 7 115-122.
Christmas, A.J., Maxwell, D.R. (1970). A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behaviour in mice and rats. Neuropharmac. 9 17-29.
Chung-Hwang, E., Van Woert, M.H. (1979). Antimyoclonic action of clonazepam; the role of serotonin. Eur. J. Pharmacol. 60 31-40.
Clarke, A., File, S.E. (1981). Neurotoxin lesions of the lateral septum and changes in social and aggressive behaviours. Brit. J. Pharmacol. 74 766p.
Clavier, R.M., Fibiger, H.C., Phillips, A.G. (1976). Evidence that self-stimulation of the region of the locus coeruleus in rats does not depend upon noradrenergic projections to telencephalon. Brain Res. 113 71-81.
Coates, J., Weetman, D.F. (1982). Effects af azepexole on the rat isolated anococcygeus preparation. Brit. J. Pharmacol. 77 541p.
Collinge, J., Pycock, C.J., Taberner, P.V. (1982). Gaba and diazepam-induced reduction of cerebral 5-hydroxytryptamine turnover. Brit. J. Pharmacol. 75 45p.
Colpaert, F.C., Janssen, P.A.J. (1983). The head twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, an LSD antagonist. Neuropharmac. 22 993-1000.
Colpaert, F.C., Niemgeers, C.J.E., Janssen, P.A.J. (1979). In vivo evidence of partial agonist activity of purported 5-hydroxytryptamine antagonists. Eur. J. Pharmacol. 58 505-509.
Colpaert, F.C., Niemgeers, C.J.E., Janssen, P.A.J. (1982). A drug discrimination analysis of lysergic acid diethylamide (LSD): in vivo agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone,an LSD antagonist. J. Pharmacol. Exp. Ther. 221 206-214.
Commisaris, R.L., Rech, R.H. (1982). Interactions of metergoline with diazepam, quipazine, and hallucinogenic drugs on a conflict behaviour in the rat. Psychopharmac. 76 282-285.
Conrad, L.C.A., Leonard, C.M., Pfaff, O.W. (1974). Connections of the median and dorsal raphe nuclei in the rat: An auto-

radiography and degeneration study. J. Comp. Neurology 156 179-206.

Cook, L., Davidson, A.B. (1973). Effects of behaviourally active drugs in a conflict-punishment procedure in rats.In The Benzodiazepines Ed S Garratini, E Mussini & L O Randall Raven Press N.Y.

Cook, L., Sepinwall, J. (1975). Behavioural analysis of the effects and mechanisms of action of benzodiazepines. In Mechanisms of action of benzodiazepines Ed E Costa & P Greengard. Raven Press N.Y.

Cooper, S.J., Crummy, Y.M.T. (1978). Enhanced choice of familiar food in a food preference test after chlordiazepoxide administration. Psychopharmacol. 59 51-56.

Coppen, A., Prange, A.J.Jr., Whybrow, P.C., Noguera, R. (1972). Abnormalities of indoleamines in affective disorders. Arch. Gen. Psychiat. 26 474-478.

Corrodi, H., Fuxe, K., Hokfelt, T. (1967). The effect of some psychoactive drugs on central monoamine neurons. Eur. J. Pharmacol. 1 363-368.

Cortes, R., Palacios, J.M., Pazos, A. (1984). Visualisation of multiple serotonin receptors in the rat brain by autoradiography. Brit. J. Pharmacol. 82 202p.

Cox, R.H.Jr., Perhach, J.L.Jr. (1973). A sensitive, rapid and simple method for the simultaneous spectrofluorometric determinations of norepinephrine, dopamine, 5-hydroxytryptamine and 5-hydroxyindole acetic acid in discrete areas of brain. J. Neurochem. 20 1777-1780.

Crawley, J.N., Skolnick, P., Paul, S. (1984). Absence of intrinsic antagonist actions of benzodiazepine antagonists on an exploratory model of anxiety in the mouse. Neuropharmacol. 23 531-538.

Crow, T.J., Deakin, J.W.F., File, S.E., Longden, A., Wendlandt, S. (1978). The locus coeruleus noradrenergic system- Evidence against a role in attention, habituation, anxiety, and motor activity. Brain Res. 155 249-261.

Curzon, G., Green, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Brit. J. Pharmacol 39 643-655.

Dahlstrom, A., Fuxe, K. (1964). Evidence for the existence of monoamine containing neurones in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurones. Acta Physiol. Scand. 62 (suppl 232). 1-55.

Dalrymple, H.W., Hamilton, C.A., Hannah, J.A.M., Reid, J.L. (1982). Cardiovascular effects of RX781094 in the rabbit: possible partial agonist effect in addition to α_2-adrenoceptor antagonism. Brit. J. Pharmacol. 78 128p.

Dandiya, P., Patni, S. (1973). Influence of substances acting on the central adrenergic receptor on open field behaviour in rats. Ind. J. Med. Res. 61 891-895.

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

Davis, M. (1979b). Morphine and naloxone: effects on conditioned fear as measured by the potentiated startle para-

digm. Psychopharmacol. 54 341-347.
Davis, M., Astrachan, D.I. (1981). Spinal modulation of acoustic startle: opposite effects of clonidine and d-amphetamine. Psychopharmacol 75 219-225.
Davis, M., Astrachan, D.I., Gendelman, P.M., Gendelman, D.S. (1980). 5-Methoxy-N, N, -dimethyltryptamine: spinal cord and brain stem mediation or excitatory effect on acoustic startle. Psychopharmacol. 70 123-130.
Davis, M., Cedarbaum, J.M., Aghajanian, G.K., Gendelman, D.S. (1977). Effects of clonidine on habituation and sensitization of acoustic startle in normal, decerebrate and LC lesioned rats. Psychopharmacol. 51 243-253.
Davis, M., Redmond, D.E.Jr., Baraban, J.M., (1979). Noradrenergic agonists and antagonists: effects on conditioned fear as measured by the potentiated startle paradigm. Psychopharmacol. 65 111-118.
Davison, G.C., Neale, J.M. (1974). Abnormal Psychology: An experimental and clinical approach. Wiley International, N.Y.
De Carvalho, L.P., Grecksch, G., Chapouthier, G., Rossier, J. (1983). Anxiogenic and non-anxiogenic benzodiazepine antagonists . Nature 301 64-66.
De Jonge, A., van Meel, J.C.A., Timmermans, P.B.M.W.M., van Zweiten, P.A. (1981). A lipophilic, selective α_1-adrenoceptor agonist: 2-(2-chloro-5-trifluoromethyl phenylimino) imidazolidine (St 587). Life Sci. 28 2009- 2016.
Delbarre, B., Schmitt, H. (1971). Sedative effects of α-sympathomimetic drugs and their antagonism by adrenergic and cholinergic blocking drugs. Eur. J. Pharmacol. 13 356-363.
Dews, P.B. (1964). Factors modifying behavioural effects of drugs: Scnedules of reinforcement. In Animal behaviour and drug action. Ed H Steinberg. J & A Churchill Ltd. London. 191-206.
Doxey, J.C., Roach, A.G., Smith, C.F.C. (1983). Studies on RX781094; a selective, potent and specific antagonist of α_2 -adrenoceptors. Brit. J. Pharmacol. 78 489-506.
Doxey, J.C., Smith, C.F.C., Walker, J.M. (1977). Selectivity of blocking agents for pre- and postsynaptic α-adrenoceptors. Brit. J. Pharmacol. 60 91-96.
Drew, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located alpha adrenoceptors. Eur. J. Pharmacol. 36 313-320.
Drew, G.M. (1982). Evidence in favour of a selective α_1-adrenoceptor blocking action of WB4101 in vivo. Naunyn Schmied. Arch. Pharmacol. 319 222-225.
Drew, G.M., Whiting, S.B. (1979). Evidence for two distinct types of postsynaptic α-adrenoceptors in vascular smooth muscle in vivo. Brit. J. Pharmacol. 67 207-215.
Dunn, A.J. (1980). Neurochemistry of learning and memory: an evaluation of recent data. Ann. Rev. Psychol. 31 343-390.
Ellison, D.E. (1977). Animal models of psychopathology.The low-norepinephrine and low-serotonin rat. American Psychologist. December 1036-1045.
Engel, G., Gothert, M., Muller-Schweintzer, E., Schlicker, E., Sistonen, L., Stadler, P.A. (1983). Evidence for common pharmacological properties of [^{3}H] 5-hydroxytryptamine bin-

ding sites, presynaptic 5-hydroxytryptamine autoreceptors in the CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn Schmied. Arch. Pharmacol. 324 116-124.

Ennis, C., Cox, B.(1982). Pharmacological evidence for the existence of two distinct serotonin receptors in the rat brain. Neuropharmacol. 21 41-44.

Essman, W.B. (1978). Serotonin in learning and memory. In Serotonin in health and disease Vol 3. Ed W B Essman. Spectrum Publications, N.Y.

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

Evans, W.O. (1961). A new technique for the investigation of some analgesic drugs on a reflexive behaviour in the rat. Psychopharmacol. 2 318-225.

Farhoumand, N., Harrison, J., Pare, C.M.B., Turner, P., Wyn, S. (1979). The effect of high dose oxprenolol on stress-induced physical and psychological variables . Psychopharmacol. 64 365-369.

Feldberg, H.C., Myers, R.D. (1964). Effects on temperature of monoamines injected into the cerebral ventricles. A new concept of temperature regulation. J. Physiol. 173 226-237.

Fernstrom, J.D., Shabshelowitz, H., Faller, D.V. (1974). Diazepam increases 5-hydroxy indole concentrations in rat brain and spinal cord. Life Sci. 15 1577-1584.

File,S.E., Day, S. (1972). Effects of time of day and food deprivation on exploratory activity in the rat. Animal Behav. 20 758-762.

File, S.E., Deakin, J.F.W., Longden, A., Crow, T.J. (1979a). An investigation of the role of the locus coeruleus in anxiety and agonistic behaviour. Brain Res. 169 411-420.

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

File, S.E., Hyde, J.R.G., Macleod, N.K. (1979b). 5, 7-dihydroxytrptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. J. Affective Disorders 1 115-122.

File, S.E., Hyde, J., Pool, M. (1976). Effects of ethanol and chlordiazepoxide on social interactions in rats. Brit. J. Pharmacol. 58 465p.

File, S.E., Lister, R.G. (1983). Anxiogenic action of picrotoxin and pentylenetetrazole: reversal by chlordiazepoxide. Brit. J. Pharmacol. 79 286p.

File, S.E., 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.

File, S.E., Vellucci, S.V. (1979). Behavioural and biochemical measures of stress in hooded rats from different sources. Physiol. & Behav. 22 31-35.

Fink, M. (1979). Anxiety, anxiolytics and the human EEG. In Phenomenology and treatment of anxiety. Ed W E Fann, I Karacan, A D Pokorny & R L Williams. Spectrul Publications. N.Y.

Fozard, J.R. (1983). Mechanism of the hypotensive effect of ketanserin. J. Cardiovasc. Pharmacol. 4 829-838.

Frances, H., Lecrubier, Y., Puech, A.J., Simon, P. (1980). Evidence for the role of noradrenaline in some effects of quipazine. Psychopharmacol. 67 307-310.

Gallag er, D.W. (1978). Benzodiazepines: Potentiation of a GABA inhibitory response in the dorsal raphe nucleus. Eur. J. pharmacol. 49 133-143.

Gallager, D.W., Kehne, J.H., Wakeman, E.A., Davis, M. (1983). Developmental changes in pharmacological responsivity of the acoustic startle reflex: effects of picrotoxin. Psychopharmacol. 79 87-93.

Gardella, J.L., Isquierdo, J.A., Izquierdo, J. (1970). The analgesic action of catecholamines and of pyrogallol. Eur. J. Pharmacol. 10 87-90.

Gardner, C.R., Piper, D.C. (1982). Effects of α-adrenoceptor modulation on drinking conflict in rats. Brit. J. Pharmacol. 75 50p.

Geller, I., Bacnman, E., Seifter, J. (1963). Effects of reserpine and morphine on behaviour suppresed by punishment. Life Sci. 2 226-231.

Geller, I., Blum, K. (1970). The effect of 5-HTP on pCPA attenuation of conflict behaviour. Eur. J. Pharmacol. 9 319-324.

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

Gessa, G.L., Tagliamonte, A., (1979). Serotonin and sexual behaviour.In Serotonin in health and disease. Vol 5. Ed W B Essman. Spectrum Publications, London.

Geyer, M.A. (1976). Heterogeneous functions of discrete serotonergic pathways in the brain. In Biochemistry of mental disorders Vol 13. Ed E Usdin & A J Mandell. Marcel Dekker Inc. N.Y.

Geyer, M.A., Segal, D.S., Mandell, A.J. (1976). Opposite behavioural effects produced by locus coeruleus and median raphe lesions. Neurosci. Abstracts 2 488.

Glowinski, J., Iversen, L.L. (1966). Regional studies of catecholamine in the rat brain. I. The disposition of [3H]-norepinephrine, [3H]-dopamine and [3H]-dopa in various regions of the rat brain. J. Neurochem. 13 655-669.

Goldenberg, M., Snyder, C.H., Aranon, H.Jr. (1947). New test for hypertension due to circulating epinephrine. JAMA 135 971-976.

Goldstein, J.M., Knobloch, L.C., Malick, J.B. (1983). Electrophysiological demonstration of both α_2-agonist and antagonist properties of RX781094. Eur. J. Pharmacol. 91 101-106.

Gould, S.E. (1979). Some effects of steroid hormones and kynurenine on tryptophan and its conversion to 5-hydroxytryptamine. Ph.D. Thesis. University of Aston in Birmingham.

Grabowska, M., Antkiewicz, L., Michaluk, J. (1974a). The influence of quipazine on the turnover rate of serotonin. Biochem. Pharmacol. 23 3211-3212.

Grabowska, M., Antkiewicz, L., Michaluk, J. (1974b). A possible interaction of quipazine with central dopamine structures. J. Pharm. Pharmacol. 26 74-76.

Graeff, F.G. (1974). Tryptamine antagonists and punished behaviour. J. Pharmacol. Exp. Ther. 189 344-350.
Grahame Smith, 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. 8 1053-1066.
Grant, S.J., Huang, Y.H., Redmond, D.E.Jr. (1980). Benzodiazepines attenuate single unit activity in the locus coeruleus. Life Sci. 27 2231-2236.
Gray, J.A. (1981). The neuropsychology of anxiety. An enquiry into the functions of the hippocampal system. Oxford Clarendon Press, Oxford.
Green, A.R., Curzon, G. (1968). Decrease of 5-hydroxytryptamine in brain provoked by hydrocortisone and its prevention by allopurinol. Nature(Lond.) 220 1095-1097.
Green, A.R., Grahame-Smith, D.G. (1974). The role of brain dopamine in the hyperactivity syndrome produced by increased 5-HT synthesis in rats. Neuropharmacol. 13 949-959.
Green, A.R., Grahame-Smith, D.G. (1976). Effects of drugs on processes regulating the functional activity of brain 5-hydroxytryptamine. Nature. 260 487-491.
Green, A.R., Johnson, P., Nimgaonkar, V.L. (1983). Interactions of adrenoceptor agonists and antagonists with the 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors. Neuropharmac. 22 656-660.
Green, A.R., Youdim, M.B.H., Grahame-Smith, D.G. (1976). Quipazine: its effects on rat brain 5-HT metabolism, monoamine oxidase activity and behaviour. Neuropharmac. 15 173-179.
Haefely, W., Schaffner, R., Polc, P., Pieri, L. (1981). General pharmacology and neuropharmacology of propanediol carbamates.In Handbook of Experimental Pharmacology Vol 55 Psychotropic Agents. Ed. F Hoffmeister & G StilleSpringer-Verlag berlin Heidelberg N.Y.
Haeusler, G. (1974). Conidine-induced inhibition of sympathetic nerve activity: no indication for a central presynaptic or indirect sympathomimetic mode of action. Naunyn Schmied. Arch. Pharmacol. 286 97-111.
Halliday, M. (1968). Exploratory behaviour. In Analysis of behavioural change Ed 1 Weiskranz. Harper & Row N.Y.
Hallstrom, C., Treasden, I., Guy-Edwards, J., Lader, M. (1981). Diazepam, propranolol, and their combination in the management of chronic anxiety. Brit. J. Psychiatry 139 417-421.
Hamon, M., Bourgoin, S., Enjalbert, A., Bockaert, J., Hery, F., Ternaux, J.P., Glowinski, J. (1976). The effects of quipazine on 5-HT metabolsm in the rat brain. Naunyn Schmied. Arcn. Pharmacol. 294 99-108.
Hammond, D.L., Levy, R.A., Proudfit, H.K. (1980). Hypoalgesia induced by microinjection of a norepinephrine antagonist in the raphe magnus: Reversal by intrathecal administration of a serotonin antagonist. Brain Res. 210 475-479.
Handley, S.L., Brown, J. (1982). Effects on the 5-hydroxytryptamine-induced head-twitch of drugs with selective actions on alphal and alpha2 adrenoceptors. Neuropharmacol. 21 507-510.
Harris, R.A., Snell, D., Loh, H.H. (1978). Effects of

stimulants anorectics and related drugs on schedule-controlled behaviour. Psychopharmac. 56 49-56.

Hatton, D.C., Tanke, R., Lanthorn, T., Meyer, M.E. (1978). Serotonin and tonic immobility in the rat. Behav. Biol. 24 97-100.

Hawkins, M., Monti, J.M. (1979). Effects of pretreatment with 6-hydroxydopamine or noradrenergic receptor blockers on the clonidine-induced disruption of conditioned avoidance responding. Eur. J. Pharmacol. 58 53-58.

Herman, Z.S., Brus, R., Drybanski, A., Szkilnik, R., Slominska-Zurek, J. (1976). Influence of 6-hydroxydopamine on the behavioural effects induced by apomorphine or clonidine in rats. Psychopharmacol. 50 73-80.

Hodges, J.R., Sadow, J.(1967).Impairment of pituitary adrenocorticotrophic function by corticosterone in the blood. Brit. J. Pharmacol. 30 385-391.

Hoebel, B.G. (1977). The psychopharmacology of feeding. In Handbook of Psychopharmacology Vol 8 Ed L L Iversen, S D Iversen & S H Snyder Plenum Press N.Y.

Hoehn-Saric, R. (0983). Effects of THIP on chronic anxiety. Psychopharmacol. 80 338-341.

Hoehn-Saric, R., Merchant, A.F., Keyser, M.C., Smith, V.K. (1981). Effects of clonidine on anxiety disorders. Arch. Gen. Psychiatry. 38 1278-1286.

Holmberg, G., Gershon, S. (1961). Autonomic and psychic effects of yohimbine hydrochloride. Psychopharmacol. 2 93-106.

Hunter, B., Zernetzer, S.F., Jarvick, M.E., McLaugh, J.L. (1977). Modulation of learning and memory: effects of drugs influencing neurotransmitter. In Handbook of Psychopharmacology. Vol 8 Ed L L Iversen, S D Iversen & S H Snyder. Plenum Press N.Y.

Iversen, S.D. (1980). Animal models of anxiety and benzodiazepine actions. Arzheimithelforsch. 30 862-868.

Iversen, S.D. (1984). 5-HT and anxiety. Invited lecture at the 5-HT symposium at Univ.of Birmingham. 10th April 1984, to be published in Neuropharmacol.

Jarrott, B., Louis, W.J., Summers, R.J. (1979). The effect of a series of clonidine analogues on ^{3}H-clonidine binding in rat cerebral cortex. Biochem. Pharmacol. 27 141-144.

Jefferson, J.W. (1974). Beta adrenergic receptor blocking drugs in psychiatry. Arcn. Gen. Psychiatry 31 681-691.

Jenner, P., Chadwick, D., Reynolds, E.H., Marsden, C.D. (1975). Altered 5-HT metabolism with clonazepam, diazepam and diphenylhydantoin. J. Pharm. Pharmacol. 27 707-710.

Joseph, L.H., Kennett, G.A. (1981). Brain tryptophan and 5-HT function in stress. Brit. J. Pharmacol. 73 267p.

Jouvet, M. (1973). Serotonin and behaviour. Ed Barchas & E Usdin. 385-400. Academic Press, N.Y.

Jouvet, M. (1977). Serotonin and the sleep waking cycle. Neuropharmacology of the sleep-waking cycle. In Handbook of Psychopharmacology. Vol 8.Ed L L Iversen, S D Iversen & S H Snyder Plenum Press N.Y.

Kalkman, H.O., Harms, Y.M., Van Gelderen, E.M., Batink, H.D., Timmermans, P.B.M.W.M., Van Zweiten, P.A. (1983). Hypotensive activity of serotonin antagonists; correlation

with α_1 adrenoceptor and serotonin receptor blockade. Life Sci. 32 1499-1506.

Kalsner, S., Quilllan, M. (1984). A hypothesis to explain the presynaptic effects of adrenoceptor antagonists. Brit. J. Pharmacol. 82 515-522.

Kaumann, A.J. (1983). Yohimbine and rauwolscine inhibit 5-hydroxytryptamine-induced contractions of large coronary arteries of calf through blockade of 5-HT_2receptors. Naunyn Schmied. Arch. Pharmacol. 323 149-154.

Kennett, G.A., Joseph, M.H. (1981). The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. Neuropharmacol. 20 39-43.

Kilian, M., Frey, H.H. (1973). Central monoamines and convulsive thresholds in mice and rats. Neuropharmacol. 12 681-692.

Kilts, C.D., Commisaris, R.L., Cordo, J.J., Rech, R.H. (1982). Lack of central 5-hydroxytryptamine influence on the anti-conflict activity of diazepam. Psychopharmacol. 78 156-164.

Kobinger, W., Pichler, L. (1977). Pharmacological characterisation of BHT 933 (2-amino 6 ethyl-4, 5, 7, 8, -tetrahydro 6H-oxazolo-[5, 4-d]-azepin dihydrochloride as a hypotensive agent of the clonidine type. Naunyn Schmied. Arch. Pharmacol. 300 39-46.

Kobinger, W., Picnler, L. (1980a). Investigation into different types of post- and presynaptic α-adrenoceptors at cardiovascular sites in rats. Eur. J. Pharmacol. 65 393-402.

Kobinger, W., Pichler, L. (1980b). Relation between central sympathoinhibitory and peripheral pre- and postsynaptic α-adrenoceptors as evaluated by different clonidine-like substances. Naunyn Schmied. Arch. Pharmacol. 315 21-27.

Kobinger, W., Pichler, L. (1982). Presynaptic activity of the imidazoline derivative St 587 a highly selective α_1-adrenoceptor agonist. Eur. J. Pharmacol. 82 203-206.

Koob, G.F., Strecker, R.E., Roberts, D.C.S., Bloom, F.E. (1980). Failure to alter anxiety or the anxiolytic properties of chlordiazepoxide and ethanol by the destruction of the dorsal noradrenergic system. Abstracts Soc.for Neurosci., 10th annual meeting, Cincinnati, Ohio, 9-14 Nov. 6 108.

Korf, J., Aghajanian, G.K., Roth, R.H. (1973). Increased turnover of norepinephrine in the rat cerebral cortex during stress; role of the locus coeruleus. Neuropharmacol. 12 933-938.

Koss, M.C. (1983). Analysis of CNS sympatho-inhibition produced by guanabenz. Eur. J. Pharmacol. 90 19-28.

Kostowski, W. (1980). Noradrenergic interactions among central neurotransmitters. In Neurotransmitters, receptors and drug action. Ed W B Essman Spectrum Publications.

Kovacs, G.L., Kishoti, J., Lissak, K., Telegdy, G. (1975). Dose- dependent dual effect of cortisone on cerebral 5-hydroxytryptamine metabolism. Neurochem. Research 2 311-322.

Kruse, H., Dunn, R.W., Theurer, K.L., Novick, W.J., Shearman, G.T. (1981). Attenuation of conflict induced suppression by clonidine: Indication of anxiolytic activity. Drug Dev. Res. 1 137-143.

Lands, A.M., Lirdena, F.P., Buzzo, H.J. (1967). Differentiation of receptors responsive to isoproterenol. Life Sci. 6 2241-2249.

Langer, S.Z. (1974). Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23 1793-1800.

Langer, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. Brit. J. Pharmacol. 60 481-491.

Langer, S.Z., Enero, M.A., Adler-Graschinsky, E., Dubocovich, M.L., Celuchi, S.M. (1975). Presynaptic regulatory mechanisms for noradrenaline release by nerve stimulation. In Central actions of drugs on blood pressure regulation. Ed D S Davis & J L Reid. Pitman Medical.

Lauener, H. (1963). Conditioned suppression in rats and the effect of pharmacological agents thereon. Psychopharmacol. 4 311-325.

Lazarova, M., Bendotti, C., Samanin, R. (1983). The role of different types of adrenergic receptors in pentylene-tetrazole- induced seizures and the effect of di-n-propylate in the rat. Psychopharmacol. 81 177-182.

Lazarova, M., Samanin, R. (1983). Serotonin mediation of the protective effect of clonidine against pentylenetetrazole-induced seizures in rats. Life Sci. 32 2343-2348.

Leger, L., Desarries, L. (1978). Serotonin nerve terminals in the LC of the adult rat: A radioautographic study. Brain Res. 145 1-13.

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vanenberk, J., Janssen, P.A.J. (1981). Receptor profile of R41468 (ketanserin), a novel antagonist at 5-HT_2 receptors. Life Sci. 28 1015-1022.

Lidbrink, P., Corrodi, H., Fuxe, K.(1974) Benzodiazepines and barbiturates: turnover changes in central 5-hydroxytryptamine pathways. Eur. J. Pharmacol. 26 35-40.

Lindvall, O., Bjorkland, A. (1974). The organisation of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta. Physiol. Scand. 92 (suppl.412) 1-48.

Lloyd, K.G., Worms, P. (1982). Differential effect of yohimbine in several chemical models of convulsions in mice. Brit. J. Pharmacol. 77 340p.

Lorden, J.F., Rickert, E.J., Dawson, R., Pelleymountes, M.A. (1980). Forebrain norepinephrine and the selective processing of information. Brain Res. 190 569-573.

Maickel, R.P., Maloney, G.J. (1973). Effects of various depressant drugs on deprivation-induced water consumption. Neuropharmacol. 12 777-782.

Maj, J., Baran, L., Grabowska, M., Sowinska, H. (1973). Effect of clonidine on the 5-hydroxytryptamine and 5-hydroxyindole acetic acid brain levels. Biochem. Pharmacol. 22 2679-2683.

Margules, D.L., Stein, L. (1968). Increase of "antianxiety" activity and tolerance of behavioural depression during chronic administration of oxazepam. Psychopharmacol. 13 74-80.

Marriott, A.S., Smith, E.F. (1972). An analysis of drug effects in mice exposed to a novel environment. Psychopharmacol. 24

397-406.
Marriott, A.S., Spencer, P.S.J. (1965). Effects of centrally acting drugs on exploratory behaviour in rats. Brit. J. Pharmacol. 25 432-441.
Martin, L.L., Sanders-Bush, E. (1982). Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn Schmied. Arch. Pharmacol. 321 165-170.
Mason, S.T., Fibiger, H.C. (1978). Evidence for a role of brain noradrenaline in attention and stimulus sampling. Brain Res. 159 421-426.
Mason, S.T., Fibiger, H.C. (1979a). Current concepts I. Anxiety: The locus coeruleus disconnection . Life Sci. 25 2141-2147.
Mason, S.T., Fibiger, H.C.(1979b) Noradrenaline, fear and extinction. Brain Res. 165 47-56.
Mason, S.T., Iversen, S.D.(1975). Learning in the absence of forebrain noradrenaline. Nature 258 424-426.
Mason, S.T., Iversen, S.D. (1977). Effects of selective forebrain noradrenaline loss on behavioural inhibition in the rat. J. Comp. Physiol. Psychol. 91 165-173.
Mason, S.T., Roberts, D.C.S., Fibiger, H.C. (1978). Noradrenaline and neophobia. Physiol. Behav. 21 353-361.
Massari, V.J., Tizabi, Y., Jacobwitz, D.M. (1979). Potential noradrenergic regulation of serotonergic neurones in the median raphe nuclei. Exper. Brain Res. 34 177-182.
Maynert, E.W., Levi, R. (1964).Stress induced release of brain norepinephrine and its inhibition by drugs. J. Pharmacol. Exp. Ther. 143 90-95.
McCleary, P.E., Leander, D.J. (1981). Clonidine analgesia and suppression of operant responding: dissociation of mechanism. Eur. J. Pharmacol. 69 63-69.
Meldrum, B. (1979). Convulsant drugs, anti-convulsants and Gaba-mediated neuronal inhibition. In Gaba-neuro-transmitters. Pharmacochemical, biochemical and pharmacological aspects. Ed P Krogsgaard-Larsen, J Scheel-Kruger, H Kofod. Munksgaard, Copenhagen.
Menkes, D.B., Baraban.J.M., Aghajanian, G.K. (1981). Prazosin selectively antagonises neuronal responses mediated by α_1-adrenoceptors in brain. Naunyn Schmied. Arch. Pharmacol. 317 273-275.
Michel, A, D., Nahorski, S.R., Whiting, R.L. (1981). Selectivity of RS21361 for α_2-adrenoceptors as determined by in vitro, in vivo and ligand binding studies. Brit. J. Pharmacol. 74 845p.
Millard, S.A., Costa, E., Gal, E.M. (1972). On the control of brain serotonin turnover by end product inhibition. Brain Res. 40 545-551.
Millenson, J.R., Leslie, J. (1974). The conditioned emotional response(CER) as a baseline for the study of antianxiety drugs. Neuropharmacol. 13 1-9.
Montgomery, K.C. (1953). The effect of hunger and thirst drives on exploratory behaviour. J. Comp. Physiol. Psychol. 46 315-319.

Montgomery, K.C. (1955). The relation between fear induced by novel stimulation and exploratory behaviour. J Comp. Physiol. Psychol. 48 254-260.

Moore, R.Y. (1980). The reticular formation: monoamine neuron systems. In The reticular formation revisited: specifying function for a nonspecific system. Ed J A Hobson & M A B Brazier Raven Press N.Y.

Morgan, W.M., Rudeen, P.K., Pfeil, K.A. (1975). Effect of immobilisation stress on serotonin content and turnover in regions of the rat brain. Life Sci. 17 143-150.

Morgane, P.J., Jacobs, L. (1979). Raphe projections to the locus coeruleus in the rat. Brain Res. Bull. 41 519-534.

Nakamura, M., Fukushima, H.(1977). Effect of fludiazepam on turnover or serotonin in mouse brain. Japan. J. Pharmacol. 27 905-908.

Nassif, S., Kempf, E., Cardo, B., Velley, L. (1983). Neurochemical lesioning of the locus coeruleus of the rat does not suppress the sedative effect of clonidine. Eur. J. Pharmacol. 91 69-76.

Nolan, N.A., Parkes, M.W. (1973). The effects of benzodiazepines on the behaviour of mice on a hole-board. Psychopharmacol. 29 277-288.

Nomura, S., Watanabe, M., Ukei, N., Nakazawa, T. (1981). Stress and β adrenergic receptor binding in the rat brain. Brain Res. 224 199-203.

Ogren, S.O., Archer, T., Ross, S.B. (1980). Evidence for a role of the locus coeruleus noradrenaline system in learning. Neurosci. Letters 20 351-356.

Paciorek, P.M., Shepperson, N.B. (1983). α_1-Adrenoceptor agonist activity of α_2-adrenoceptor antagonists in the pithed rat preparation. Brit. J. Pharmacol. 79 12-14.

Papanicolaou, J., Summers, R.J., Vajda, F.J.E., Louis, W.J. (1982). Anticonvulsant effects of clonidine mediated through central α_2 adrenoceptors. Eur. J. Pharmacol. 77 163-166.

Papeschi, R., Sourkes, T.L., Youdim, M.B.H. (1971). The effect of yohimbine on brain serotonin metabolism, motor behaviour and body temperature of the rat. Eur. J. Pharmacol. 15 318-326.

Pawlowski, L., Przegaliński, E., Hano, J. (1983). Antiserotonergic and antidopaminergic action of pirenperone - a new S_2-serotonin receptor antagonist. Naunyn Schmied. Arch. Pharmacol. 324 Suppl. R20.

Peroutka, S.J., Snyder, S.H. (1979). Multiple serotonin receptors: differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]-lysergic acd diethylamide and [^{3}H]spiroperidol. Molecular Pharmacol. 16 687-699.

Pickel, V.M., Joh, T.H., Reis, D.J. (1978). Immunocytochemical evidence for serotonergic innervation of noradrenergic neurones in the nucleus L.C. In Interactions between putative neurotransmitters in the brain. Ed S Garratini, J F Pujol & F Samanin. Raven Press N.Y.

Plaznik, A., Danysz, W., Kostowski, W., Bidzinski, A., Hauptmann, M. (1983). Interaction between noradrenergic and serotonergic brain systems as evidenced by behavioural and biochemical effects of microinjections of adrenergic

agonists and antagonists into the median raphe nucleus. pharmacol. Biochem. Behav. 19 27-32.
Prange, A.J.Jr., Wilson, I.C., Lynn, C.W., Alltop, L.B., Stikeleather, R.A. (1974). L-tryptophan in mania. Cnntribution to a permissive hypothesis of affective disorders. Arch. Gen. Psychiatry 30 56-62.
Rastogi, R.B., Agarwal, R.A., Lapierre, Y.D., Singhal, R.L. (1977). Effects of acute diazepam and clobazam on spontaneous motor activity and central amine metabolism in rats. Eur. J. Pharmacol. 43 91-98.
Redmond, D.E.Jr. (1977). Alterations in the function of the nucleus locus coeruleus: a possible model for studies of anxiety. In Animal models in Psychiatry and Neurology Ed I Hannin & E, Usdin Pergammon Press, Oxford.
Redmond, D.E.Jr. (1979). New and old evidence for the involvement of a brain norepinephrine system in anxiety. In Phenomenology and treatment of anxiety. Ed W E Fann, I Karacan , A D Pokorny & R L Williams. Spectrum Publications, N.Y.
Redmond, D.E.Jr. (1982). Does clonidine alter anxiety in humans? T.I.P.S. 3 477-479.
Redmond, D.E., Huang, Y.H. (1979). Current Concepts II: New evidence for a locus coeruleus- norepinephrine connection with anxiety. Life Sci. 25 2149-2162.
Reinhard, J.F., Roth, R.H.(1982). Noradrenergic modulation of serotonin synthesis and metabolism.1.Inhibition by clonidine in vivo. J. Pharmacol. Exp. Therap. 221 541-546.
Robbins, T.W. (1981). Behavioural determinants of drug action: rate dependency revisited. In Theory of Psychopharmacology. Vol 1. Ed S J Cooper. Academic Press, London.
Robichaud, R.C., Sledge, K.L. (1969). The effects of p-CPA on experimentally induced conflict in the rat. Life Sci. 8 965-969.
Rochette, L., Bralet, J. (1975). Effect of the norepinephrine receptor stimulating agent "clonidine" on the turnover of 5-hydroxytryptamine in some areas of the rat brain. J. Neural Trans. 37 259-267.
Rodriguez, R., Rojas-Ramirez, J.A., Drucker-Colin, R.R. (1973). Serotonin-like actions of quipazine on the central nervous system. Eur. J. Pharmacol. 24 164-171.
Roth, K.A., Mefford, I.M., Barchas, J.D. (1982). Epinephrine, norepinephrine, dopamine and serotonin: differential effects of acute and chronic stress on regional brain amines. Brain Res.239 417-424.
Rothballer, A.B. (1959(. The effects of catecholamines on the central nervous system. Pharmac. Rev. 11 494-547.
Rushton, R., Steinberg, H.(1966). Combined effects of chlordiazepoxide and dexamphetamine on activity of rats in unfamiliar environment. Nature. 211 1312-1313.
Sahgal, A., Iversen, S.D., Lewis, M.E., Trimnell, L.E.(1979). Effects of ACTH 4-10 on a conflict schedule in pigeons. Commun. Psychopharmacol. 3 211-216.
Sakai, K., Touret, M., Salvert, D., Leger, L., Jouvet, M. (1977). Afferent projections of the cat locus coeruleus as visualised by the horse radish peroxidase technique. Brain

Res. 119 21-41.
Sakakibara, Y., Muramatsu, I., Fujiwara, M., Nagasaka, Y. (1981). Effects of guanabenz on the adrenergic mechanisms in rabbit arterial strips. Jap. J. Pharmacol. 31 1029-1036.
Samanin, R., Bernasconi, C., Quathrone, A. (1976). Antinociceptive action of quipazine: relation to central serotonergic receptor stimulation. Psychopharmacol. 46 219-222.
Sanghvi, I., Gershon, S., (1974) Yohimbine: Behavioural and biochemical effects in mice. Arch. Int. Pharmacodyn. 210 108-120.
Scheel-Krueger, J., Hasselager, E. (1974). Studies of various amphetamines, apomorphine and clonidine on body temperature and brain 5-hydroxytryptamine metabolism in rats. Psychopharmacol. 36 189-202.
Schildkraut, J.J., Kety, S.S. (1967). Biogenic amines and emotion. Science 156 21-30.
Schlicker, E., Gothert, M. (1981). Antagonistic properties of quipazine at presynaptic receptors and α-adrenoceptors in rat brain cortex slices. Naunyn Schmied. Arch. Pharmacol. 317 204-208.
Sepinwall, J., Cook, L. (1978). Behavioural pharmacolgy of antianxiety drugs: In Handbook of Psychopharmacology Vol 13 Ed L L Iversen, S D Iversen & S H Snyder Plenum Press N.Y.
Sepinwall, J., Cook, L. (1981). Studies on clonidine in animal tests for antianxiety activity. Psychopharmacol. Bulletin 17 24-26.
Sepinwall, J., Grodsky, F.S., Sullivan, J.W., Cook, L. (1973). Effects of propranolol and chlordiazepoxide on conflict behaviour in rats. Psychopharmacol. 31 375-382.
Setler, P.E. (1977). The Neuroanatomy and Neuropharmacology of drinking. In Handbook of Psychopharmacology Vol 8 Ed L L Iversen, S D Iversen & S H Snyder Plenum Press N.Y.
Setoguchi, M., Takehara, S., Nakajima, A., Tsumagari, T., Takigawa, Y.(1978). Effects of 6-(o-chlorophenyl)-8-ethyl-1-methyl-4H-s-triazolo [3, 4-C] thieno [2, 3-e]Z1, 4] diazepine(Y-7, 131) on the metabolism of biogenic amines in brain. Drug Res. 28 1165-1169.
Shephard, R.A., Buxton, D.A., Broadhurst, P.L. (1982). Drug interactions do not support reduction in serotonin as the mechanism of action of benzodiazepines.Neuropharmacol. 21 1027-1032.
Sladek, J.R., Walker, P. (1977). Serotonin-containing neuronal perikarya in the primate locus coeruleus and subcoeruleus. Brain Res. 134 359-366.
Sorensen, C.A., Gordon, M. (1975). Effects of 6 hydroxydopamine on shock elicited fighting and maternal behaviour in female rats. Pharmacol. Biochem. Behav. 3 331-335.
Soubrie, P., De Angelis, L., Simon, P., Boissier, J.R. (1976). Effets des anxiolytiques sur la prise de boisson en situation nouvelle et familiere. Psychopharmacol. 50 41-45.
Starke, K. (1972). Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: evidence for an α-receptor mediated feedback inhibition of noradrenaline release. Naunyn Schmied. Arch. Pharmacol. 275 11-23.

Starke, K., Borowski, E., Endo, T. (1975) Preferential blockade of presynaptic α-adrenoceptors by yohimbine. Eur. J. Pharmacol. 34 385-388.

Stein, L., Berger, B.D. (1969). Paradoxical fear-increasing effects of tranquillisers: evidence of repression of memory in the rat. Science 166 253-256.

Stein, L., Wise, C.D., Belluzi, J.D. (1975) Effects of benzodiazepines on central serotonergic mechanisms. In Mechanisms of action of Benzodiazepines Ed E Costa & P Greengard. Raven Press N.Y.

Stein, L., Wise, C.D., Belluzi, J.D. (1977). Neuropharmacology of reward and punishment. In Handbook of Psychopharmacology Vol 8. Ed L L Iversen, S D Iversen & S H Snyder. Plenum Press, N.Y.

Stein, L., Wise, D., Berger, B.D.(1973) Antianxiety action of benzodiazepines: Decrease in activity of serotonin neurons in the punishment system. In The Benzodiazepines Ed S Garratini, E Mussini & L O Randall. Raven Press, N.Y. 299-326.

Stjarne, L., Brundin, J. (1975). Dual adrenoceptor-mediated control of noradrenaline secretion from human vasoconstrictor nerves: facilitation by β-receptors and inhibition of α-receptors. Acta. Physiol. Scand. 94 139-141.

Stone, E.A., Platt, J.E. (1982). Brain adrenergic receptors and resistance to stress. Brain Res. 237 405-414.

Strombom, U. (1975). Effect of low doses of catecholamine receptor agonists on exploration in mice. J. Neural Trans. 37 229-235.

Sullivan, H.S. (1964). The fusion of psychiatry and social sciences Introduction and commentary by H S Perry. Norton, N.Y.

Sulser, F., Vetulani, J., Mobley, Ph.L. (1978). Mode of action of antidepressant drugs. Biochem. Pharmacol. 27 257-261.

Svensson, L., Ahlenius, S. (1983) Enhancement by the putative 5-HT receptor agonist 8-OH-2-(di-n-propylamino) tetralin of the acoustic startle response in the rat. Psychopharmacol. 79 104-107.

Tanaka, T., Starke, K. (1980). Antagonist /agonist-preferring or α_1/α_2 receptors? Eur. J. Pharmacol. 63 191-194.

Tanaka, M., Kohno, Y., Nakagawa, R., Ida, Y., Takeda, S., Nagasaki, N. (1982). Time related differences in noradrenaline turnover in rat brain regions by stress. Pharmacol. Biochem. Behav. 16 315-319.

Taube, H.D., Starke, K., Borowski, E. (1977). Presynaptic receptor systems on the noradrenergic neurones of rat brain. Nuanyn Schmied. Arch. Pharmacol. 229 123-141.

Tenen, S.S. (1967). The effects of PCPA, a serotonin depletor, on avoidance acquisition pain sensitivity and related behaviour in the rat. Psychopharmacol. 10 204-219.

Thiebot, M.H., Hamon, M., Soubrie, P. (1982). Attenuation of induced-anxiety in rats by chlordiazepoxide- role of raphe dorsalis benzodiazepine binding sites and serotoninergic neurones. Neurosci. 7 2287-2294.

Thiebot, M.H., Soubrie, P., Doore, L., Simon, P. (1984a). Evidence against the involvement of a noradrenergic mechanism

in the release by diazepam of novelty-induced hypophagia in rats. Eur.J.Pharmacol. 100 201-206.

Thiebot, M-H., Soubrie, P., Hamon, M., Simon, P. (1984b). Evidence against the involvement of serotonergic neurons in the antipunishment activity of diazepam in the rat. Psychopharmacol. 82 355-359.

Thierry, A.M., Fekete, M., Glowinski, J.(1968) Effects of stress on the metabolism of noradrenaline, dopamine and serotonin(5-HT) in the central nervous system of the rat. II Modifications of serotonin metabolism . Eur. J. Pharmacol. 4 384-389.

Tye, N.C., Everitt, B.J., Iversen, S.D. (1975). 5-hydroxytryptamine and punishment Nature. 268 741-743.

Uhde, T.W., Post, R.M., Siever, L., Buchsbaum, M.S., Jimerson, D.C., Silberman, E.K., Murphy, D., Bunney, W.E.Jr. (1981). Clonidine: effects on mood, anxiety and pain. Psychopharmacol. Bulletin 17 125-126.

Ungerstedt, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. Scand.82 (Suppl.367) 1-48.

Upritchard, D.C., Greenberg, D.A., Snyder, S.H. (1978). The labelling of two α-adrenergic receptors in the CNS by catecholamines and other ligands:ion and nucleotide interactions. In Recent advances in the Pharmacology of Adrenoceptors. Elsevier, North Holland.

Valzelli, L. (1978). Affective behaviour and serotonin. In Serotonin in Health and disease Vol 3. Ed W B Essman. Spectrum Publications, N.Y.

van Meel, J.C., DeJonge, A.A., Timmermans, P.B.M.W.M., van Zweiten, P.A. (1981). Selectivity of some alpha adrenoceptor agonists for peripheral alpha-1, and alpha-2 adrenoceptors in the normotensive rat. J. Pharmacol. Exp. Ther. 219 760-767.

Vogel, J.R., Beer, B., Clody, D.E. (1971). A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacol. 21 1-7.

Wallnau, L.B., Gallup, G.G. (1977). A serotonergic mid-brain raphe model of tonic immobility. Biobehavioural reviews. 1 35-43.

Warner, R.S. (1965). Management of the office patient with anxiety and depression. Psychosomatics. 6 347-351.

Whitaker, P.M., Seaman, P. (1978). High affinity ^{3}H-serotonin binding to caudate: inhibition by hallucinogens and serotonin drugs. Psychopharmacol. 59 1-5.

Winter, J.C. (1972). Comparison of chlordiazepoxide and cinanserin as modifiers of punished behaviour and as antagonists of N, N, dimethyltryptamine. Arch. Int. Pharmacodyn. Ther.197 147-159.

Wise, C.D., Berger, B.D., Stein, L.(1972) Benzodiazepines: Anxiety reducing activity by reduction of serotonin turnover in the brain. Science 177 180-183.

Wise, C.D., Berger, B.D., Stein, L. (1973). α-noradrenergic receptors for reward and serotonergic receptors for punishment in the rat brain. Biol. Psychiatry 6 3-22.

Wise, R.A., Dawson, V. (1974). Diazepam-induced eating and

lever pressing for food in sated rats. J. Comp. Physiol. Psychol. 86 930-941.

Young, III, W.S., Kuhar, M.O., (1981). Anatomical mapping of clonidine (α2-noradrenergic) receptors in rat brain: Relationship to function. In: Psychopharmacology of Clonidine Ed. H. Lal & S. Fielding. Alan R. Liss Inc. N.Y.