ASPECTS OF TIC LIKE BEHAVIOUR AND SEROTONERGIC CONTROL.

ANDREW CHRISTOPHER McCREARY B.Sc. (Hons)

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF ASTON IN BIRMINGHAM
SEPTEMBER 1995

Dedicated to Andrea and Alice

University of Aston in Birmingham.

Aspects of tic like behaviour and serotonergic control.

Andrew Christopher McCreary

Submitted for the Degree of Doctor of Philosophy, 1995.

Summary

Tic-like movements in rodents bear close similarities to those observed in humans both pharmacologically and morphologically. Pharmacologically, tics are modulated by serotonergic and dopaminergic systems and abnormalities of these systems have been reported in Tourette's Syndrome (TS). Therefore, serotonergic and dopaminergic modulation of tics induced by a thyrotrophin-releasing hormone (TRH) analogue were studied as possible models for TS.

The TRH analogue MK771 induced a variety of tic like movements in mice; blinking fore-paw-licking and fore-paw-tremor were quantified and serotonergic and dopaminergic modulation was investigated. The selective dopamine D1 receptor antagonists SCH23390 and SCH39166 and dopamine D2 antagonists raclopride and sulpiride had no effect on MK771 induced blinking. The D1 antagonists attenuated fore-paw-tremor and -licking while the D2 antagonists were generally without effect on these behaviours. Ketanserin (5-HT2A/ alpha-1 antagonist) and ritanserin (5-HT2A/2C antagonist) were able to attenuate MK771-induced blinking and ketanserin, ritanserin, mianserin (5-HT2A/2C antagonist) and prazosin (alpha-1 adrenoceptor antagonist) were able to attenuate MK771-induced fore-paw-tremor and -licking. The 5-HT2C/2B antagonist SB200646A was without effect on blinking and fore-paw-licking but dose-dependently potentiated fore-paw-tremor. The 5-HT1A agonists 8-OH DPAT and buspirone attenuated blinking at the lower doses tested but were ineffective at the higher doses, the converse was found for fore-paw-licking and -tremor behaviours. The effects of these ligands appeared to be at a postsynaptic 5-HT1A site since para-chlorophenylalanine was without effect on the manipulation of these behaviours. (S)-WAY100135 was without effect on MK771-induced behaviours, spontaneous and DOI-induced head shakes.

Because kynurenine potentiates head shakes and plasma concentrations are raised in TS patients the effects of kynurenine on the 5-HT2A/2C agonist DOI mediated head shake were established. Kynurenine potentiated the DOI head shake. Attempts were made to correlate serotonergic unit activity with tic like behaviour in cats but this proved unsuccessful. However, the pharmacological understanding of 5-HT1A receptor function has been hampered because of the lack of selective antagonists for this site. For this reason the effects of the novel 5-HT1A antagonists (S)-WAY-100135 and WAY-100635 were tested on 5-HT single-unit activity recorded from the dorsal-raphe-nucleus in the behaving cat. Both drugs antagonised the suppression of unit activity caused by 8-OH DPAT. (S)-WAY-100135 reduced unit activity whereas WAY-100635 increased it. This suggests that WAY-100635 is acting as an antagonist at the 5-HT1A somatodendritic autoreceptor and that (S)-WAY-100135 acts as a partial agonist at this site.

Aspects of tic like behaviour and serotonergic control are discussed.

Key Words: Tourette's Syndrome, thyrotrophin-releasing hormone, tic like movements, 5-HT receptors, 5-HT single-unit activity, 5-HT1A antagonists.

ACKNOWLEDGEMENTS

I am extremely grateful to Dr. S.L. Handley for her help, encouragement and patience.

I also acknowledge the help of the staff in the Department of Pharmaceutical and Biological Sciences, especially Mr. A. Richardson, Mr. G. Smith, Mr. M. Gamble and all the members of the Animal Unit and the secretaries.

I would like to thank Dr. D. Heal, Mr. M. Prow and Ms. S. Butler for assaying monoamines (Chapter 4).

I would like to thank Prof. B.L. Jacobs at Princeton University for allowing me to spend some time in his laboratory. I would also like to thank all in Prof. Jacobs' laboratory for making my stay so enjoyable and makining me feel so welcome.

I would like to thank Dr. C.A. Fornal for his advise during the preparation of this manuscript.

I would like to thank The James Watt Fellowship and SERC (BBSRC) for their financial support of my visit to Princeton.

I would like to thank Dr. Serdar Dursun for his initial guidance, advise and encouragement at the beginning of these studies.

I would like to thank my parents for their support throughout my studies (both financial and moral).

Finally, I would like to thank my wife for her care, understanding, encouragement and assistance with the preparation of this manuscript.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

5-HIAA 5-hydroxyindoleacetic acid
5-HT 5-hydroxytryptamine
5-HTP 5-hydroxytryptophan
5-MeODMT 5-methoxy-N-N"-dimethyl-tryptamine
8-OH DPAT 8-hydroxy-2-(di-n-propyl-amino) tetralin
ACTH Adrenocorticotrophic hormone
BMC back-muscle contractions
CNS central nervous system
CSF cerebral spinal fluid
DA dopamine
DOI 1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane
DOPAC 3, 4-dihydroxyphenylacetic acid
DRN dorsal raphe nucleus
FPL Fore-paw-licking
FPT Fore-paw-tremor
GABA gamma-aminobutyric acid
GTS Gilles de la Torette's Syndrome
HS head shake
HVA homovanillic acid
i.p. intra-peritoneal
i.t. intr-athecal
mCPP m-chlorophenylpiperazine
NA noradrenaline
OCD obsessive-compulsive disorder
p.o. *pars-orale*
s.c. sub-cutaneous
TRH thyrotrophin-releasing hormone
WDS wet-dog shakes

GENERAL INTRODUCTION.

GENERAL INTRODUCTION.

1. General

Gilles de la Tourette's Syndrome (GTS) was described late in the last century (de la Tourette, 1885) and is still poorly understood owing to a lack of animal models available to study the disease. However, a series of behaviours observed in animals may offer possible models for the study of the tics observed in GTS patients (Handley and Dursun, 1992) in being rapid, recurrent, non-rhythmic and stereotyped (Diagnostic and Statistical Manual of Mental Disorders Third Edition (Revised) (DSM-III-R), 1987). The experiments reported herein explore the pharmacology of tic like behaviours, particularly those induced by the thyrotrophin-releasing hormone analogue MK771 (Veber *et al.*, 1976). The effects of two novel 5-HT1A antagonists upon serotonergic unit activity in the dorsal raphe nucleus (DRN) were also studied in a freely moving behaving animal model.

2. 5-HT in the Central Nervous System.

2.1 Initial Discovery

Serotonin (5-hydroxytryptamine (5-HT)) was originally discovered in the intestine (Vialli and Erpsamer, 1933) and 15 years later in serum (Rapport and Green, 1947). In 1953 Twarog and Page identified the monoamine in the brain.

2.2 Serotonergic pathways in the CNS

Nine clusters of serotonergic cell bodies have been identified in or near raphe regions of the medulla and upper brain stem using histochemical techniques. The clusters were arbitrarily assigned B1-B9 (Dahlstrom and Fuxe, 1965). B1 to B3 project to the spinal cord and form a descending serotonergic pathway. The rostral groups B6 to B9 project extensively to the fore brain with B7, B8 and B9 (the dorsal and median raphe and raphe magnus) providing the majority of innervation to the fore brain (Dahlstrom and Fuxe, 1965; Kosofsky and Molliver, 1987).

2.3 5-HT Receptors.

Studies have indicated a multiplicity of serotonergic receptors, complete characterisation data are available for some of these but the more recently described receptors still lack a full classification. Complete discussion of the full characterisation of 5-HT receptors is out of the scope of this Introduction and has recently been discussed in detail elsewhere (see Humphrey *et al.*, 1993; Hoyer *et al.*, 1994 for reviews). Thus, information presented here is intended as an overview of the subject. The effector systems for each of the receptors is displayed in *table* 1.2.3. The appellation 5-ht5, 5-ht6 and 5-ht7 receptors is, at the moment only tentative, until functional correlates are available, Hoyer and colleagues (1994) suggested that the lower case terminology be used.

Gaddum and Picarelli (1957) classified two distinct 5-HT receptors in the isolated guinea pig ileum on the basis that only a portion of its contractile response to 5-HT could be blocked by high concentrations of morphine but the remainder of the response could be antagonised by dibenzyline (phenoxybenzamine). Conversely when maximally effective concentrations of dibenzyline were present the remainder of the contractile response to 5-HT was antagonised by low concentrations of morphine. From these observations the existence of two serotonergic receptor sub-types (in ileum) was speculated - 5-HTD and 5-HTM.

Using the then relatively new radioligand binding techniques Peroutka and Snyder (1979) demonstrated two separate binding sites, one having a high affinity for tritiated 5-HT and a low affinity for spiperone (designated the 5-HT1 receptor) and another binding site with the reverse profile (designated the 5-HT2 site). Fozard and colleagues (1979), described a receptor which mediated noradrenaline (NA) release in the rabbit heart, at which cocaine displayed weak antagonist action, this receptor was similar to the 5-HTM receptor and was characterised as the 5-HT3 receptor (Bradley *et al.*, 1986). Pedigo *et al.* (1981), using tritiated spiperone displacement curves, demonstrated a high and a low affinity site which were designated 5-HT1A and 5-HT1B sites, respectively. Another sub-type of the 5-HT receptor was classified using mesulergine which showed high affinity binding to a site ascribed as 5-HT1C (Pazos *et al.*, 1984), wheras 5-HT1A and 5-HT1B receptors displayed low affinity for mesulergine. Because of the increased interest in research into 5-HT receptors and confusion as to the exact nomenclature of such receptors Bradley *et al.* (1986) set about correcting this and reported a more flexible classification which attempted "to accommodate the current state of knowledge and to provide a unifying concept on which to build and elaborate." They proposed the characterisation of three main sites: 5-HT1 sites be ascribed "5-HT1-like" receptor until different functionalities could be identified; 5-HT2 and 5-HT3 receptors were also characterised. 5-HT2 receptors were described as being similar to the classical 5-HTD receptor described by Gaddum and Picarelli (1957). Since this time a multiplicity of 5-HT1-like sites have been characterised. The 5-HT1D receptor has been identified in human and calf caudate nucleus (Heuring and Peroutka, 1987; Hoyer 1988b). 5-ht1E receptors were revealed with tritiated 5-HT in the presence of excess 5-carboxyamidotryptamine (to block 5-HT1A and 5-HT1D binding) in the frontal cortex (Leonhardt *et al.*, 1989). 5-ht1F has been identified in the mouse (Amlaiky *et al.*, 1992; initially called 5-ht1Eß receptors) and human tissue (Adham *et al.*, 1993). The 5-HT2 family of receptors has, like the 5-HT1 family, been found to be a heterogeneous family. The initial work by Peroutka and Snyder (1979) has been expanded on considerably and a number of radioligands with affinity for the 5-HT2 (now called the 5-HT2A receptor, Humphrey *et al.*, 1993)

have been identified but at present the most selective radioligand is ketanserin (Leysen *et al.,* 1982; see also Hoyer *et al.,* 1994; Humphrey *et al.,* 1993). Cloning of the stomach fundus receptor (5-HT2B) (Foguet *et al.,* 1992; Kursar *et al.,* 1992) clarified that this receptor was indeed a distinct receptor sub-type (see Hoyer *et al.,* 1994). The 5-HT1C (now 5-HT2C, see later) was initially characterised following the selectivity in autoradiographic studies of tritiated lysergic-acid-di-ethylamide (LSD), 5-HT and mesulergine but not other 5-HT2 ligands (Meibach *et al.,* 1980; Pazos *et al.,* 1984). However, Humphrey and colleagues (1993) laid down new criteria for the definition of 5-HT receptors based on operational, structural and transductional data, at this point the classical 5-HT1C receptor was re-named as the 5-HT2C receptor, based on signal transduction data (see later, *table* 1.2.3) and the classical 5-HT2 receptor was re-named as the 5-HT2A receptor. The existence of a fourth serotonergic receptor family has been reported, with the existence of the 5-HT4 receptor in mouse and guinea pig brain and ileum (Dumuis *et al.,* 1988, 1989a and b) and more recently specific agents have become available to study this receptor (Gale *et al.,* 1994). 5-ht5 receptors cannot at this point be fully characterised since their transduction mechanisms are unknown (Hoyer *et al.,* 1994), but two genes encoding for two sub-types (5-ht5A and 5-ht5B) have been isolated (Plassat *et al.,* 1992; Matthes *et al.,* 1993; Erlander *et al.,* 1993). Receptor genes have recently been reported to encode for a receptor protein which has tentatively been called the 5-ht6 receptor (Monsma *et al.,* 1993; Ruat *et al.,* 1993a). Receptor genes for the 5-ht7 receptor have been cloned (Bard *et al.,* 1993; Lovenberg *et al.,* 1993a; Plassat *et al.,* 1993; Ruat *et al.,* 1993b; Meyerhof *et al.,* 1993; Shen *et al.,* 1993).

Receptor	Effector Pathway	References (*eg.*)	Central location (*eg.*)
5-HT1A	+ cAMP + K^+ channel	Devivo and Maayani; 1985, 1986; Weiss *et al.*, 1986 Andrade *et al.*, 1986	Septum, hippocampus, raphe nuclei
5-HT1B	+ cAMP	Bouhelal *et al.*, 1988	S. nigra, globus pallidus
5-HT1D	+ cAMP	Hoyer and Schoeffter, 1988; Schoefter *et al.*, 1988	Substantia nigra, globus pallidus
5-ht1E	+ cAMP	McAllister *et al.*, 1992; Levy *et al.*, 1992a	Cortex
5-ht1F	+ cAMP	Amlaiky *et al.*, 1992; Adham *et al.*, 1993	DRN, hippocampus, striatum, cortex
5-HT2A	IP3/DAG	Conn and Sanders-Bush, 1984, 1985	Cortex, limbic areas
5-HT2B	IP3/DAG	Foguet *et al.*, 1992a; Kursar *et al.*, 1992	Amygdala, cerebellum, cortex ?
5-HT2C	IP3/DAG	Conn *et al.*, 1986; Conn and Sanders-Bush, 1986	Choroid plexus, limbic areas
5-HT3	Ligand gated ion channel	Derkach *et al.*, 1989	Brainstem, cortex, hippocampus
5-HT4	- cAMP	Hoyer *et al.*, 1994 (review)	Olfactory tuburcles, nucleus accumbens
5-ht5	?	-	cortex
5-ht6	- cAMP	Monsma *et al.*, 1993; Ruat *et al.*, 1993a	Striatum, cortex
5-ht7	- cAMP	Lovenberg *et al.*, 1993a; Plassat *et al.*, 1993	Hypothalamus, amygdala, thalamus, *etc.*

Table 1.2.3. Demonstrating the effector pathways of and locations of 5-HT receptors. + cAMP, inhibition of adenylyl cyclase; - cAMP induction of adenylyl cyclase and IP3/DAG, stimulation of inositolphosphates via phospholipase C. The 5-ht5 transduction system has not yet been isolated. A full list of areas and citations are given in the text.

2.4 Central Distribution of 5-HT Receptors

A brief summary is displayed in *table* 1.2.3, above.

It would appear from autoradiographic studies that there is a very heterogeneous distribution of serotonin receptors in the brain. The 5-HT1A receptor is most concentrated in the septum and hippocampus, the dorsal raphe nucleus is also twice as densely populated as the median raphe and the amygdala (Marcinkiewicz *et al.*, 1984; Pazos *et al.*, 1987a; Hoyer, 1986a). 5-HT1B receptors are primarily localised in the basal ganglia with the substantia nigra pars reticulata displaying high concentrations (Pazos and Palacios, 1985). More recently studies have indicated that the distribution of 5-HT1B sites is similar to that of 5-HT1D sites, being located in the substantia nigra, globus pallidus, dorsal subiculum and superior colliculi (Segu *et al.*, 1991; Boulengeuez *et al.*, 1992; Palacios *et al.*, 1992; Waeber *et al.*, 1988). The 5-ht1E receptor has been found in cortical homogenates (Leonhardt *et al.*, 1989). 5-ht1F receptor expression has been indicated in the DRN, hippocampus, striatum and cortex (Adham *et al.*, 1993; Amlaiky *et al.*, 1992; Lovenberg *et al.*, 1993b). 5-HT2A receptors have been detected in a variety of areas and were found in high density in the cortex such as layers I and IV (rat) and III and V (human) of the neocortex, in the limbic system (especially the olfactory nuclei), parts of the basal ganglia, the pyriform cortex and the claustrum (Hoyer *et al.*, 1986b; Pazos *et al.*, 1987b). The 5-HT2B receptor has not been found in the rat brain (Kursar *et al.*, 1992). However, 5-HT2B mRNA has been isolated (Flanigan *et al.*, 1995, in press) along with the 5-HT2B protein in the medial amygdala, Purkinje cells in the cerebellum, frontal cortex, hippocampus and septum (Duxon *et al.*, 1995). High densities of 5-HT2C binding sites have been identified in the choroid plexus (Meibach *et al.*, 1980; Pazos *et al.*, 1984), in lower densities in the limbic system and areas associated with locomotor behaviour (Pazos and Palacios, 1985), in high densities in the globus pallidus and substantia nigra in humans (Pazos *et al.*, 1987a) and 5-HT2C mRNA has been isolated in the olfactory nucleus, cingulate cortex, lateral habenula and subthalamic nucleus (Mengod *et al.*, 1990a). 5-HT3 receptors have been detected in areas of the lower brainstem and the spinal cord in high concentrations (Hamon *et al.*, 1989; Pratt *et al.*, 1990), cortical areas, the hippocampus, the amygdala, the median habenula (Kilpatrick *et al.*, 1987; Jones *et al.*, 1992) and the area postrema (Jones *et al.*, 1992; Barnes *et al.*, 1990). 5-HT4 receptors have been reported in the rat and guinea pig olfactory tubercles, nucleus accumbens, globus pallidus, septum, sustantia nigra, superior colliculus, hippocampus, interpenduncular nucleus (Grossman *et al.*, 1993) (although the distribution was not homogeneous in the two species) and human cerebral cortex (Monferini *et al.*, 1993). The 5-ht5A receptor mRNA was present in the cerebral cortex, hippocampus, habenula, olfactory bulb and the granular layer of

the cerebellum, and CA1 field of the hippocampus (Plassat *et al.,* 1992). The location of the 5-ht6 receptor is currently unknown but mRNA was found in the striatum, olfactory tubercle, cerebral, cortex and hippocampus (Ruat *et al.,* 1993a; or Monsma *et al.,* 1993). 5-ht7 receptors are located in the hypothalumus, thalamic nuclei, CA1-CA3 regions of the hippocampus, the neocortex, the cerebellum, the medial amygdala, dentate gyrus, entorhinal cortex, substantia nigra, superior colliculus, lateral septum, medial hypothalamic nuclei, pallidum, DRN and suprachiasmatic nucleus (Lovenberg *et al.,* 1993b; Plassat *et al.,* 1993; Ruat *et al.,* 1993b; Shen *et al.,* 1993; Tsou *et al.,* 1994; To *et al.,* 1995).

2.5 Behavioural Effects of 5-HT

Following stimulation of central serotonergic mechanisms a series of behaviours have been reported which are known as the "5-HT behavioural syndrome" (Jacobs, 1976). The syndrome includes resting tremor, forepaw treading, flattened body posture, head weaving, hind limb abduction, Straub tail and head shakes (HS). Certain features of this syndrome appear to be connected with particular 5-HT receptor sub-types. For example, the HS response has been blocked by 5-HT2A antagonists (Colpaert and Janssen, 1983a; Leysen, 1983; Peroutka *et al.,* 1981; Yap and Taylor, 1983) and is almost certainly due to activation of 5-HT2A receptors (Kennett and Curzon, 1991; Kennett *et al.,* 1994a). HS may also be modulated by 5-HT1A agonists (*eg.* Arnt and Hyttel, 1989; Goodwin *et al.,* 1986; Dursun and Handley, 1993; Schreiber *et al.,* 1995). Hypothermia was seen in mice and rats following administration with 5-HT1A agonists (*eg.* Bill *et al.,* 1991), but this response appears to be mediated at postsynaptic 5-HT1A sites in rats and at autoreceptors in the mouse (Bill *et al.,* 1991). Hyperphagia was demonstrated after administration of 5-HT1A agonists and these effects were probably due to their action at somatodendritic autoreceptors (Dourish, 1992). 5-HT1A activation facilitates seminal emissions and ejaculations (Kwang *et al.,* 1986).

The ear scratch response in mice has been reported as a behavioural correlate of 5-HT2A receptor activation (Heaton and Handley, 1989; Darmani *et al.,* 1990). 5-HT2C receptors have attracted considerable interest, since 5-HT2C antagonists have given an anxiolytic profile in anxiety models (*eg.* Kennett *et al.,* 1994a; Kennett, 1992; Kennett *et al.,* 1989). Conversely 5-HT2C agonists are anxiogenic (*eg.* Kennett *et al.,* 1989; Kennett *et al.,* 1994a and b). Agonist administration also induces hypolocomotion and hypophagia (*eg.* Kennett and Curzon, 1988; Kennett *et al.,* 1994; Lucki *et al.,* 1984) and such effects were antagonised by antagonists displaying 5-HT2C receptor antagonist properties (metergoline, mianserin, cyproheptadine and SB200646A) (Kennett *et al.,* 1994a; Kennett and Curzon, 1988).

3. 5-HT and Human Disease States

Central serotonergic neurotransmission dysfunction has been suggested as underlying the aetiology of a vast number of psychiatric illnesses including generalised anxiety disorder, obsessive compulsive disorder (OCD) (Pauls *et al.*, 1990), depression and GTS (Schweitzer and Friedhoff, 1988; Comings, 1990a), but the role of 5-HT in such disorders still remains unclear. This section is intended as an overview and a preliminary guide to indicate in which psychiatric conditions perturbation of 5-HT is thought to play a role, owing to the multiplicity of literature available on each of the subjects covered.

Affective and personality disorders . Reduction in cerebro-spinal-fluid (CSF) 5-hydroxy-indole-acetic acid (5-HIAA) has been found in a number of studies carried out on depressed patients (Siever *et al.*, 1991), but this reduction has been suggested to be caused by a sub-group of patients (Gibbons and Davis, 1986). Reduced CSF levels of 5-HIAA have been suggested to occur in a population of depressives who attempt suicide by self-aggressive means (Asberg *et al.*, 1976, 1987; Agren *et al.*, 1986; Banki *et al.*, 1981; Stanley and Stanley, 1990) and moderate increases in levels of this major metabolite of 5-HT occurr on recovery from depression (Traskman-Benz *et al.*, 1984).

Plasma tryptophan levels, an index of the availability of tryptophan which may be converted into 5-HT, and hence a measure of central 5-HT activity, were reduced in studies of depressed patients (Meltzer and Lowy, 1987), exhibiting evidence for a serotonergic underactivity in depression.

CSF 5-HIAA has also been shown to be diminished in patients showing high aggression scores - results which were correlated with a history of attempted suicide (Brown *et al.*, 1979). Later Brown *et al.* (1982) demonstrated a negative correlation between 5HIAA in the CSF and a life history of physical aggression and psychopathic deviance. Data which was confirmed by increases in plasma tryptophan levels in alcoholics with a history of aggressive or suicidal behaviour (Branchey *et al.*, 1984).

Anxiety disorders. A recent review on the subject eloquently discusses the role of 5-HT and 5-HT receptors in anxiety models (Handley, 1995). A hypothesis was presented that 5-HT systems rather than acting as input or output channels for brain aversive systems provide information regarding the arousal level of the organism which is crucial for the response to a threatening stimuli. Different terminal regions may make use of this information in different ways, such that higher centres appear to facilitate information processing, wheras brainstem centres may provide restraint (Handley, 1995).

The involvement of 5-HT in panic disorder has been suggested. Treatment with m-chlorophenylpiperazine (mCPP) induced panic and anxiogenic effects in patients

with panic disorder and normal controls (Charney *et al* ., 1987). Orally, however, a dose of 0.50 mg/kg was not found to be anxiogenic in normal subjects (Muellar *et al.*, 1986; Zohar *et al.*, 1987; Kahn *et al.*, 1990), but at an even lower dose (0.25mg/kg p.o) it was found to be anxiogenic in panic disorder patients but not normal controls (Kahn *et al.*, 1988). Fenfluramine, a 5-HT releasing agent, has been found to be anxiogenic and panic inducing and augmented the cortisol and prolactin levels in female patients with panic disorder (Targum and Marshall, 1990). Administration of mCPP augmented cortisol release only in patients with panic disorder (Kahn *et al.* 1988) but at a higher dose mCPP induced cortisol release in both patients and controls (Charney *et al.*, 1987). Tryptophan challenge, however, caused no change in prolactin responses in patients with panic disorder (Charney and Henninger, 1986). The fact that fenfluramine and mCPP produce augmented behavioural responses in panic disorder patients but tryptophan does not has been postulated as representing a presynaptic 5-HT deficit (Kahn and Van Praag, 1988, 1990).

Evidence for a dysfunction of serotonergic systems in obsessive-compulsive-disorder (OCD) is also apparent. Kahn *et al.* (1988) demonstrated amelioration of both obsessional and compulsive behaviours in placebo controlled studies using clomipramine (a selective 5-HT reuptake inhibitor). Compounds having less efficacy for 5-HT reuptake such as: desmethylimipramine (Zohar and Insel, 1987); nortriptyline (Thoren *et al.*, 1980) and amitriptyline (Ananth *et al.*,1981) were without effect. In double blind studies clomipramine significantly reduced symptoms to a greater extent than the noradrenaline reuptake inhibitor desipramine (Leonard *et al.*, 1988; Zohar and Insel, 1987). Furthermore, since a relationship was observed between clinical improvement and clomipramine levels and not levels of its noradrenergic metabolite desmethylclomipramine, its therapeutic effect is most likely due to its action on 5-HT and not the nor-noradrenergic systems (Stern *et al.*, 1980; Insel *et al.*, 1983). Fluvoxamine, a selective serotonin re-uptake inhibitor, has beeen used in the treatment of OCD (Goodman *et al.*, 1989; Perse *et al.*, 1987) and provides further evidence to the under-utilisation of 5-HT in OCD . Handley and McBlane (1991) proposed that OCD might be a "failure of disengagement" of serotonergic systems but that this might be due to reduced serotonergic function.

Schizophrenia. There is growing evidence suggesting a role of the serotonergic system in the pathogenesis of schizophrenia. The selective 5-HT2A/2C antagonist ritanserin reduces the negative symptoms in schizophrenic patients either alone or with neuroleptic treatment (Ceulemans *et al.*, 1985a; Gelders *et al.*, 1986). Mixed dopamine D2/5-HT2A antagonists which have *in vivo* binding affinities for the D2 receptor 20-fold those for the 5-HT2 receptor also yield a reduction (Ceulemans *et al.*, 1985a; Gelders *et al.*, 1986). Cyproheptadine, a mixed 5-HT1/2 antagonist, has

proved effective at alleviating both negative and positive symptoms in an open pilot study (Silver *et al.* 1989). Clozapine, a potent 5-HT2 antagonist (but non-selective), has proven to be effective in schizophrenic patients refractory to treatment (Kane *et al.,* 1988). Its is more efficacious than conventional neuroleptics in treating non-refractory patients (Fischer-Cornelssen and Ferner, 1976; Shopsin *et al.* 1979; Leon, 1979). These effects observed with clozapine would appear to be independent of any action of clozapine on dopamine (DA) receptors and an mCPP probe for adrenocorticotrophic hormone (ACTH) in drug free patients who responded to clozapine (*cf.* non-responders) revealed an augmented ACTH response in clozapine responders indicating an increased 5-HT receptor function (probably via 5-HT2A and/or 5-HT2C receptors) (Kahn *et al.*, 1993). Binding studies in the pre-frontal cortex of post-mortem schizophrenics has demonstrated that 5-HT1A receptor binding was increased and 5-HT2A binding decreased (Hashimoto *et al.,* 1993).

Serotonin has also been implicated in various other psychiatric conditions. For example, the importance of 5-HT in the regulation of dietary intake has been reported by Garattini and Samanin (1976). Interestingly bulimia has been treated using a 5-HT1A agonist SM-3997 (Tamai *et al.,* 1990).

An involvement of serotonin has been reported in a number of patients with movement disorders such as Parkinson's disease, tardive dyskinesia, Huntingdon's chorea, myoclonus and dystonia (Sandyk and Fisher, 1988). In GTS, the most severe of the tic disorders, the direct involvement of serotonergic systems has been suggested (Crosley, 1979; Shapiro and Shapiro, 1981; Van Woert *et al.,* 1982; Cohen *et al.,* 1984; Caine, 1985; Robertson, 1989; Comings, 1990a; Singer and Walkup, 1991; see later), but this is an area which requires much more research.

4. Human Tic Disorders

The first clear reports of GTS date from 1825 when Itard reported the case of a French noblewoman, the Marquis de Dampierre, who was reported to have developed GTS symptoms at the age of seven. The socially unacceptable nature of her vocalisations (copralalia at times) compelled her to live her life as a recluse until her death.

The first clear description of GTS was 9 documented patients with multiple tics, involuntary movements, copralalia and echolalia (copying speech) described by de la Tourette (1885).

4.1 Diagnosis of GTS and other tic disorders

Transient Tic Disorder. The diagnostic criteria laid down by DSM-III-R (1987) for Transient Tic Disorder (or habit tics, acute simple tics):

1. Single or multiple and/or vocal tics.

2. The tics occur many times a day, nearly every day for at least two weeks, for no longer than twelve consecutive months.

3. No history of Tourette's or Chronic Motor or Vocal tic disorder.

4. Onset before age of twenty-one.

5. Occurrence not exclusively during psychoactive substance intoxication, known central nervous system disease, such as Huntingdon's chorea or post-viral encephalitis.

The average occurrence is about 5-24% in school children (for review see Shapiro *et al.,* 1988) and is the most common and mildest form of tic disorders. This disorder usually arises in childhood and is typically characterised by motor tics such as eye blinking or facial movements.

If motor or phonic tics are present for more than twelve months then a diagnosis of chronic multiple tic disorder can be made. Tics fall into two categories, all motor or, less commonly solely vocal. Evidence suggests that chronic multiple tic disorder may be a milder form of GTS and that both are familial (Golden, 1978; Kurlan *et al.,* 1987; Pauls *et al.,* 1990). The criteria laid down for the chronic multiple tic disorder are:

1. Either motor or vocal tics, but not both, have been present at some time during the illness.

2. The tics occur many times a day, nearly every day, or intermittently throughout a period of one year.

3. Onset before the age of twenty-one.

4. Occurrence not exclusively during psychoactive substance intoxication or known central nervous system disease, such as Huntingdon's chorea and post-viral encephalitis.

GTS is the most severe form of tic disorders and again the diagnostic criteria are those of criteria laid down by DSM-III- R (1987):

1. Both multiple motor and one or more vocal tics have been present at some time during the illness, although not necessarily concurrently.

2. The tics occur many times a day (usually in bouts), nearly every day or intermittently throughout a period of more than one year.

3 The anatomic location, number, frequency, complexity and severity of the

4. Onset before age 21. *

5. Occurrence not exclusively during psychoactive substance intoxication or known central nervous system disease, such as Huntingdon's chorea and post viral encephalitis.

* The median age of onset is given as 7 years and symptoms must be present for a duration of at least one year.

However, a number of different classifications have been suggested by individual investigators (Singer and Walkup, 1991). The classification which Singer and Walkup (1991) use is the one adapted by the Tourette Syndrome Group and endorsed by the Tourette Syndrome Association (Kurlan, 1989). This classification divides disorders into either "transient" or "chronic" depending on whether there is a history of tics for either less than or more than one year (respectively). Further subdivision also takes place within the chronic category into chronic single tic disorder or chronic multiple tic disorder (based on the presence of either single or multiple tics, respectively), and further whether the tics are motor, phonic or a combination of both (GTS).

Singer and Walkup (1991) believe that tic disorders represent a "clinical spectrum" ranging from mild transient tic disorders to full blown GTS.

Although GTS criteria includes onset of before age 21- a few cases have been reported of adult onset. For example, Fliman *et al.* (1991) have reported a late-adult-onset of a 66 year old suffering from GTS symptoms. Other diagnostic criteria include waxing and waning of the course of the disease; the gradual replacement of old symptoms with new ones and the absence of other medical explanations for tics. Singer and Walkup (1991) emphasise that diagnosis is not dependent on coprolalia or echolalia (in contrast to de la Tourette's original description, 1885), or intellectual deterioration. Similarly palilalia and echokinesis (imitation of another's movement) are confirmatory but not essential diagnostic findings (Singer and Walkup, 1991). Tics are usually first apparent as a simple eye blink, facial grimace, or head twitch in about 50% of patients (Singer and Walkup, 1991). Apparently and wholly inappropriately, patients are often referred to ophthalmologists because of abnormal blink patterns.

To be absolutely certain of a diagnosis of GTS at least one vocalisation must be apparent (DSM-III-R; Singer and Walkup, 1991). These vocalisations may be present as throat clearing, grunting, barking or coughing, all symptoms which may sometimes be mis-interpreted as representing allergic, bronchial or sinus disease, or less commonly as attention-getting behaviour (Singer and Walkup, 1991).

GTS was originally thought to be a life long disease, but evidence suggests that it may not be. Estimations suggest that of all the children with GTS in 30-40% of the cases all the tic symptoms will disappear by late adolescence, in another 30% tics diminish markedly and the remaining patients will have symptoms which persist into adulthood (Erenburg *et al,*. 1987; Golden, 1987).

A currently accepted figure is of the order of about 0.5 patients per thousand (Bruun, 1984), but Robertson (1989) believed this figure to be a gross underestimation. Indeed the prevalence was reported as 1 in 169 (Comings, 1990b). In support of this high incidence Kurlan and colleagues (1987) suggested

that many cases of GTS may be mild and do not come to medical attention, a reason for apparent underestimation of the prevalence of GTS.

Evidence indicates a genetic predisposition for GTS but the genetics do seem to be unclear (Zausmer and Dewey, 1987) as sporadic cases occur (Baraister, 1982). Numerous genetic studies have been carried out and it has been indicated that family members of GTS sufferers may present motor, vocal (or both) or GTS symptoms. Chronic multiple tic disorder and GTS may be genetically related (Robertson, 1989). The pattern of inheritance of GTS has been proposed as fitting an autosomal dominant pattern of inheritance (*eg.* Devor, 1984; Price *et al.,* 1984; Pauls and Leckman, 1986; Kurlan *et al.,* 1986,1987; Robertson and Gourdie, 1990). In the latter study the autosomal dominant pattern is only evident when chronic multiple tic symptoms and obsessive compulsive behaviours are included in the phenotypical analysis, and even then penetrance is incomplete. Comings and Comings (1986) suggested the possibility of an X-related modifying gene to account for the increased incidence in males.

One study looked at blood serotonin and tryptophan levels in the patients with GTS and found a significant decrease in 5-HT : platelet ratio and in tryptophan (no difference was found in parents with and without the symptom) (Comings, 1990a and b). He summarised that the low serum 5-HT and tryptophan levels in GTS were consistent the gene encoding for tryptophan oxygenase as possibly being defective in GTS. However, tryptophan levels remained unchanged in fasting GTS patients (Dursun *et al.,* 1994), but serum kynurenine was elevated. Comings and Comings (1992) have proposed that the primary genetic defect in GTS may lie on the tryptophan oxygenase gene and that this affects 5-HT metabolism and that secondary modifying gene (such as the DA D2 allele) might be mutated such that both genes are necessary for the expression of GTS symptoms. Comings and colleagues (1993) have suggested that there may be a mutation on the D3 receptor gene, which, when homozygous and present with a mutation of the D2 receptor gene may reveal the symptoms of GTS. However, more recently Robertson and colleagues have proposed that the tryptophan oxygenase gene is normal in GTS sufferers (Robertson, 1995 in press).

5. Clinical Features of human tics

The age of onset of GTS ranges from 2-15 years with a mean of seven years being most commonly reported (Robertson, 1989). In 96% of cases symptoms have appeared by the age of eleven years, the most frequent initial symptom being tics involving the eyes in 38-59% of cases; for summary see *table* 1.4.1 and cumulative lifetime symptoms are displayed in *table* 1.4.2.

Table 1.4.1. Showing initial symptoms of GTS

Initial Symptom	**Range (%)**	**Incidence (%)**	**Reference**
Eye blinking	38-59	36	Lees *et al.*, 1984
		48	Comings& Comings,1985
Head and face			
Vocalisations	12-37		
a/Throat clearing		Most frequent	Comings& Comings,1985
			Regeur *et al.*, 1986
b/Coprolalia	2-6		
c/Mouth opening		7.6	Comings & Comings,1986

Table 1.4.2. Showing the cumulative lifetime tic symptoms (Robertson, 1989).

Symptom (tics)	**Percentage**
Face	94-97
Head, neck & shoulder	89-92
Upper arms	51-81
Legs	40-55
Body	41-54

Comings and Comings (1985) described the most common tic of the head and neck as "hair out of the eye tic ", licking has been described in 20% and spitting in 9% of patients (Regeur *et al.*, 1986; Robertson *et al.*, 1988). In about 50% of patients there are more complicated movements, for example stamping or uncontrollable touching of people or objects (Shapiro *et al.*, 1978; Lees *et al.*, 1984; Shapiro *et al.*, 1973). For a review see *table* 1.4.3 below.

Table 1.4.3. Showing development of complex movements in GTS patients (reviewed by Robertson, 1989).

Tic	**Percentage**
Touching	38-61
Hitting or striking	35-36
Jumping	28
Smelling of the hands	12
Smelling objects	11
Stamping	9
Squatting	7
Complexities of gait:	
a/Retracting steps	5
b/Twirling	5
c/Deep knee bends	4
Complicated movements:	7-11 (of all patients)
a/Complicated hand movements	61
b/Complicated foot movements	27

In most cases the onset of vocalisations usually occurs later than that of the motor tics (mean age 11 years). The usual utterances being: grunting, coughing, throat clearing, barking, snorting, explosive noises, screaming, word accentuation, humming, hissing, clicking, colloquial emotional exclamations, low and high pitched noises and inarticulate sounds (Robertson, 1989). Robertson *et al.* (1988) reported the mean cumulative number of vocalisations as 4.8 per person.

Coprolalia was first described by de la Tourette (1885), but does not occur in all GTS patients and is not a diagnostic criterion. Coprolalia usually has a mean onset of 13-14.5 years and later disappears in up to a third of patients. In 18-21% of samples in the U.K. copropraxia (inappropriate and obscene gestures) has been demonstrated, the most common gesture being the "V-sign" (Lees *et al.*, 1984; Robertson *et al.*, 1988).

GTS symptoms appear to be aggravated by anxiety, stress, boredom, fatigue and excitement. Stress has been proposed to be a precipitating factor of the illness (Robertson, 1989) and chronic stress is present in some sufferers (Eisenberg *et al.*, 1959; Stevens, 1964; Faux, 1966). However, sleep, alcohol, orgasm, fever, relaxation or concentration on an enjoyable task usually lead to a transient disappearance of symptoms (Robertson, 1989). Tics are still present during sleep (Glaze *et al.*, 1982; Incagnoli and Kane, 1983).

6. Pathophysiology of GTS

Dysfunction of central neurotransmitter systems has been suggested in GTS based upon the response of patients to specific medications, studies of neurotransmitter metabolites in the CSF and analysis of post-mortem brain tissue. So far dopaminergic, serotonergic, noradrenergic gamma-aminobutyric acid (GABA) and opioid systems have shown abnormalities (see Singer and Walkup, 1991).

The DAergic hypothesis, which proposes a DAergic over-activity as underlying the symptomatology seen in GTS, is an area which appears to have received the greatest amount support thus far. The non-specific dopamine D2 receptor antagonists such as haloperidol, fluphenazine and pimozide suppress motor and phonic tics in most GTS patients (Jankovic, 1984).

The dopamine hypothesis would suggest that there is an excessive amount of DA or an increased sensitivity to the neurotransmitter. A significant elevation in the number of dopamine uptake carrier sites has been identified in the striatum (Singer *et al.,* 1990), but the concentration of 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were normal in the 3 subjects studied suggesting that there was an increase in the number of DA terminals. DA levels, although reduced, remained significantly unchanged in other post-mortem studies (Anderson *et al.,* 1992) and tyrosine hydroxylase activity to be normal in GTS patients in all but the anterior lateral thalamus (Anderson *et al.,* 1992), suggesting that DA overproduction does not underly the symptomatology of the disease, furthermore it would suggest that the synthesis modulating autoreceptor functions normally. Normal HVA levels were found (Sweet *et al.,* 1976; Anderson *et al.,* 1992) but this is in contrast to the diminished CSF HVA levels found in other studies (Butler *et al.,* 1979; Singer *et al.,* 1982). Schweitzer and Friedhoff, 1988 suggested that the reduction in DAergic turnover might reflect a compensatory effect of DA over activity. Positron Emission Tomography scans using [^{11}C]N-methylspiperone have found an increased density for striatal DA D2 receptors (Wong *et al.,* 1989). However, no mention of the drug naiveté of the patients was given, indeed if the patients were receiving dopamine D2 antagonists such results would be expected, D2 density remained unchanged in un-medicated GTS patients (Brooks *et al.,* 1992) or was low (10.5% of patients), normal (47%), elevated (31%) or very elevated (31%) in 19 patients, 15 of which were un-medicated (Singer *et al.,* 1992). Moreover, no difference was observed between D1 and D2 receptor binding in the post-mortem caudate and putamen of GTS sufferers *cf.* control membranes (Singer *et al.* 1990).

Evidence for the involvement of noradrenergic mechanisms is limited and is based on the efficacy of clonidine (*eg.* Goetz *et al.,* 1987; Goetz, 1992). Urinary

excretion of noradrenergic metabolites may be reduced, (Baker *et al.,* 1991) but levels remain unchanged in CSF (Butler *et al.,* 1979) as do noradrenaline (NA) concentrations in all but the ventral lateral thalamus where they were reduced (Anderson *et al.,* 1992). Lower 5-HIAA concentrations have been revealed in the CSF (Cohen *et al.,* 1979; Butler *et al.,* 1979). More recently reduced 5-HT concentrations in 11 of 13 brain areas studied was found (although significance was not reached) but tryptophan and 5-HIAA concentrations were significantly reduced (Anderson *et al.,* 1992). The same group displayed a reduction in 5-HT and 5-HIAA concentrations in cortical regions of post-mortem GTS patients (primary motor cortex, auditory association cortex, middle and temporal inferior gyri and the cingulate gyrus) along with an increase in 5-HT1A receptor binding in all but the auditory association cortex (maximal changes were found in the middle temporal gyrus (161% of control)), 5-HT2A receptor binding was increased most in the primary motor cortex (131%) (Akbari *et al.,* 1993).

Comings (1990a) demonstrated lower levels of blood tryptophan (and 5-HT platelet binding) in GTS patients. A genetic defect in tryptophan oxygenase was suggested, resulting in higher levels of synthesis or hyper-inducibility of the liver and/or the brain enzyme. This would lead to an alteration of blood kynurenine concentration, which would tend to increase (Comings, 1990a). This would correlate with lower tryptophan and 5-HT levels, as a greater proportion of tryptophan would be diverted to kynurenine production. Indeed increased kynurenine concentrations have been detected, but corresponding tryptophan concentrations remained unchanged (Dursun *et al.,* 1994). More recently Comings and Comings (1992) have proposed that the primary genetic defect in GTS (see earlier) may lie on the tryptophan oxygenase gene and that this affects 5-HT metabolism and that secondary modifying gene (such as the DA D2 allele), may cause the symptoms observed in GTS. However this has not been supported by a more recent study (Robertson, 1995 personal communication). Comings and Comings (1987) suggested that the reductions in 5-HT (as in DA) turnover might reflect a compensatory mechanism of the disease. Treatment with GABA agonists has proved inconsistent (Gonce and Barbeau, 1977; Shapiro *et al.,* 1981; Shapiro and Shapiro, 1981). Furthermore, glutamate decarboxylase (a highly specific presynaptic marker for GABAergic interneurones in the cerebral cortex) remains normal in post-mortem brain studies (Singer *et al.,* 1984) as are GABA levels in CSF, whole blood and sub-cortical areas (Van Woert *et al.,* 1982; Anderson *et al.,* 1992).

Glutamine concentrations were reduced in the substantia nigra pars reticulata, lateral and medial globus pallidus as were glycine concentrations in the substantia nigra pars reticulata, aspartate was unchanged (Singer *et al.,* 1992).

Increased CSF dynorphin-A (1-8) concentrations has been demonstrated (Leckman *et al.*, 1988) along with decreased dynorphin-A (1-17) (Haber *et al.*, 1986). Increased plasma alpha-melanocyte-stimulating-hormone, along with decreased luteinising hormone concentrations have been found (Sandyk , 1989).

It has also been proposed that infection with ß-haemolytic streptococci causes an immune-reaction which might lead to the production of anti-neuronal antibodies producing the symptoms GTS (Swedo *et al.*, 1994).

In summary it would appear the reported abnormalities are complex. However, many of the studies conducted have not yet been reproduced and have commonly been conducted with small sample numbers and the choice of controls has left something to be desired.

7. Animal Models of GTS

The study of GTS in animals is somewhat difficult as no validated animal models have been shown to exist. In order to validate an animal model one must provide face (similarities between the disease state and the model including pharmacological considerations), construct (theoretical) and predictive (the prediction of drug efficacies in the clinic based on the animal model) validity before such model may be accepted as modelling a human disease state (Abramson and Seligman, 1977; McKinney and Bunney, 1969).

Currently construct validity cannot be assessed for a model of GTS since the cause of GTS is not fully understood (Handley and Dursun, 1992). Predictive validity has not yet been carried out to any great extent.

Most approaches in the past have been based on construct validity, such that a hyperdopaminergic state was sought due to the reported efficacy of neuroleptics in GTS. Hyperdopaminergia was induced by administration of L-3,4-dihydroxytryptamine (L-DOPA) (Knott and Hutson, 1982; Schweitzer and Friedhoff, 1988) or dopaminergic super-sensitivity following 6-hydroxydopamine (Friedhoff, 1982; Shaywitz *et al.*, 1982; Weiner *et al.*, 1982). However, such models have not included the production of tics such as those observed in GTS (Handley and Dursun, 1992). Tic like movements have been modelled in isolated mice who displayed head shakes (and other behavioural changes) (Essman and Essman, 1982). Iminodiproprionitrile induced a series of behavioural stereotypies including tic like head jerks (Diamond *et al.*, 1982). Such 'models' display face validity for GTS. The tail pinch test has also been proposed (Knott and Hutson, 1982). However, Handley and Dursun (1992) have proposed that tic like behaviours may model GTS, since they display face validity for the tics observed in GTS.

Phenomenological similarity between GTS tics and tic like movements in animals

Schelkunov *et al.* (1986) proposed that phenomenological analysis of the movements seen in GTS patients reveal similarities in movements between GTS patients and certain features of the serotonergic syndrome such as those seen with large doses of monoamine oxidase inhibitors, 5-hydroxytryptophan (5-HTP) or by 5-HT releasers such as fenfluramine or combinations of such drugs (*eg.* Corne *et al.*, 1983; Green and Grahame-Smith, 1976: Jacobs *et al.*, 1982). Such serotonergic movements have been described as relatively simple, abrupt, rapid and swift which agrees with DSM-III-R (1987) criteria for GTS (Handley and Dursun, 1992) and in so being are distinct from those observed after amphetamine or apomorphine administration which have been described as smooth and well co-ordinated movement sequences. Such dopaminergic stereotypies as induced by apomorphine or amphetamine are perhaps a better model for some of the associated features of GTS, such as kicking, touching, slapping, auto-aggressive acts and vocalisations (Schelkunov *et al.*, 1986).

Perhaps the most tantalising of these "serotonergic" models is the head shake tic (HS) first described in mice by Corne *et al.* (1963) and proposed as a model for the study of central 5-HT activity. The relevance to GTS patients is that they bear a somewhat similar HS. Administration of the 5-HT precursor 5-HTP produces this HS response in both mice (Corne *et al,* 1963) and rats (Matthews and Smith, 1980) through a central action on 5-HT receptors (Nakamura and Fukushima, 1978; Matthews and Smith, 1980). 5-HTP or citalopram (a 5-HT re-uptake inhibitor) administration induced a shudder of the head and trunk which resembled the shaking behaviour observed in dogs when wet and has so been termed the "wet-dog shake" (WDS) (Bedard and Pycock, 1977; Arnt *et al.*, 1984) and may be a more fully expressed version of the HS response (Wilkinson and Dourish, 1991). WDS may represent a model for GTS since they may be comparable with the shoulder shrugs and trunk/shoulder rotations observed in GTS patients (Handley and Dursun, 1992) and see earlier.

Head shakes and wet-dog-shakes as models for GTS

Amphetamine along with other stimulants has been shown to exacerbate GTS symptoms (Erenberg *et al.*, 1986). However, Corne *et al.*, (1963) reported that high doses of amphetamine attenuated the 5-HTP HS but sub-chronic administration of amphetamine sensitised mice to the 5-HTP HS (Karler *et al.*, 1990). Low doses of amphetamine can potentiate the 5-HT2A/2C agonist-induced 1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane (DOI) induced HS but antagonism is seen at higher doses (Dursun and Handley, unpublished).

5-HTP. Van Woert *et al.* (1982) found that 5-HTP plus carbidopa had no consistent effect on tics or vocalisations. However, five of the nine patients with attention deficit disorder symptoms developed exacerbation of their symptoms. Other behaviours such as generalised hyperactivity, floor pacing and aggressiveness increased. Head banging, emotional irritability, agitation, anxiety and rapid speech were also worsened. Although administration of 5-HTP to one patient has been reported to be effective in reducing tics and lip biting (Shapiro and Shapiro, 1981).

5-HT agonists. One patient was successfully treated with LSD (Smith, 1969), data which would seem to contrast with HS data but only after chronic treatment, thus suggesting receptor down-regulation. Of 25 patients seen by Shapiro some patients reported benefit, but others did not (Shapiro and Shapiro, 1981). However, the HS is induced by LSD (Corne and Pickering, 1967) and also by other 5-HT2A agonists: quipazine (Mallick *et al.,* 1977), 5-methoxy-dimethyl tryptamine (5-MeODMT) (Friedman and Dallob, 1979) and the selective 5-HT2A/2C agonist DOI (Ogren and Fuxe, 1979). The 5-HT1A agonist buspirone has been used to treat GTS (Dursun *et al.,* 1995; Burke *et al.,* 1995) which correlates with the action of this and other 5-HT1A agonists to inhibit HS in mice (*eg.* Dursun *et al.,* 1993 and see later).

5-HT antagonists. Treatment with serotonergic antagonists has been sparse which is surprising. Two GTS patients were treated with the non-selective antagonist methysergide (Shapiro *et al.,* 1978). Similarly the 5-HT antagonists metergoline, cinanserin, cyproheptadine, methysergide, 2-bromo-LSD and ritanserin attenuate the HS response (Peroutka *et al.,* 1981; Corne *et al.,* 1963; Colpaert and Janssen, 1983a; Green *et al.,* 1983; Goodwin and Green, 1985). Data for the attenuation of the HS behaviour is also very closely correlated with the affinity of these compounds for 5-HT2A receptors (Peroutka *et al.,* 1981; Niemegers *et al.,* 1983; Lucki *et al.,* 1984; Kennett and Curzon, 1991). WDS were attenuated by methysergide, metergoline, cyproheptidine, ketanserin, pirenperone and spiperone (*eg.* Matthews and Smith, 1980; Yap and Taylor, 1983; Skingle *et al.,* 1991).

Neuroleptics. The first choice medication in the treatment of GTS is haloperidol and non-responders are given pimozide (Shapiro and Shapiro, 1981). However, at the doses required there can be a rejection rate of up to 80% (Robertson, 1989). Sulpiride was suggested as a possible alternative to haloperidol (Robertson *et al.,* 1990). In animals tic reduction of HS behaviours (both serotonin and spontaneous) and WDS has been found (Schelkunov, 1979; Matthews and Smith, 1980; Arnt *et al.,* 1984; Dursun and Handley, 1992; Handley and Singh, 1986a). However their potency to inhibit tics in animals is correlated to their potency at 5-HT2A and alpha-1 sites (Dursun, 1992).

Clonidine. In GTS administration of clonidine has provided clinicians with a useful alternative to haloperidol-non-responders or for patients who cannot tolerate the side effects of haloperidol (Cohen *et al.,* 1979; Bruun, 1984). Clonidine and other alpha-2 agonists attenuate spontaneous and drug induced HS in mice (Handley and Brown, 1982; Bednarczyk and Vetulani, 1978; Handley and Singh, 1986a; Dursun and Handley, 1992a) and antagonists potentiate them (Handley and Brown, 1982; Heal *et al.,* 1986).

Calcium antagonists. Nifedipine has been useful (Goldstein, 1984; Berg, 1985) in GTS and chronic multiple tic (Walsh *et al.,* 1986) and verapimil and flunarizine (GTS only) have also proven useful (Walsh *et al.,* 1986; Michelli *et al.,* 1990). Such data is consistent with the action of nifedipine on tics in rodents (Green *et al.,* 1990).

Uptake inhibitors. Uptake inhibitors have been used on the basis of their effectiveness in OCD. Desipramine, a tricyclic antidepressant with predominant NAergic reuptake blocking action has been shown to lack efficacy both in GTS (Caine *et al.,* 1979) and against the HS response (Handley and Singh, 1986b). However, in GTS, tricyclics that act exclusively on 5-HT reuptake such as clomipramine have been shown to be useful (Ciprian, 1980; Yaryura-Tobias, 1975; Yaryura-Tobias and Neziroglu, 1977) although Caine *et al.* (1979) demonstrated no effect and even an exacerbation in one case, consistent with the worsening of symptoms reported with fluvoxamine (Riddle *et al.,* 1988). However, the amelioration of symptoms (non-significantly) has been noted with fluvoxamine (George *et al.,* 1993). Fluoxetine is either ineffective, worsens or improves symptoms (Riddle *et al.,* 1988). In the above studies small n-numbers were used. Chronic citalopram in mice initially exacerbated spontaneous HS, a subsequent reduction was observed and after 20 days HS count returned to basal levels (Handley and Dursun, 1992).

Lithium. Lithium treatment has been suggested as effective (Messiha *et al.,* 1976) after 3 weeks of treatment and follow up indicated improvement after 3.5 months, but others have not demonstrated significant therapeutic effect (Shapiro and Shapiro, 1981). Other authors have supported the amelioration, noted a lack of effect or seen an exacerbation (Erickson *et al.,* 1977; Borison *et al.,* 1982; Yassa and Ananth, 1980). It appears that the beneficial effects (if seen at all) appear after long term treatment (Messiha, 1988). In pre-clinical studies lithium induced shaking behaviour at high doses (*eg.* 200mg/kg) (see Handley and Singh, 1986a).

Withdrawal from morphine is also capable of inducing shaking behaviour, effects which may be antagonised by ritanserin (Handley *et al.* 1986) and similarly opiate withdrawal may unmask GTS symptoms (Lichter *et al.,* 1988).

Kynurenine. Plasma kynurenine is elevated in GTS patients (Dursun *et al.,* 1994) and administration of kynurenine and the low dose of the kynurenine pathway metabolite 3-hydroxykynurenine caused potentiation of the 5-HTP HS whereas high doses inhibited HS (Handley and Miskin, 1977). Moreover, kynurenine also potentiated the DOI HS (McCreary and Handley, 1995) (data also presented here).
Bliss *et al.* (1980) by way of a patient's attempt to describe GTS from a patient's point of view indicated the presence of a sensory pre-cursor. Sensory processing is implicated in shaking behaviour. Hallucinogens and other shake inducers, when given to man (in sufficient doses) may cause sensory disturbances as a sensory side effect (Handley and Dursun, 1992). In mice the 5-HTP HS is inhibited by distracting stimuli and local anaesthesia to the pinna (Boulton and Handley, 1973).

Other evidence for the involvement of 5-HT in GTS

Knott and Hutson (1982) in proposing a possible animal model for GTS (the tail-pinch test) commented "that an animal model of GTS is of necessity limited, in as much as the verbal component is often a major characteristic of the disorder." Thus it would seem that an animal model which demonstrated both components - a vocal tic (which are frequently present with motor tics (Ludlow *et al.,* 1982)) and a motor tic would offer an excellent phenomenological similar model for GTS. Up until recently this was not possible but administration of RX336-M to mice provided such a model. The compound induced a complex of simultaneous tic-like movements consisting of body-jerk or HS together with one or more back muscle contractions (BMC), a fore-paw movement and a tail "wriggle" which coincided with single squeak vocalisations, which was antagonised by the 5-HT2A/2C antagonists ritanserin, ICI169,369 and the D2 antagonist haloperidol (Dursun and Handley, 1994; Dursun, 1992).

8. TRH and Tic Like Movements

TRH and its analogues also induce a series of rapid-phasic-movements (Dursun and Handley, 1991; Fone *et al.,* 1987,1988, 1989a and b) which have been described as relatively simple, abrupt, swift and arrhythmic which agrees with DSM-III-R (1987) criteria for GTS (Handley and Dursun, 1992).
Administration of TRH-amide to mice induced a behavioural profile consisting of eye blinking, Straub-tail, tail tremor, fore-paw-tremor (FPT), scratching and head shaking (Dursun and Handley, 1991), which were attenuated with ritanserin. Haloperidol only antagonised Straub-tail, tail tremor, scratching and HS (but to a lesser extent than ritanserin). Blinking and scratching behaviour were also found to occur with a bursting nature which has also been found to be the case with blinking in GTS patients (Dursun, Rickards and Handley, unpublished observation). In GTS patients blinking is one of the first symptoms observed (Bonnett, 1982).

Pretreatment with phenoxybenzamine (non-selective alpha-adrenoceptor antagonist) and prazosin (alpha-1antagonist) blocked WDS and FPL elicited by CG3509 whereas idazoxan (alpha-2-antagonist) (Fone *et al.*, 1987). Clonidine potently attenuated WDS behaviour and FPL induced by CG3509 was not significantly affected but a biphasic effect was observed with 0.2 and 0.5 μg intra-thecal (i.t.) diminishing FPL and 2.0μg of clonidine potentiating it, but none of these effects reached significance. The actions of clonidine on WDS are consistent with its actions in GTS (see above).

Similar behaviours have also been demonstrated in rats by other authors. For example, Heal *et al.* (1981) demonstrated WDS by central (nucleus accumbens) administration of TRH or the analogues CG3509 or CG3703 and WDS were also evident by peripheral administration of CG3509 or CG3703. Administration of the TRH analogues RX77368, CG3509, CG3703 induced FPL and WDS behaviours, which were antagonised by prazosin (Johnson *et al.*, 1989). Fone *et al.* (1989a) demonstrated that i.t. administration of the TRH analogue CG3509 produced WDS and FPL which were antagonised by pretreatment with ritanserin. Jackson (1990) also demonstrated the induction of WDS with RX77368 in rat pups.

WDS and FPL behaviours were described following administration of the TRH analogue MK771 (Simaska and Horita, 1985). With chronic treatment of MK771 tolerance to WDS occurred which was paralleled by a decrease in TRH receptor binding. Pranzatelli (1988) compared the behaviours elicited by MK771 and 5-HTP. MK771 and 5-HTP evoked reciprocal fore-paw tapping, Straub-tail, hunching, hind limb abduction and shaking behaviour. MK771 elicited both HS and WDS; ipsapirone, 8-OH DPAT and pirenperone all antagonised the WDS behaviour, consistent with the actions of 5-HT1A agonists and 5-HT2 antagonists (such as methysergide) in GTS (see above).

BMC appear to fit the DSM-III-R (1987) criteria for tic like movements in being rapid, recurrent and stereotypic in nature and would seem like a possible model for GTS. I.t. administration of 5-HT2 agonists (2,5-dimethoxy-alpha,4-dimethylbenzene ethamine hydrchloride (DOM) and 5-MeODMT induced BMC which were attenuated by ritanserin. BMC in response to prior treatment with DOM were enhanced following intrathecal administration of 5,7-dihydroxytryptamine (Fone *et al.*, 1989a). However, an inhibitory tone is thought to be exerted by 5-HT2C receptors (Fone and Sharma, 1993).

In conclusion TRH or TRH analogue-induced tics may offer a useful model for the study of tics observed in GTS, especially blinking given its phenomenological similarity to blinking tics observed in GTS.

9. The Pharmacology of TRH

Quite apart from the actions of TRH in the hypothalamo-pituitary axis on the stimulation of thyroid-stimulating-hormone TRH seems to play a role in central neurotransmission / neuromodulation.

TRH is co-localised with 5-HT and substance-P in the spinal cord and parts of the brain. For example, TRH-like immunoreactivity has been isolated in the raphe pallidus, obscurus and magnus where it is co-localised with 5-HT, substance-P or both (Johansson *et al.,* 1981; Johannson *et al.,* 1983; Sasek *et al.,* 1990). In the spinal cord 5-HT, substance-P and TRH showed very similar patterns of distribution in the ventral horn and a very similar pattern of depletion after treatment with the serotonergic neurotoxin 5,7-dihydroxytryptamine. In the dorsal spinal cord TRH-like immunoreactivity was restricted to areas adjacent to the central canal which, along with 5-HT were depleted after 5,7-dihydroxytryptamine. Neurotransmitter levels were higher in the lumbar spinal cord than the cervical cord and TRH, 5-HT and substance-P were depleted following 5,7-dihydroxytryptamine in the ventral lumbar cord but not dorsal (Gilbert *et al.,* 1982; Lighton *et al.,* 1984). Harkness and Brownfield (1986) have reported that TRH is located in GABA interneurones, which is consistent with the co-localisation of TRH with GABA in dorsal cord neurones (Fleming and Todd, 1994).

TRH has been localised in a number of discrete extra-hypothalamic brain regions (*eg.* raphe nuclei (above), striatum, septum, nucleus accumbens, hippocampus, occipital cortex, median eminence amygdala and pyriform nucleus), however the levels in the median and dorsal raphe nuclei, occipital cortex, suprachiasmatic nucleus and hippocampus were relatively low (Johansson *et al.,* 1981; Johannson *et al.,* 1983; Sasek *et al.,* 1990; Lighton *et al.,* 1984; Przegalinski and Jaworska, 1990; Przegalinski *et al.,* 1990).

TRH receptors have also been isolated in a number of regions and show a heterogeneous distribution. The use of an autoradiographical technique verified the presence of TRH receptors in a number of brain areas with differing densities, to briefly summarise: olfactory system (heterogeneous distribution in different nuclei, accessory olfactory bulb enriched, olfactory bulb-low levels), amygdaloid complex (heterogeneous among different nuclei), septum (moderate density), basal ganglia (highest levels in the nucleus accumbens), hippocampus (heterogeneous distribution), thalamus, hypothalamus (heterogeneous distribution), cerebral cortex (relatively low levels, but studies indicated high levels in the guinea pig), mid-brain (heterogeneous distribution), pons/medulla (enriched in motor nuclei and spinal trigeminal), and spinal cord (spinal cord dorsal grey enriched) (Sharif, 1989).

The TRH receptor has been cloned and sequenced from rat (de la Pena *et al.,* 1992; Zhaou *et al.,* 1992) and mouse (Straub *et al.,* 1990) pituitary cells. However, de la Pena and colleagues failed to demonstrate the existence of DNA encoding this

receptor in the CNS, suggesting that central TRH receptors protein differs considerably from that of the pituitary receptors.

In pituitary the TRH receptor is linked to the phospoinositide second messenger system (Gershengorn, 1986), this appears to be linked to either a fast transduction system via Gq/11 G-protein or a slow transduction mechanism (G-protein not-identified) (Brady *et al.*, 1994). The failure to find DNA encoding for the 'pituitary-like' receptor might be indicative of different receptor sub-types. In support of this Funatsu and colleagues (1985) reported multiple binding sites and in spinal-trauma studies only certain analogues were active (Faden *et al.*, 1988).

TRH and TRH-analogues have been demonstrated to have potent effects on behaviour (see above and later). Chronic administration of TRH (10 days) caused an increase in brain stem tryptophan hydroxylase and 5-HT, but not 5-HIAA, at 15 days tryptophan hydroxylase was unaffected and 5-HT and 5-HIAA levels were augmented (Agarwal *et al.*, 1977). In acute studies with the TRH analogue MK771 5-HT and 5-HIAA was increased in the hippocampus, hypothalamus, mid-brain and pons/medulla (Rastogi *et al.*, 1981). Conversely, fenfluramine increased TRH concentrations in the striatum which was blocked by citalopram and metergoline (Przegalinski *et al.*, 1990). In the lumbar spinal cord 5-HT induced TRH release, effects which were attenuated by ketanserin and methysergide, the effects of veratidine were attenuated by 8-hydroxy-2-(di-n-propyl-amino) tetralin (8-OH DPAT), it was therefore suggested that agonist action (of the endogenous agonist - 5-HT) at 5-HT2A receptors promoted the release of TRH and that accumulated 5-HT could reduce TRH release via 5-HT1A receptors (Ono *et al.*, 1991).

Other neurotransmitter systems have been implicated to interact with TRH. For example microdialysis studies have indicated that acetylcholine release was increased in the cortex and hippocampus following TRH and MK771 (Hutson *et al.*, 1990; Giovanini *et al.*, 1991). Similarly, TRH interacts with NA behaviourally (see above) and neurochemically, this has been reviewed extensively elsewhere (Bennett, 1990).

10. Blinking and GTS

Cohen *et al.* (1980) reported an increase in blink rate in a GTS patient which was ameliorated by clonidine. Later Bonnett (1982) in a quantitative study reported an increase in spontaneous blink rates in unmedicated GTS patients of approximately 3 times when compared to controls. In patients medicated with dopamine antagonists the blink rate was attenuated by approximately 50%. An increase in blink rate was also noted as occurring in close relatives of GTS patients. However, any increase was not substantiated in 19 patients (Karson *et al.*, 1985). Schelkunov *et al.* (1986) also noted an increase in GTS patients. Final confirmation of an abnormal blink

reflex in GTS was demonstrated with an increase in duration of the R2 (see later) component of the blink reflex and an abnormality of its recovery cycle. The R1 (see later) component was, however, unaltered (Smith and Lees, 1989).

11. The Pharmacology of Spontaneous Blinking

Dopamine. Stevens (1978) hypothesised that spontaneous blinking is modulated by central DA activity. Indeed, the reduction in blink rate observed in Parkinson's disease is correlated with a depletion of striatal DA (Adams and Victor, 1981). Such a hypothesis appears consistent with animal data in which there has been found to be an increase in blink rate in monkeys after administration of the dopamine agonists apomorphine and bromocriptine (Karson, 1983; Casey *et al.,* 1980). These effects have also been shown to be independent of peripheral action of these drugs and, furthermore, pretreatment with sulpiride attenuated this response (Karson, 1988). Administration of the DA D1 antagonist SKF 38393 was without effect on blinking (Karson, 1989), although he concluded that this may be due to poor brain penetrance of the drug. However, a role for both the D1 and D2 receptor subtypes was indicated (Taylor *et al.,* 1991; Elsworth *et al.,* 1991). Further evidence comes from the study of increased blink rates observed in schizophrenics. However, studies were mainly carried out in patients who had undergone prolonged neuroleptic treatment (Karson *et al.,* 1981, 1982, 1983; Kleinmann *et al.,* 1984; Muesser *et al.,* 1984; Helms and Goodwin, 1985) and long-term neuroleptic therapy could induce post-synaptic DA receptor supersensitivity and thus increase blink rates (Mackay *et al.,* 1982). Moreover, Karson *et al.* (1983) found the highest blink rates in schizophrenics with tardive dyskinesia and since DA receptor supersensitivity is the most widely accepted explanation of tardive dyskinesia (Klawans *et al.,* 1980; Bannet and Belmaker, 1983) it is impossible to say whether the increased blinking is due to schizophrenia or rather a consequence of neuroleptic treatment (Mackert *et al.,* 1991). This incongruity was addressed by Mackert *et al.* (1991) who found a significant increase in blinking in drug naive schizophrenics, which was attenuated with neuroleptic treatment; in the previously neuroleptic treated schizophrenics no attenuation was seen, indeed there was a slight (non-significant) increase. In a review article Karson (1988) hypothesised that "... the failure to associate dyskinesias (except L-DOPA induced dyskinesia) with increased blinking indicates that the pathophysiology of these conditions may not involve hyperactivity of CNS dopamine systems.' Thus, it appears that it would be pertinent to address the possibility of the involvement of other neurotransmitter systems which may be involved in the pharmacology of blinking.

Other Neurotransmitter Systems and Spontaneous Blinking. The alpha-1-agonist phenylephrine, the alpha-2 agonist clonidine and phentolamine (mixed alpha-

adrenergic antagonist) were without effect on blinking; as was LSD (Karson, 1983). However, Dursun and Handley (1991) demonstrated antagonism of TRH-induced blinking with ritanserin but not haloperidol.

In primates diazepam antagonised apomorphine induced blinking (Karson, 1983). Schizophrenics were treated with clonazepam for a few weeks and mean blink rates were attenuated (Karson, 1989). It is possible, though, that sedation may have played a role in the blink suppressing action of these benzodiazepines (Karson, 1989). Valproic acid caused an, albeit non-significant, reduction in spontaneous blink rate.

The cholinergic antagonist atropine dose dependently increased blinking in primates, conversely physostigmine (an acetylcholineesterase inhibitor) decreased blink rate (Karson, 1989; Schelkunov *et al.*, 1986), although this data is difficult to interpret because of the marked autonomic effects of these drugs on the eyes.

12. The Blink Reflex

Two components of the blink reflex have been identified - the R1 and R2 reflexes. Stimulation of supraorbital nerve elicits these two reflexes components (Kugelberg, 1952), an early R1 and late R2 component. The R1 component appears to be a simple pontine pathway (Shahani and Young, 1972) whereas the R2 reflex is a more complex reflex involving the spinal pathway of the trigeminal nerve (Kimura and Lyons, 1972). The locus of direct anatomical control appears to be mediated between the seventh cranial nerve and the rostral medulla (Kimura, 1988).

Discharges of the pontine reticular formation precede each spontaneous blink (Cohen and Feldman, 1968) and it has been suggested that this area may modulate rhythmic brain function (Garcia-Rill and Skinner, 1990).

Cell loss within the substantia nigra correlates with the symptoms observed in Parkinson's disease (Adams and Victor, 1981; Forno, 1981), one of which is reduced blink rate, thus it may be postulated that the substantia nigra facilitates blinking.

Blink rates in progressive supranuclear palsy are lower than that of Parkinson's disease, in which the periaqueductal grey, superior colliculus and pretectal area have all been implicated (Adams and Victor, 1981).

Of these structures it has been hypothesised that the superior colliculus facilitates blinking (Karson, 1989), since ablation of this area in subhuman primates causes a staring behaviour which lasts up to 30 seconds (Brown and Chambers, 1976).

Discharges from the lateral geniculate bodies occur soon after a blink (Cohen and Feldman, 1968).

A case report of a man with blepharospasm associated with lesions of the thalamus may impact blinking (Powers, 1985).

In cerebellectomised rats the blink rate was quadrupled relative to controls (Freed *et al.*, 1981) but the application of such data to humans is questionable because of the relatively low rate of blinks normally observed in rats (Karson *et al.*, 1989). However, a similar increase has been observed in humans with cerebella tumours (Karson *et al.*, 1989). Evidence also indicates a role for cortical structures in blink modulation. Activation of the visual cortex by visual tasks (Phelps and Kuhl, 1981) or targeted saccades (such as reading) (Fox *et al.*, 1985) are also associated with decreased blinking (Karson, 1989). During blinking both visual activity, electrical activity and the activity of the visual cortex are suppressed (Volkman *et al.*, 1979; Buisseret and Maffei, 1982).

Blinks also result in pulsed stimulation of the occipital cortex as indicated by the evoked potentials following each blink (Armington, 1981) but it is not clear whether this is related to the phenomena of blink or eye closure seizures (Darby *et al.*, 1980; Terzano *et al.*, 1983).

13. 5-HT1A antagonists

A selective 5-HT1A agonist has been available as a tool to probe 5-HT1A function for some time now (Gozlan *et al.*, 1983) but until recently selective 5-HT1A silent antagonists have not been available. (A silent antagonist has been described as an antagonist which is devoid of intrinsic agonist activity (Boddeke *et al.*, 1992).)

Research compounds available have either partial agonist profiles or lack specificity for 5-HT1A receptors (see Fletcher *et al.*, 1993a for review). Non-selective ß-adrenoceptor antagonists display antagonist activity at 5-HT1A receptors. For example in post synaptic models of 5-HT1A receptor function ß-blockers have shown efficacy, such that (-)-pindolol antagonised 8-OH DPAT induced-inhibition of adenylate cyclase activity (Schoeffter and Hoyer, 1988), (-)-propranolol, (-)-pindolol and (-)-tertatolol attenuated 8-OH DPAT-induced syndrome behaviours (Millan *et al.*, 1993; Tricklebank *et al.*, 1984) and (-)-penbutalol, (±)-pindolol and (-)-tertatolol antagonised hypothermia in rats following treatment with 5-HT1A agonists (Millan *et al.*, 1993; Hjorth 1992). In somatodendritic autoreceptor models (-)-propranolol, (-)penbutalol, (-)-pindolol, (±)-pindolol and (-)-tertatolol acted as antagonists at the autoreceptor (using electrophysiological and behavioural paradigms) (*eg.* Sharp *et al.*, 1989; Sprouse and Aghajanian, 1986; Hjorth and Sharp, 1993; Hjorth, 1992; Dursun and Handley, 1993; Prisco *et al.*, 1993; Jackson and Nutt, 1992). However, Fornal and colleagues reported a lack of antagonist effect of (-)-propranolol in DRN (Fornal *et al.*, 1994b) and (-)-pindolol has been reported as a partial agonist (Hjorth and Carlsson, 1986). Spiperone was demonstrated as having post-synaptic actions on the behavioural syndrome induced by 8-OH DPAT (ambulation, head weaving, flat body posture and fore-paw-

treading, hypothermia (rat); Tricklebank *et al.*, 1984; Millan *et al.*, 1993) and antagonised the 8-OH DPAT inhibition of adenylate cyclase in the calf hippocampus (Schoeffter and Hoyer, 1988). Spiperone also acts as an antagonist at the somatodendritic autoreceptor (*eg.* Fornal *et al.*, 1994a; Blier *et al.*, 1989 and 1993; Lum and Piercey, 1988; Gobert *et al.*, 1995; Millan *et al.*, 1995). However, spiperone is 165 times more potent at the DA D2 site and 39 times more potent at the 5-HT2A site than the 5-HT1A receptor (Hoyer, 1988; Leysen *et al.*, 1981). A number of compounds display partial agonist efficacy. For example, BMY7378, NAN190 and SDZ216525 all display antagonist actions at postsynaptic receptors (Schoeffter and Hoyer, 1988; Sharp *et al.*, 1990; Hjorth and Sharp, 1990; Przegalinski *et al.*, 1990; Moser, 1991; Claustre *et al.*, 1991; Millan *et al.*, 1992; Schoeffter *et al.*, 1993). However, at the 5-HT1A autoreceptor studies have demonstrated that BMY7378, NAN190 and SDZ216525 act as partial agonists (Millan *et al.*, 1994; Claustre *et al.*, 1991; Sharp *et al.*, 1993; Greuel and Glasser, 1992; Sharp *et al.*, 1990; Gurling *et al.*, 1993; Hjorth and Sharp, 1990; Williams and Dourish, 1992; Mundey *et al.*, 1994a; Fletcher *et al.*, 1993b; Fornal *et al.*, 1994b). However, some authors have hypothesised that such effects may be due antagonist action at the alpha-1 adrenoceptor (Claustre *et al.*, 1991; Gurling *et al.*, 1993). (S)-UH301 was demonstrated as an antagonist at the postsynaptic site (Bjork *et al.*, 1991) and also acts as an antagonist at the somatodendritic autoreceptor (Nomikos *et al.*, 1992). However, the major problem with (S)-UH301 is its lack of selectivity, it is only 8 times more potent for the 5-HT1A receptor than the D2 receptor (Hillver *et al.*, 1990).

More recently a selective 5-HT1A silent antagonist WAY-100135 was described (Cliffe *et al.*, 1993; Fletcher *et al.*, 1993b; Routledge *et al.*, 1993) and later a structural analogue of this compound WAY-100635 (Fletcher *et al.*, 1994).

14. Aims of the Project

The initial aim of the project was to extend on previous work conducted in Dr. Handley's laboratory and assess TRH analogue-induced behaviours in the mouse as possible *in vivo* models for GTS. This included testing a range of serotonergic and dopaminergic agents on TRH analogue (MK771)-induced blinking, fore-paw-licking and fore-paw-tremor. A secondary aim was to explore the pharmacology of MK771-induced blinking. Tic like behaviours were also to be explored in the cat and simultaneous measurements of DRN unit activity were to be recorded. However, this was not feasible so instead the selective 5-HT1A antagonists (S)-WAY-100135 and WAY-100635 were tested for intrinsic activity and ability to antagonise the selective 5-HT1A agonist 8-OH DPAT upon serotonergic unit activity in the DRN of the chronically implanted behaving cat.. These experiments

were conducted in Prof. Barry Jacobs laboratory at Princeton University. The effects of the (S)-WAY-100135 were assessed on the spontaneous and DOI-induced HS response in mice. The effects of kynurenine were assessed on the DOI HS response.

GENERAL METHODS.

EXPERIMENTAL METHODS.

1. Animals, Animal Husbandry and Laboratory Conditions

1.1 Mice

Experiments reported were carried out on Aston-bred male mice of MF1 strain which weighed between 20 and 30g. Subsequent to weaning, mice were kept in groups of 20-30 (from the same birth cohort) in polypropylene cages in the Animal House at an ambient temperature of 21 ± 2°C. These animals were fed on a conventional 41B cube diet (Pilsbury's Ltd., Birmingham) and received tap water *ad libitum.* Mice were transferred to the quiet experimental room (where they were kept in groups of 10) at least 2 days prior to experimentation. The experimental room was maintained at 21 ± 2°C, relative humidity between 50-60% and the animals were exposed to a 12 hour light-dark cycle with the light cycle corresponding to normal daylight hours (lights on 08:00-20:00), and all experimental sessions were carried out between 08:30 and 19: 00 hours.

1.2 Cats

Experiments were carried out on male domestic short-haired cats (Biomedical Associates, Friedensberg, PA, USA, and Liberty Labs, NY, USA) weighing between 3.5-5.4kg. Upon arrival at the laboratory, cats were individually housed in stainless steel cages at an ambient temperature of 22 ± 1°C under incandescent lighting conditions (lights on 07:00-21:00 hours). These animals were fed on a conventional dry diet (Purina cat chow; Ralston-Purina Co., St. Lois, MO) available *ad libitum*, and given treats of tinned cat meat and baby food approximately every other day. They received tap water *ad libitum.* Cats were transferred from the colony to the experimental room (which was maintained at 20 ± 2°C)for screening of microelectrodes and all experimental sessions were conducted during the light portion of the light-dark-cycle.

2. Injection Techniques

2.1 Subcutaneous (s.c.) Injection

Subcutaneous injections were made by insertion of the hypodermic needle under the loose skin at the back of the neck of mice and cats. In experimental sessions where mice received more than one s. c. injection, the second injection was made by inserting the hypodermic needle under the skin in the flank of the animal. The injection volume was 10.0ml/kg for mice and 1.0ml/kg for cats.

2.2 Intraperitoneal (i.p.) Injection

Intraperitoneal injections were made by inserting the hypodermic needle through the abdominal wall pointing towards the diaphragm. Care was taken not to penetrate too deeply and thereby damage 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 10.0 ml/kg for mice and 1.0 ml/kg for cats.

2.3 Oral Administration of Drugs (p.o.)

Mice: administration by this route was carried out using an oral gavage dosing needle (21gauge x 38mm; A.R. Horwell Ltd.). The gavage needles had round bulbous tips to prevent oesophageal damage and were slightly bent to minimise oesophageal tissue damage and the chance of introducing drugs into the lungs.

Cats: oral administration of tablets (lubricated with mineral oil) was carried out using a veterinary tablet dispenser inserted into the mouth and aimed towards the throat. After administration the throat was massaged to encourage swallowing and to ensure that the cat had swallowed the tablet.

2.4 Intravenous (i.v.) Injection

Cats: injections were made remotely from an adjacent room via an infusion line connected to the venous catheter (see later). The infusion line was constructed from Tygon tubing (0.02" internal diameter) with a filling volume of less than 0.5ml. Drug solutions were loaded into the line at the stop cock and then the stop cock was turned "on-to-the animal" (connecting the i.v. line directly to the venous catheter). Prior to drug administration the animal was left undisturbed for approximately 5-10min. for its behaviour to return to baseline. During this period baseline unit discharge activity was recorded. All i.v. injections were made while the cats were in a quiet, but alert behavioural state. Drugs were prepared in an injection volume of 0.05-0.10ml/kg and flushed with 1.5ml of sterile saline over a 3sec. period. Injections of an equivalent volume of sterile saline alone served as controls.

3. General Procedures for the Analysis of MK771 Induced Behaviour in Mice

One hour prior to experimentation, the mouse (acutely isolated) / mice (paired) from the same stock cage were placed in glass aquaria (25x20x20cm) with black fabric floors and partitioned with black card to form small observation chambers (20x9x20cm). Comparisons between paired and acutely isolated mice were carried out since differences in the number of HS have been observed depending on the number of mice placed in the same observation chamber, indeed the largest difference was observed when the number of mice was decreased from two to one (Boulton and Handley, 1973).

The behaviours elicited by the TRH analogue MK771 were all recorded on a video (Panasonic WV-KT 115E linked to a Panasonic Video Cassette Recorder NV-FS90B) and analysed later by means of a programme written for a BBC microcomputer (obtained from Birmingham University department of Psychology, written by Dr. T. Kirkham).

It was not always possible to observe each animal for the full duration of each experiment, since the animal either turned away or was obscured by another mouse. Therefore it was necessary to correct for the time that the animal was not observed or the "time out", this was done by multiplying the count for the particular behaviour by a correction term:

$$\frac{\text{bin duration}}{[\text{bin duration-time out}]}$$

Blinking, FPT and FPL behaviours were counted. Blinking was recorded as a rapid tic like squeezing of the eyes together, unilateral or bilateral blinks were recorded as one blink. FPT was recorded as a rapid vertical movement of the fore-paws and was recorded in bouts as either unilateral or bilateral. FPL was recorded as the tic like licking of the fore-paws and counted in bouts, unilateral or bilateral movements being recorded as one incident. Wherever possible all experiments were conducted with the observer blind to the treatment that each mouse had received.

4. General Procedures for the Analysis of Head Shake in Mice

Groups of 4-6 mice (head twitch frequencies are reduced with fewer than 3 mice per chamber; Boulton and Handley, 1973) from the same stock cage were habituated in small sawdust lined glass aquaria (25x20x20cm) for at least one hour prior to testing drug-induced or spontaneous HS. A HS was defined as a rapid rotational flick of the head.

Experiments were recorded on video tape and analysed later (equipment as above) with the experimenter unaware of the treatment each mouse had received.

5. In vivo Unit Recording of Serotonergic Neurones

Surgical Procedures.

5.1 Preparation of Microelectrodes

Microelectrodes were prepared prior to surgical implantation. Fine wire electrodes consisted of 32 or 64μm diameter Formvar-coated nichrome wire (Stablohm 675, stress relieved, California Fine Wire, Grover City, CA, USA). The impedences of these microwires was typically 200 to 500kΩ measured at 1kHz. Two microwire bundles were used each containing three 32μm and four 64μm microwires. Approximately 3mm of insulation was carefully burnt off from the end of the wire and any residue scraped off with a scalpel blade. The uninsulated portion of each microwire was then soldered to the gold-plated contact pins of a 25-lead connector (Amphenol 17-102500-1(390)). The contact pins were pressed into the connector

using a tool designed for this purpose and the remaining positions of the connector were filled with pins containing solder for the later connection of the gross electrodes during surgery (see later). The microwires were separated into two distinct bundles, and the junction between the microwires and pins were cemented with silicone rubber. On the day of surgery the microwire bundles were soaked in a bactericidal solution (Cetylcide; Cetylite Industries Inc., Pennsauken, NJ) for approximately 5 hours before implantation and were rinsed in sterile saline immediately prior to implantation.

5.2 Surgical implantation of electrodes

Food deprived (16-20 hours) cats were pre-treated with atropine sulphate (0.2mg/kg s.c.) to reduce airway secretions and 30 min later anaesthetised with sodium pentobarbitone (40 mg/kg i.p.). After a sufficient depth of anaesthesia had been reached (*ie.* loss of muscular tension of the limbs and absence of the pedal and corneal reflexes) the cats were treated with Polyflex (ampicillin, 70mg/kg i.m.), the head and neck were shaved, the claws were clipped and the ears were cleaned (to ensure correct placement in the ear bars). The cat was then placed in a stereotaxic frame (Kopf Instruments, CA, USA) and a topical anti-infective (10% pividone-iodine solution; IDE, Plainview, NY) was applied to its head and rinsed 10min. later with 70% ethanol. A midline scalp incision (about 8cm long) was made and the muscles overlying the skull were retracted. The skull was cleaned of all fascia and then thoroughly dried in order for the dental cement to firmly adhere to the bone.

A hole was drilled in the bone overlying the right frontal sinus, the sinal membrane was removed and the cavity was cleaned. A stainless-steel screw electrode was threaded into the retro-orbital bone to record gross eye movement potentials (electrooculogram, EOG). The sinus was filled with an absorbable gelatine sponge (GelFoam; Upjohn, Co., Kalamazoo, MI, USA) prior to cementing with dental acrylic. Another stainless-steel screw electrode was placed into the left frontal sinus to serve as an indifferent electrode for EOG recording. In order to record the electroencephalogram (EEG) a pair of stainless-steel screw electrodes were implanted bilaterally in the skull overlying the parietal cortex. The nuchal electromyogram (EMG) was recorded from the uninsulated tips of two multi-stranded Teflon coated wires which were inserted bilaterally into the dorsal neck muscles. In addition four other stainless-steel screws were placed at the lateral margins of the skull to serve as anchor points for the entire implant assembly.

The microdrive used for chronic single-unit recording consisted of two stainless-steel inner cannulae (23 gauge) which could be lowered through two outer cannulae (19 gauge) (separated by 1.0mm). A small spring around the machine screw

provided tension for the microdrive unit. The microdrive was attached to a stereotaxic carrier via a special holder. Please refer to *figure* 1.

A hole was drilled into the skull and the tips of the inner cannulae of the microdrive were lowered into the brain with the cannulae resting 5mm above the dorsal raphe nucleus (DRN). GelFoam was packed around the cannulae to protect the underlying brain tissue before the hole was sealed and the microdrive secured to the skull with dental cement.

The microelectrode assembly was secured to a stereotaxic carrier, such that the two electrode bundles extended downwards and parallel to each other. The electrode bundles were then cut to the appropriate length with a pair of sharp sterile scissors. The bundles were lowered through each inner cannula into the brain so that the wires protruded 5mm beyond the cannulae tip. The microwires were then secured to the tops of the cannulae with Krazyglue (super glue). The stereotaxic co-ordinates for the anterior bundle were posterior 1.5mm, lateral 0.0mm and horizontal 1.0mm (*cf.* stereotaxic zero), at an angle of 45° posterior to the vertical (Berman, 1968). The electrodes were zeroed on a zeroing bar. (*N.B.* In the brain atlas (Berman, 1968) the plane 10mm to the horizontal interaural plane is defined as the horizontal zero plane and the sagittal zero plane corresponds to the midline). The microwires could then be advanced ventrally through the brain by turning the jeweller's screw on the microdrive.

The gross electrodes were soldered to the contact pins of the Amphenol connector. After the connector was placed in its final desired position, a protective housing was slipped over the microdrive assembly (a cut-down plastic centrifuge tube with a screw lid). The exposed wires were covered with silicone rubber and the entire apparatus was then anchored to the skull with dental cement.

Cats were removed from the stereotaxic apparatus and a Tygon catheter (0.040" internal diameter, 0.070" external diameter) containing heparinized saline (100 U/ml) was passed sub-cutaneously from the scalp incision to an incision made above the right external jugular vein. The catheter was inserted into the vein and advanced to the vena cava. The catheter was secured in place with three ligatures around the vein. The distal end of the catheter which had been previously fitted with a three-way stop-cock, was cemented to the implant head piece. After both incisions were sutured closed, boric acid ointment (10%) was then applied to the incision wounds.

5.3 Post-Operative care

Cats were removed from the operating room and placed in a heated recovery booth until they were fully alert (usually 1-2 days) and treated with Polyflex (amoxycillin, 30 mg i.m.) and topical antibiotic powder (neomycin) was applied to the implant

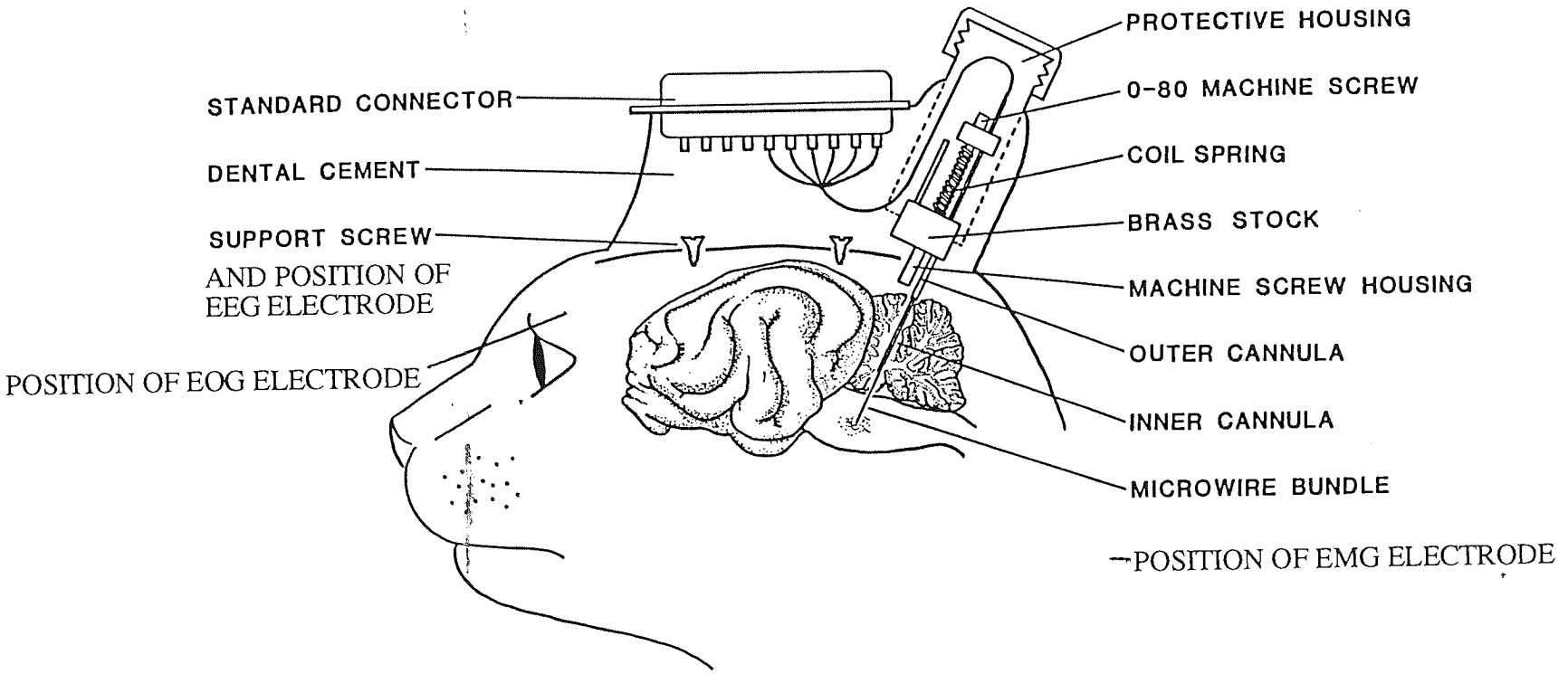


Figure 1. Demonstrating the head-set apparatus used for the recording of serotonergic unit activity.

and i.v. incisions. For the first post-operative week cats were treated with 63.5 mg b.i.d. p.o. of amoxycillin/clavulinic acid. The i.v. catheters were flushed weekly with heparinized saline (1000 U/ml) to maintain catheter patency. Cats received a single dose of antibiotics 3 hours before i.v. lines were flushed. The scalp incision was cleaned every few days with hydrogen peroxide (Consumer Value Store, Princeton, USA) and a topical antibiotic powder (neomycin) was applied around the implant to prevent infection.
Routine screening of serotonergic cells began 1 weak after surgery.

6. Unit Recording

6.1 Equipment Set Up

All experimental sessions were carried out in a double-walled, wooden sound attenuated chamber (65x65x95cm). Electrical shielding in the form of 1/8" copper mesh was present on all sides apart from a Plexiglas (perspex) door fitted to the front of the chamber. Electrical signals were led from the animal by a low noise cable (Malco, South Pasedena, CA, USA) which connected to a 24 channel counter-weighted slip ring assembly (Airflyte Electronics Company, Bayone, NJ, USA). The recording cable passed through an aperture in the roof of the chamber. The slip ring assembly permitted the animal to turn around completely (360°) without twisting the cable connections. A second low noise cable fed signals from the slip ring to an electrode switching panel located in an adjacent room.
Two 23 position selector switches were used to set one microelectrode wire as the active lead and the other as the indifferent lead to a pre-amplifier (Grass 7P511 AC). Microelectrode signals were amplified and filtered (band pass 0.1-3.0 kHz) with the output continually monitored on a storage oscilloscope. Single unit activity was separated from background noise by using a time-amplitude window discriminator (Bak Electronics model DIS-1). The electronic pulse from the discriminator was fed into an electronic spike counter and a polygraph (Grass model 7C). EEG, EMG and EOG gross potentials were amplified (Grass 7P5 or 7P511 AC pre-amp), band pass filtered and recorded on-line on the polygraph. Filter settings were: EEG, 1-35 Hz; EOG, 1-35 Hz and EMG, 30-90 Hz. Only signals displaying a signal-to-noise ratio of 3:1 or better were used for data collection. Unit activity of serotonergic neurones could usually be recorded for several hours and often for several days. Recordings were carried out in the light portion of the light dark cycle and cats were denied access to food and water during recording sessions.
Animal behaviour could be monitored using a closed-circuit television set-up with the camera aimed through the perspex door of the recording chamber.

6.2 Recording Procedure

During the post-operative care period (1weak), cats were habituated to the recording booth by placing them in the booth for 10-15min. four to five times daily and thereafter routinely screened around 4 times daily, during wakefulness. This screening procedure entailed connecting the cat to the recording cable, and then using the selector switches to examine the electrical activity of each wire. Following no sign of recordable activity on any of the leads, the microdrive jeweller's screw was turned 1/4 of a turn which advanced the microwires 75μm deeper into the brain (one complete turn=approximately 300μm). If single unit activity was present but the signal-to-noise ratio was too low, the microdrive was turned in either direction (*ie.* downwards or upwards) in order to improve the recording quality. Following each screening, animals were returned to their home cages, this procedure was then repeated at 2 hour intervals 2 to 4 times daily, until isolated unit activity was found. The 2 hour delay between screenings allowed "floating" microelectrodes to stabilise in their new positions within the brain.

6.3 Neuronal Identification

Single unit activity in the DRN was established as being serotonergic (as opposed being of another neuronal type) during quiet waking using previously established criteria (Fornal and Jacobs, 1988) *viz.* 1., slow and highly regular discharge activity (around 1-4 spikes/sec); 2., biphasic action potentials with a relatively long duration (≥ 2 msec) and 3., complete, or near complete, suppression of activity during REM sleep. Neurones were further characterised by pharmacological challenge with 8-OH DPAT (5-40 μg/kg i.v.), a selective 5-HT1A agonist which potently and completely inhibits the discharge of central serotonergic neurones by an action at the somatodendritic autoreceptor (Sprouse and Aghajanian, 1986).

6.4 Histological Verification of Recording Sites

The cats were anaesthetised with sodium pentobarbitone (50.0 mg/kg i.p.) and direct anodal current was passed through the microelectrode (20μA for 20 sec) to mark the sites from which neuronal recordings were obtained. Cats were then perfused intracardially with 0.9%, saline followed by 10% formalin and then 5% potassium ferocyanide in 10% stock formalin. The reasons for this were to 1., fix all the brain tissue and to 2., produce a Prussian blue reaction at all electrically marked sites. Brains were removed and stored in 10% formalin until 50μm thick coronal sections were cut through frozen samples of mid-brain. All sections were mounted and stained with neutral red and the recording sites (blue spots) were located using a microscope. Only cells found to be located within the DRN were included in this analysis.

6.5 Identification of Behavioural State

Following isolation of a presumed serotonergic neurone cats were allowed to go through at least one sleep-wake-cycle. This was done in order to characterise the discharge activity of the cell across different behavioural states. A unique characteristic of serotonergic neurones in behaving animals is that their activity is strongly state-dependent. Firing rates are reduced during slow-wave-sleep (SWS) and fall silent or near silent during REM (see Fornal and Jacobs, 1988; Jacobs and Fornal, 1993). The cessation of unit activity during rapid-eye-movement (REM) sleep serves as an important criterion for identifying serotonergic neurones in freely moving animals. Active waking (AW) was characterised by a low voltage fast (desynchronised) EEG, phasic EMG bursts superimposed on high amplitude tonic EMG, frequent (>20 per minute) and large (>400μV) eye-movement potentials. Quiet waking (QW) was characterised by a desynchronised EEG, absence of phasic EMG bursts and diminished overall EMG, absence of large EOG potentials with intermittent EOG potentials (<400μV) and no gross body movements (*eg.* sitting or lying with eyes open). SWS was characterised by a large amplitude (200-500μV) synchronous EEG with sleep spindles present, diminished tonic EMG, eyes closed but with small eye movement potentials present and a recumbent posture. REM sleep was characterised by intermittent EOG potentials, lack EMG potentials, a low voltage fast EEG and a stretched out recumbent posture.

All drug studies (Chapter 5) were conducted in QW.

6.6 Data Handling

Firing rate data were obtained and, where individual drug trials were carried out, comparisons were made within the same behavioural states (QW, Chapter 5), in order to control for the influence of behavioural state (since unit activity is behavioural state dependent (see above and Fornal and Jacobs, 1988; Jacobs and Fornal, 1993).

Firing rates were calculated from successive 10 second epochs over the minute prior to administration of drugs and at predetermined time points following administration of drugs. Percentage changes in firing rates were determined by comparing changes in firing rate with baseline firing rate (using the 1 min time scale). Please refer also to Chapter 5.

7. Drug, reagent sources and vehicles

7.1 Drugs

Augmentin	IDE
Atropine	IDE
Boric acid ointment (10%)	IDE

Buspirone	Sigma
DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane	RBI
(±)-8-OH DPAT (8-hydroxy-2-(di-n-propyl-amino) tetralin	RBI
Heparin	IDE
Ketanserin tartrate	Janssen
Kynurenine	Sigma
Mianserin	BDH
MK771(l-pyro-2-aminoadipyl-L-histidyl -L-thiazoloidine-4 carboxamide))	MSD
Prazosin	Sigma
pCPA (DL-p-chlorophenylalanine methyl ester)	Sigma
Raclopride	Astra
Ritanserin	Janssen
SB200646A (N-(1-methyl-5-indolyl)-N'-(3-pyridyl) urea hydrocloride)	SKB
(±) SCH23390 ((+)-7-chloro-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1H)-3-benzazepine maleate	RBI
SCH39166 ((-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro -2-hydroxy-N-methyl-5-H-benzo[2,1b]azepine)	Scherring Plough
Sodium pentobarbitone	IDE
± sulpiride	RBI
(S)-WAY100135 (N-tert-butyl-3-(-4(2-methoxyphenyl) piperazin-1-yl-2- phenylpropionamide dihydrochloride)	Wyeth
WAY-100635 (N-(2-(4-(2-methoxyphenyl-1-piperezinyl) ethyl)-N-(2-pyridinyl) cyclohexane carboxamide trihydrocloride)	Wyeth

The drugs were dissolved in saline (0.9% sodium chloride) (only sterile for i.v. administration to cats) with the following exceptions:
Ritanserin and prazosin were dissolved in 0.9% saline with the aid of 0.1M hydrochloric acid and the pH adjusted to 7.0 by addition of 0.1M sodium hydroxide.
SB200646A was ground into 1% methyl cellulose in 0.9% saline (w/v) with a drop of BRIJ 35 (Kennett *et al.*, 1994a).

7.2 List of reagents *et cetera*.

BRIJ 35	Sigma
Cetylcide	Cetylite Industries
Ethanol	IDE

GelFoam	Upjohn
Hydrochloric acid (analar)	BDH
Hydrogen peroxide	CVS
Metyhyl cellulose	Sigma
Pividone-iodine	IDE
Saline (0.9% sterile)	IDE
Sodium hydroxide (granular)	BDH
Sodium chloride (Analar)	BDH

CHAPTER 1.

CHARACTERISATION OF MK771-INDUCED BEHAVIOURS IN MICE.

CHAPTER 1.

Introduction

Administration of TRH and its analogues haS been shown to induce a wide variety of behavioural consequences (see General Introduction). For example, TRH and the TRH analogues induce blinking, WDS, HS, FPT, FPL tail-elevation and tremor *etc.* (see Introduction). It is thought that this group of behaviours might model the symptoms GTS (Handley and Dursun, 1992).

The aims of the experiments presented in this chapter are: to characterise the behaviours induced by the peripherally active TRH analogue MK771 (Veber *et al.*, 1976), to develop reproducible methods for ascertaining the frequency of these behaviours in relationship to rapid-phasic-movements, assess the time-course of drug effects and therefore find a suitable time-window for analysis of behaviours, analyse the population distribution of the frequencies of these behaviours and assess whether the number of mice per observation chamber alters the results since Boulton and Handley (1973) demonstrated that the HS response was dependent on the number of mice present in the observation chamber. Initially the aims were focused on FPL and blinking behaviours, but as studies progressed it became evident that the quantification of fore-paw-tremor (FPT) behaviour was possible and this behaviour was included in subsequent studies.

Additional Methods

Procedures for the Analysis of MK771 Induced Behaviours in Mice

Saline (10.0ml/kg) or MK771 (5.0mg/kg i.p.) was administered to either acutely isolated or paired mice (20-30g, 6 mice/treatment group) and their blink rates and FPL behaviour counted in 5min. bins for 30min. following injection.

Using the data above totals, time course and differences between paired and isolated mice could be analysed.

Dose Response Relationship

Using the technique described above, dose response relationships for blinking and FPL were ascertained in paired animals (20-30g), 2mice /treatment group) for a 5min. time period 7.5min. after treatment with MK771 (0.25 - 60.0mg/kg i.p.). Each mouse received only one injection of MK771.

Population Distribution

Values for blinking (n=152), FPT (n=146) and FPL (n=139) were taken from Saline+MK771 (2.5mg/kg i.p.) treated drug groups (positive controls - see subsequent Chapters) and tested for normality using the Minitab statistical package for the PC. Normality was checked by using a correlation test for normality whereby normal score values were correlated with the counts obtained for blinking,

FPT and FPL; significance of the correlation was calculated using statistical tables (Minitab Reference Manual - Release 8, 1991, pp. 4.8).
Correlations of blinking, FPT and FPL were performed with each other in order to establish whether each of these behaviours were related to each other.
Statistical analyses: MK771 treated animals were compared with vehicle treated mice using an un-paired t-test. Behavioural scores (population distribution data) on the three behavioural variables were correlated (Minitab - Pearson's correlation) with each other to ascertain if there was any relationship between blinking, FPT and FPL.

Results

Behaviours Elicited by MK771

Administration of MK771 to mice produced a behavioural syndrome consisting of: blinking (unilateral and bilateral, which were not differentiated on counting), FPL, FPT, tail elevation and tremor along with rapid vertical movements of the tail which appeared to be due to contractions of the abdominal musculature, ear scratch and infrequent back muscle contractions, which were more apparent as a twitch of back than the characteristic contractions observed in rats (see later).

Procedures for the Analysis of MK771 Induced Behaviours in Mice

Totals. MK771 significantly induced blinking which was equal in magnitude in paired and acutely isolated mice (*figure* 1.1). FPL, however, was higher in acutely isolated mice than in paired mice (*figure* 1.2), but this did not reach significance.
Time course. MK771 induced significant increases in blinking in paired mice each of the 6 5 minute bins tested and peaked in the 2nd and 3rd bins (5-10min and 10-15min following injection) (*figure* 1.3). In acutely isolated mice significance from vehicle controls was also seen at all time points, but peaked in the 1st and 2nd bins (*figure* 1.4). FPL was significantly increased in paired animals by prior treatment with MK771 in bins 2 and 3 (*figure* 1.5), but significance was only reached in bin 6 in acutely isolated mice (*figure* 1.6).

Dose Response Relationship

Dose-response for MK771-induced blinking revealed a bell-shaped dose response curve (fitted using a third order polynomial model on Cricket Graph (for the Apple Macintosh)) (ED_{50}= 1.72 [1.38-3.63 (95%confident limits)] for linear portion of curve, obtained using a program written for the Northstar Computer (*figure* 1.7).
FPL did not appear to be related to dose of MK771 (*figure* 1.8).

Population Distribution

Blinking: was a normally distributed population (*figure* 1.9).

FPT: was a normally distributed population (*figure* 1.10).

FPL: had a truncated normal distribution (*figure* 1.11).

Correlation coefficients for the normal score against the number of counts for each behaviour are shown in *table* 1.1, along with the significance level for the population fitting a normal distribution (Minitab Reference handbook, 1991).

BEHAVIOUR	CORR. COEFF.	SIGNIFICANCE
Blinking	0.995	$p<0.01$
FPT	0.992	$p<0.01$
FPL	0.934	NS

Table 1.1. Showing the correlation coefficient for the normal scores against the counts for each behaviour.

Statistical analyses

MK771 induced blinking, FPT and FPL showed no correlation with each other (*table* 1.2). It must be remembered that FPL and FPT are mutually exclusive.

	BLINKING	FPL
FPL	-0.048 (NS)	-
FPT	-0.065 (NS)	0.006 (NS)

Table 1.2. showing the correlation coefficients (r) between blinking, FPT and FPL in Saline+MK771(2.5mg/kg i.p.) treated mice, (NS= not significant).

Discussion

MK771 induced a behavioural profile consisting of blinking (unilateral and bilateral, which were not differentiated on counting), FPT, FPL, tail elevation and tremor (with Straub-tail in the proximal two-thirds of the tail), ear scratch, infrequent back-muscle-contractions. Very few HS were seen. Blinking and FPT were normally distributed, while FPL showed a truncated normal distribution and the behaviours appeared not to be related to each other.

The behavioural profile of MK771 in mice is consistent with that seen by other researchers. FPL has been observed following the administration of TRH and the TRH analogues RX77368, CG3509 and CG3703 (Fone *et al.*, 1987; 1988; 1989a

and b; Johnson *et al.*, 1989; Jackson, 1990). FPT has been demonstrated by many authors (Simasko and Horita, 1985; Yarborough *et al.*, 1978; Wei *et al.*, 1975; Costall *et al.*, 1979; Fone *et al.*, 1987), although only one study quantified this behaviour, but this was by means of a rating scale and FPT was not discriminated from FPL, (Dursun and Handley, 1991; Dursun, 1992). Tail elevation and tremor have been noted following TRH or the TRH analogues CG3509, CG3703 or RX77368 (Dursun and Handley, 1991; Fone *et al.*, 1987; Johnson *et al.*, 1989). Scratching behaviour has also been noted (Dursun and Handley, 1991; Costall *et al.*, 1979), but in the studies presented here MK771-induced scratching appeared to peak at around the 30 minute mark (slower than the time-course for MK771-induced blinking and FPL). BMC have been observed in rats following the administration of 5-HT2 agonists (Fone *et al.*, 1989a), but those observed here in mice following MK771 were far more difficult to see than those in rats, and were not of the characteristic type observed in response to administration of 5-HT2 agonists. Administration of TRH and the TRH analogues CG3509, CG3703, RX77368 and MK771 induced WDS and HS behaviours in rats (Johnson *et al.*, 1989; Fone *et al.*, 1988; Fone *et al.*, 1987; Fone *et al.*, 1989a and b; Simasko and Horita, 1985; Yamada *et al.*, 1984; Heal *et al.*, 1981), but in the studies presented here MK771 appeared to be without effect on HS frequency in mice, a reason for this is that no more than two animals were placed in the observation chambers and the HS frequency is diminished if fewer than three mice are present in an observation chamber (Boulton and Handley, 1973).

The number of blinks induced by MK771 was equal in both paired and acutely isolated animals (*figure* 1.1), although the number of FPL bouts appeared to be lower in acutely isolated mice this was not significant (*figure* 1.2), but not significantly. The time course for MK771-induced blinking was not dissimilar in paired and acutely isolated mice (*figures* 1.3 and 1.4), peaking within 10min. in both cases. The time course for FPL in acutely isolated mice, although similar to paired mice, only reached significance from vehicle treated mice in the final (6th) bin, but was significantly different from vehicle treated mice in bins 2 and 3 in paired animals. The standard-error-of-the-mean in acutely isolated *cf.* paired mice was greater indicating a greater variation in the population. For the above reasons, and expediency in conducting experiments, it was decided that future experiments would be carried out on paired mice and that the 7.5-12.5 minute time window (following i.p. administration of MK771) would be used for the analysis of blinking and FPL. The reductions in HS frequency when the number of mice was decreased from 2-1 per observation chamber observed by Boulton and Handley (1973), was not mirrored in the FPL and blinking behaviours induced by MK771,

although the time course and variability for FPL may have been altered in acutely isolated mice.

Dose response analysis revealed a bell-shaped dose response curve for MK771 induced blinking with an ED_{50} value for the linear portion of the curve of 1.72 [1.38-3.63], subsequent experiments were therefore carried out using a dose of 2.5mg/kg i.p. of MK771.

MK771-induced FPL was not dose-related (*figure* 1.8) which is consistent with a previous report indicating that CG3509-induced FPL was not dose related (Johnson *et al.,* 1989). However, CG3703 and RX77368-induced FPL tended to increase with dose (Johnson *et al.,* 1989).

Analysis of the population distribution for MK771-induced blinking and FPT revealed normal distributions, meaning statistical analysis of these behaviours could be carried out using parametric statistics. Analysis of the distribution for MK771-induced FPL, however, showed this behaviour to be a normal distribution which is truncated at zero, but with a non-significant correlation between the normal scores and the number of bouts of FPL (correlation co-efficient 0.934, *table* 1.1). This suggests that non-parametric statistics should be used for the analysis of MK771-induced FPL, however some authors suggest that if data is not-far from normally distributed that parametric statistics can still be used (Winer, 1971; Linton and Gallo, 1975; personal communication to ACM from Ann Spooner, Statistics Lecturer, Aston University 21/2/95).

The behaviours tested showed no relationship to each other (using Pearsons correlation coefficients *table* 1.3). This suggests that MK771 might be acting at different terminal fields *ie.* different populations of TRH receptors to induce each of the behaviours analysed.

In conclusion it has been demonstrated that the peripherally active TRH analogue MK771 induces a variety of tic like behaviours in mice. Methods have been developed to quantify these behaviours into a reproducible form and criteria have been established for the future use of the blinking, FPT and FPL paradigms in pharmacological studies.

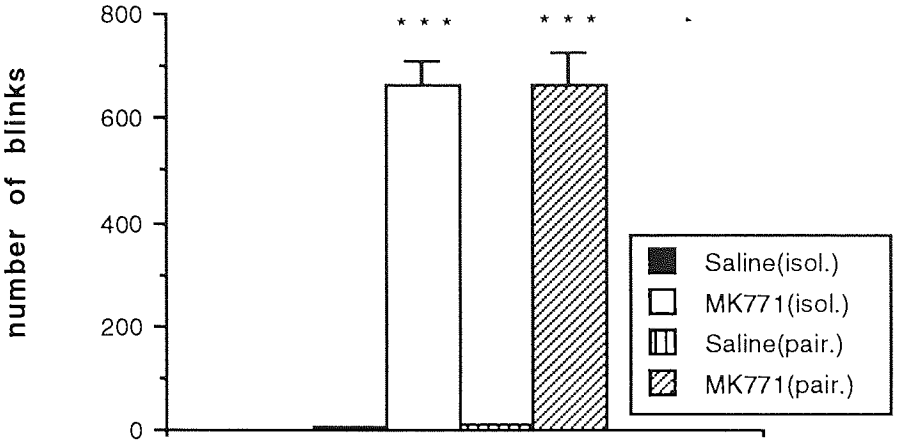


Figure 1.1. Data representing the total number of blinks over a 30min. period following administration of MK771 (5.0mg/kg i.p.) to paired and acutely isolated mice. ***, p<0.0005 *cf.* saline control (n=6, t-test).

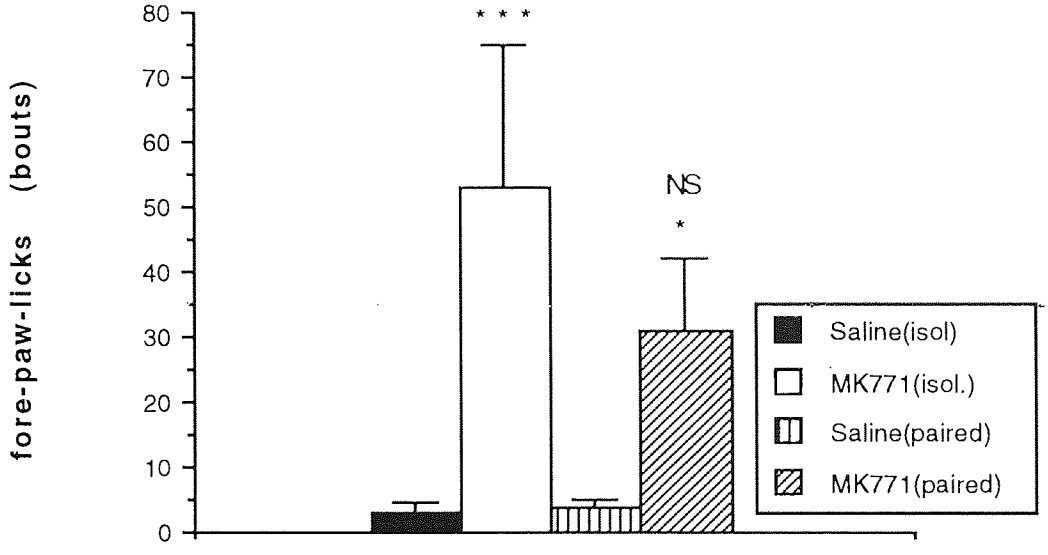


Figure 1.2. Data representing the total number of FPL bouts over a 30min. period following administration of MK771 (5.0mg/kg i.p.) to paired and acutely isolated mice. ***, p<0.0005 and *, p<0.025 *cf.* saline control animals, NS, not significantly different from acutely isolated mice (n=6, t-test).

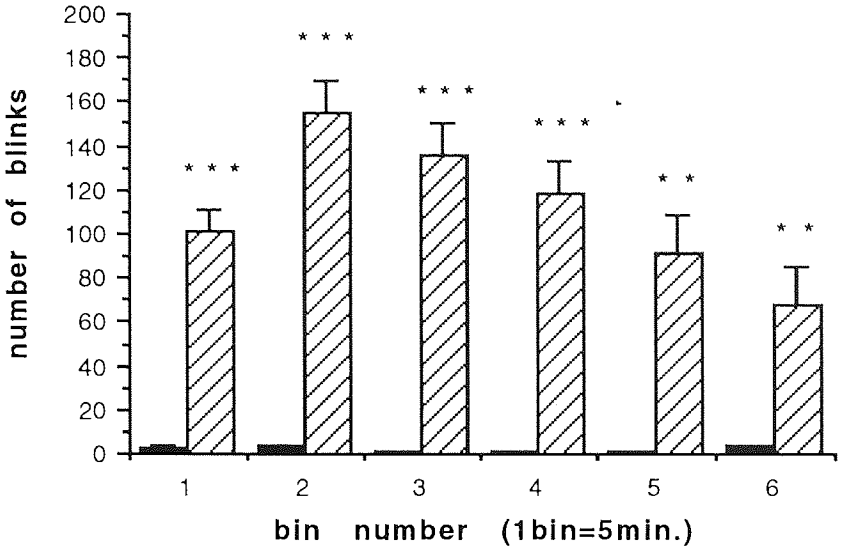


Figure 1.3. Data representing the time course for MK771-induced (5.0mg/kg i.p.) blinking in paired mice recorded in 6 successive 5min. bins. ***, $p<0.0005$ and **, $p<0.005$ *cf.* saline treated mice (n=6, t-test). Filled bars, saline-treated and hatched bars MK771-treated.

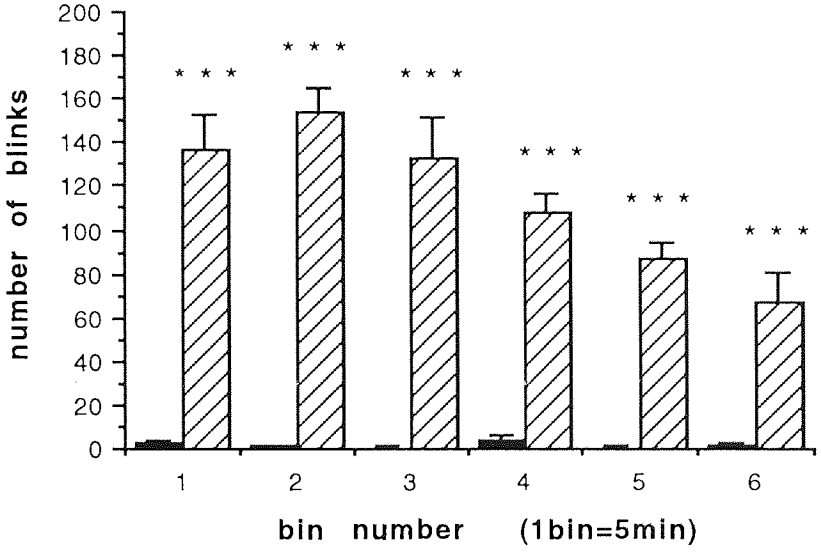
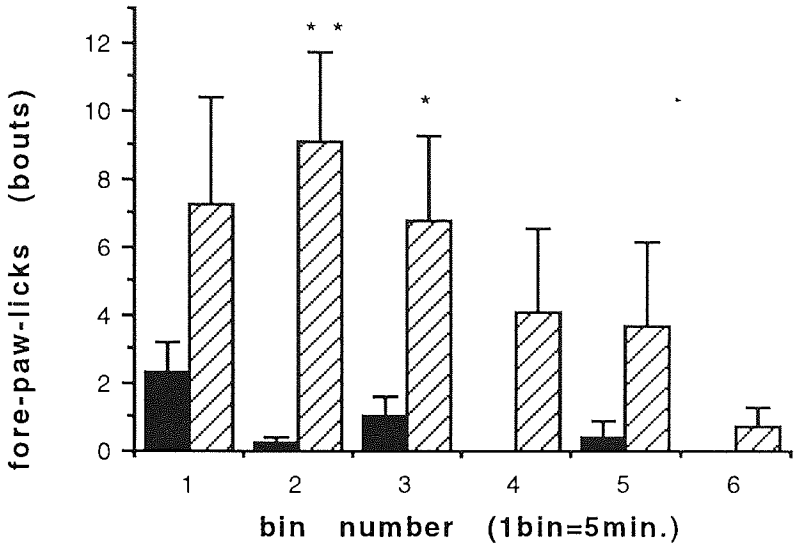


Figure 1.4. Data representing the time course for MK771-induced (5.0mg/kg i.p.) blinking in acutely-isolated mice recorded in 6 successive 5min. bins. ***, $p<0.0005$ *cf.* saline treated mice (n=6, t-test). Filled bars, saline-treated and hatched bars MK771-treated.



Figuree 1.5. Data representing the time course for MK771-induced (5.0mg/kg i.p.) FPL in paired mice recorded in 6 successive 5min. bins. **, $p<0.005$ and *, $p<0.025$ *cf.* saline treated mice (n=6, t-test). Filled bars, saline-treated and hatched bars, MK771-treated.

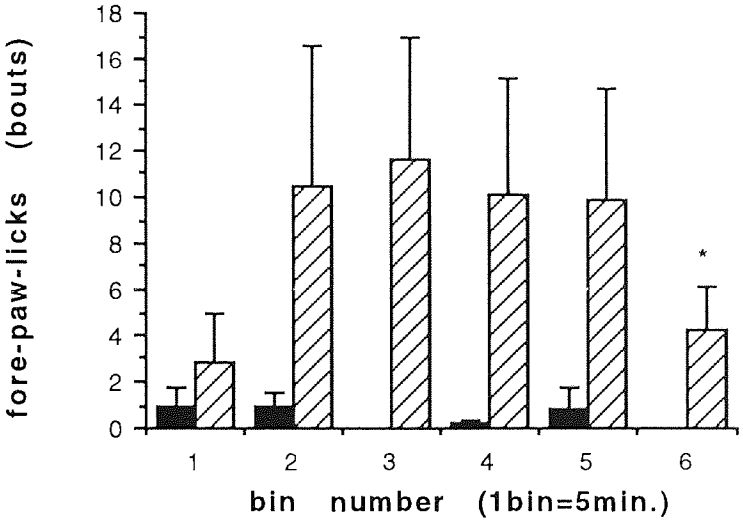


Figure 1.6. Data representing the time course for MK771-induced (5.0mg/kg i.p.) FPL in acutely isolated mice recorded in 6 successive 5min. bins. *, $p<0.05$ *cf.* saline treated mice (n=6, t-test). Filled bars, saline-treated and hatched bars MK771-treated.

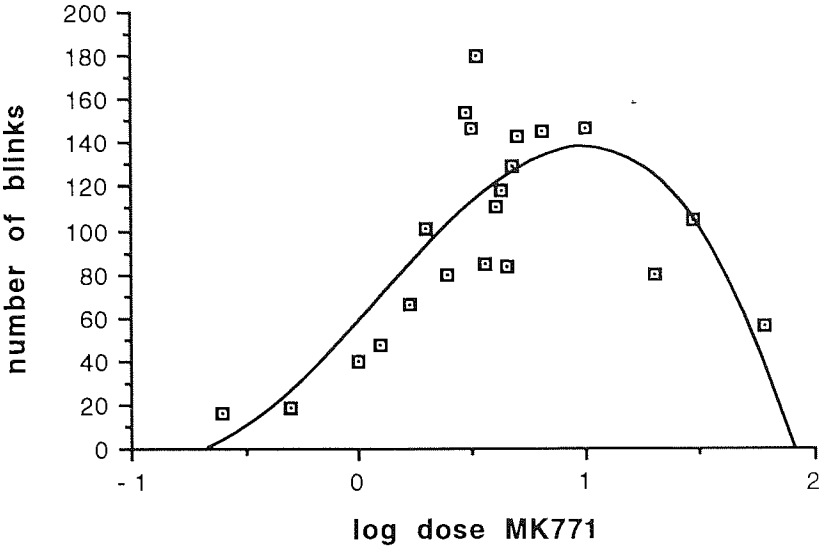


Figure 1.7. Dose response relationship of MK771-induced (0.25-60.0mg/kg i.p.) blinking in paired mice. Line fitted with a 3rd order polynomial model.

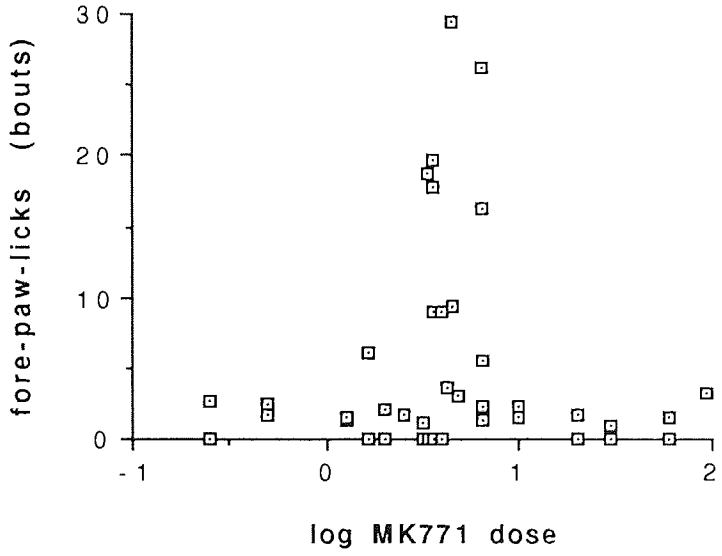


Figure 1.8. Dose response relationship of MK771-induced (0.25-60.0mg/kg i.p.) FPL in paired mice.

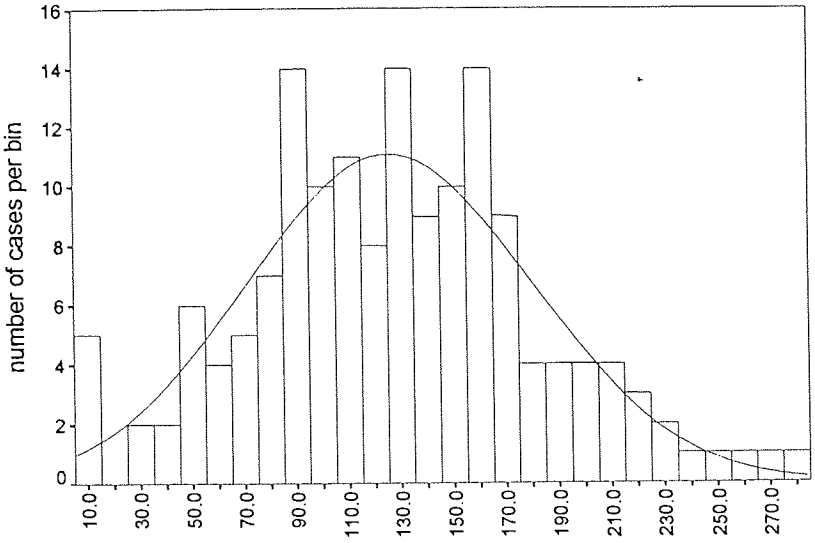


Figure 1.9. Population distribution of MK771-induced (2.5mg/kg i.p.) blinking in paired mice.

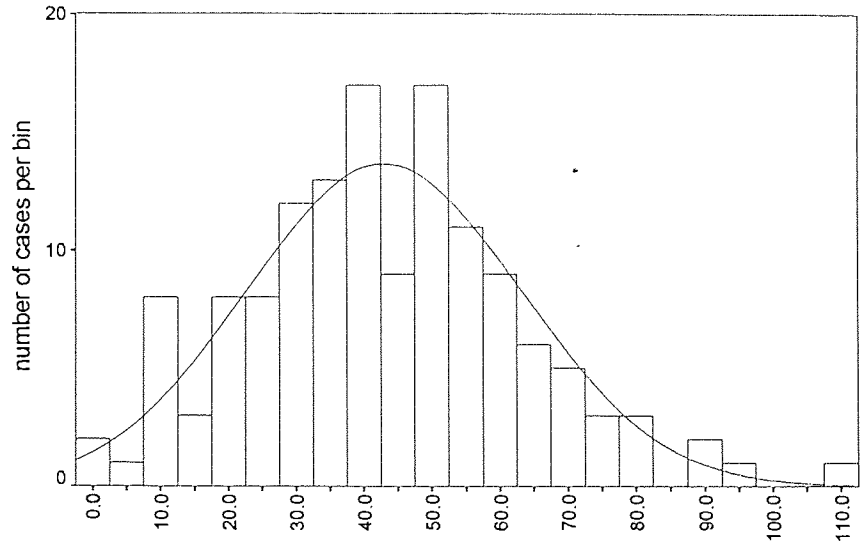


Figure 1.10. Population distribution of MK771-induced (2.5mg/kg i.p.) FPT in paired mice.

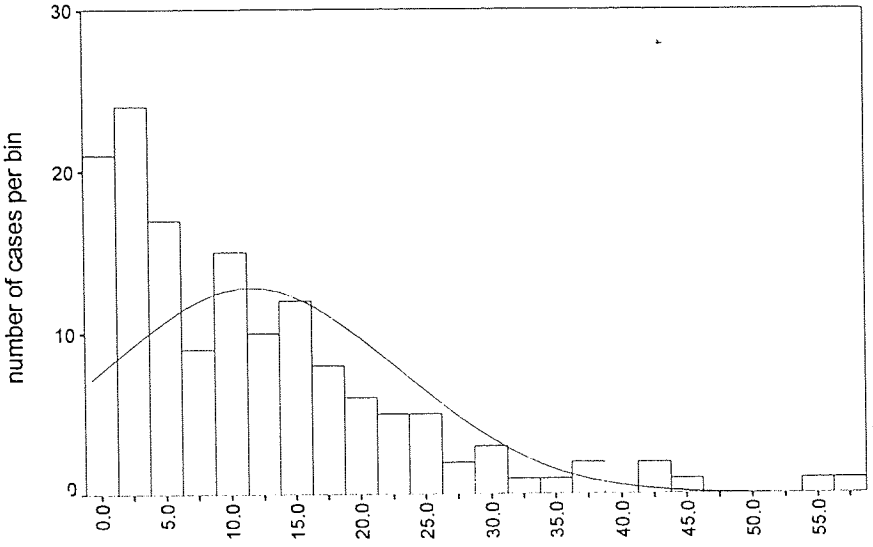


Figure 1.11. Population distribution of MK771-induced (2.5mg/kg i.p.) FPL in paired mice.

CHAPTER 2.

THE EFFECTS OF SELECTIVE DOPAMINE ANTAGONISTS ON MK771-INDUCED BEHAVIOURS.

CHAPTER 2.

Introduction

Studies with TRH and its analogues have demonstrated a variety of neuropharmacological effects on central DAergic systems. TRH potentiated the excitatory effect of L-DOPA in normal and hypophysectomized mice (Plotnikoff *et al.*, 1972; Plotnikoff *et al.*, 1975). When TRH or the TRH analogues CG3703 or CG3705 were injected into the nucleus accumbens, locomotor activity resulted (Miyamoto and Nagawa, 1977; Heal and Green, 1979; Sharp *et al.*, 1982) and it is thought that this effect was due to the DA releasing actions of TRH in this area. Prior administration of chlorpromazine, haloperidol or pimozide antagonised the effects of the TRH analogues, CG3509 or CG3703 (Heal *et al.*, 1981; Miyamoto *et al.*, 1979). However, Costall and co-workers (1979) failed to replicate the DAmimetic properties of TRH. In the caudate nucleus unilateral injections of TRH were without effect on rotational behaviour (Miyamoto *et al.*, 1977). These data were supported by *in vitro* studies demonstrating DA release by TRH in the nucleus accumbens but not the caudate nucleus (Kerwin and Pycock, 1979). However, TRH was without effect on DA release in combined nucleus accumbens lateral septal slices (Mendez *et al.*, 1993) and MK771 failed to induce the accumulation of L-DOPA in nucleus accumbens where TRH did (*ie.* increased the synthesis of dopamine) (Yamada *et al.*, 1984). In the striatum a variety of different effects have been noted: TRH increased L-DOPA accumulation, DA, HVA and DOPAC concentrations (Xu *et al.*, 1990; Crespi *et al.*, 1986; Yamada *et al.*, 1984) the TRH analogue YM-14673 increased DA, DOPAC and HVA concentrations (Xu *et al.*, 1990), MK771 produced a rise in HVA, DOPAC and tyrosine hydroylase activity with a reduction in striatal DA (Rastogi *et al.*, 1991) but RX77368 and CG3509 were without effect (Sharp *et al.*, 1982). It is probable that the increases in DA release and metabolism were mediated by cholinergic mechanisms since the effects of the TRH analogue YM-14673 were antagonised by prior treatment with pirenzepine, an M1 muscarinic receptor antagonist (Xu *et al.*, 1990). In tuberoinfundibular neurones TRH released DA (Nishikawa *et al.*, 1993; Annuzinto *et al.*, 1984). Reductions were observed in the hypothalamus and the pons/medulla (Rastogi *et al.*, 1981). Cortical DA was increased following systemic TRH administration (Agarwal *et al.*, 1977).

Studies of the pharmacological basis of spontaneous blinking suggest that DA modulated this behaviour (for reviews see Elsworth *et al.*, 1991; Karson, 1989; General Introduction), particularly the D1 and D2 receptor sub-types (Karson, 1989; Elsworth *et al.*, 1991). Pre-clinical studies in non-human primates demonstrated that the selective DA D1 agonist dihydrexidine significantly induced

blinking (Taylor *et al.*, 1991; Elsworth *et al.*, 1991) which was reversed by SCH23390, but D2 agonist induced blinking was not attenuated by SCH23390 (Elsworth *et al.*, 1991). The lack of effect of the D1 agonist SKF38393 has been reported (unpublished data, Karson, 1988), which was accounted for by poor brain penetrance (Karson, 1988). However, Elsworth and co-workers (1991) suggest that this may be due to the partial agonist effects of the drug, but it has also been suggested that SKF38393 has no agonist action in the primate striatum *cf.* the rat striatum (Pifl *et al.*, 1991). The DA D2 agonists bromocriptine, apomorphine and 4-propyl-9-hydroxy naphazine induced blinking (Karson, 1983; Casey *et al.*, 1980; Karson *et al.*, 1980; Elsworth *et al.*, 1991; Lawrence *et al.*, 1991) and these effects were reversed by prior administration with D2 antagonists (Elsworth *et al.*, 1991; Karson *et al.*, 1983; Lawrence *et al.*, 1991). Administration of D2 antagonists alone caused a reduction in blinking and this was be reversed by D2 agonists (*eg.* Lawrence *et al.*, 1991). However, the DA D2 receptor antagonist haloperidol was without effect on TRH-induced blinking in mice while the 5-HT2A/2C antagonists ritanserin and ICI 169,369 attenuated this behaviour (Dursun and Handley, 1991; Dursun 1992). Clinical studies demonstrate that central DA is involved in the pharmacology of abnormal blinking in a number of psychiatric and neurological conditions. Parkinson's disease patients display a characteristic reduction in spontaneous blinking which was correlated with a reduction in substantia nigral DA (Adams and Victor, 1981). In schizophrenics neuroleptics can reduce the elevated blink rates (Karson *et al.*, 1981, 1982, 1983; Kleinmann *et al.*, 1984; Muesser *et al.*, 1984; Helms and Goodwin, 1985). Elevation of the blink rate is thought to be a consequence of the disease rather than a consequence of the treatment (Mackert *et al.*, 1991). In one of the above studies (Dursun and Handley, 1991; Dursun 1992) haloperidol significantly reduced scratching behaviour but was without effect on FPT behaviour. Behavioural investigations suggest that D1 receptors were involved in grooming, since administration of the D1 agonists SKF38393, SKF77434, SKF75670, SKF82958, SKF81297 and CY 208-243 induced grooming (Murray and Waddington, 1989,1990; Molloy and Waddington, 1984; Arnt *et al.*, 1987; Eilam *et al.*, 1992; Molloy and Waddington, 1987; Starr and Starr, 1986a and b) and these effects were attenuated by prior administration of SCH23390 and SKF83566 (Murray and Waddington, 1989; Starr and Starr, 1986a). SCH23390 also inhibited novelty induced grooming (Linthorst *et al.*, 1992) and increased (low doses) or attenuated grooming (higher dose) (Starr and Starr, 1986b). It must , however, be stated that FPT and FPL are not strictly speaking 'grooming', but are being considered as 'grooming-like' here.

The aims of the experiments demonstrated in this chapter were to analyse the effects of DA D1 and D2 blockade on MK771-induced blinking, FPL and FPT using the

selective D1 antagonists SCH23390 and SCH39166 and the D2 antagonists raclopride and sulpiride. In order to ascertain the function of DA in the modulation or mediation of these behaviours.

The choice of doses used in the studies presented here were based on previous studies conducted in our laboratory (Dursun, 1992).

Additional Methods

Saline (10.0 ml/kg i.p.), the DA D1 antagonists SCH23390 and SCH39166 (1.0 and 5.0 mg/kg i.p.) or the DA D2 antagonists raclopride (3.0 and 5.0 mg/kg i.p.) or sulpiride (5.0 and 10.0 mg/kg i.p.) were administered 22.5 minutes prior to MK771 (2.5 mg/kg i.p.) and the number of blinks, FPT bouts and FPL bouts were recorded 7.5 minutes later for a 5 minute period. Each mouse was used for one experiment and then sacrificed.

Statistical analyses two statistical tests were performed i., between Drug+Saline and Saline+Saline groups and ii., Drug+MK771 groups and Saline+MK771 control groups using an un-paired t-test and data are presented as mean ± SEM.

Results

Effects of drugs on MK771-induced behaviours

SCH23390

SCH23390 (1.0 and 5.0 mg/kg) was without effect on MK771-induced blinking (*figure* 2.1). However, both doses of SCH23390 significantly attenuated MK771-induced FPT (1.0mg/kg, -67.60%, $p<0.05$; 5.0mg/kg, -43.10%, $p<0.01$ (Student's unpaired t-test); *figure* 2.2) and FPL (1.0mg/kg, -71.51%, $p<0.025$; 5.0mg/kg, -69.11%, $p<0.05$ (Student's unpaired t-test); *figure* 2.3).

SCH39166

SCH39166 (1.0 and 5.0 mg/kg) was without effect on MK771-induced blinking in saline pre-treated mice (*figure* 2.4). However, both doses of SCH39166 significantly attenuated MK771-induced FPT (1.0mg/kg, -93.74%, $p<0.0005$; 5.0mg/kg, -59.42%, $p<0.05$ (Student's unpaired t-test); *figure* 2.5) and FPL (1.0mg/kg, -44.06%, $p<0.025$; 5.0mg/kg, -91.55%, $p<0.005$ (Student's unpaired t-test); *figure* 2.6).

Raclopride

Raclopride (3.0 and 5.0mg/kg) was without effect on any of the behaviours tested (*figures* 2.7,.8 and .9) with the exception of FPT, which was potentiated by the high (5.0mg/kg) dose (+178.82%, $p<0.05$ (Student's unpaired t-test); *figure* 2.8).

Sulpiride

Sulpiride (5.0 and 10.0mg/kg) was without effect on any of the behaviours tested (Student's unpaired t-test) (*figures* 2.10, 2.11, and 2.12).

Discussion

The experiments presented in this chapter suggest that DA D1 and D2 receptors do not modulate MK771-induced blinking and that D1 receptors modulate FPT and FPL. The failure of both SCH23390 and SCH39166 (1.0 and 5.0mg/kg, *figures* 2.1 and 4) to attenuate MK771-induced blinking is surprising, given the effects of dihydrexidine and SCH23390 (Taylor *et al.*, 1991; Elsworth *et al.*, 1991; see Introduction).

Raclopride and sulpiride were without effect on MK771-induced blinking (*figures* 2.7 and 10), consistent with a lack of response of haloperidol on TRH (icv) induced blinking (Dursun and Handley, 1991; Dursun, 1992). Pre-clinical and clinical studies have demonstrated the role of DA D2 receptors in the modulation of spontaneous blinking, but not TRH induced blinking (Dursun and Handley, 1991). It is therefore apparent from the results presented here that TRH receptor mediated blinking is not related to DA receptor mediated blinking and it is feasible to suggest that the population(s) of receptors involved in TRH receptor stimulated blinking are not under the control of central DAergic sites or projections from those sites. Furthermore, it is apparent that TRH mediated blinking is not a consequence of DA release, given the DAmimetic properties of TRH and TRH analogues (see earlier).

Pretreatment of MK771 treated mice with SCH39166 or SCH23390 significantly attenuated FPT and FPL behaviours (*figures* 2.2, 2.3, 2.5 and 2.6). Previous studies suggest that D1 receptors are involved in grooming (see Introduction for references and details). The D2 antagonists raclopride and sulpiride were without effect on FPL and FPT except at the higher dose of raclopride (5.0mg/kg) which potentiated MK771-induced FPT (*figure* 2.8). This lack of effect is consistent with the failure of haloperidol to attenuate TRH-induced FPT in mice (Dursun and Handley, 1991; Dursun, 1992), but haloperidol has DA D1 and 5-HT2A affinity (Meltzer *et al.*, 1989). The potentiation of MK771-induced FPT by raclopride (5.0mg/kg) is consistent with the action of D2 agonist inhibition of grooming (Linthorst *et al.*, 1992; Starr and Starr, 1986a and b; Eilam *et al.*, 1992). Furthermore, sulpiride enhanced intense grooming (Waddington *et al.*, 1986; Molloy and Waddington 1987b). However, piquindone antagonised grooming induced by SKF77434 (Murray and Waddington, 1989), but D1 agonist induced grooming was attenuated by D2 agonists (Eilam *et al.*, 1992). Taken together these results suggest that there is a D1: D2 interplay in the mediation of grooming behaviour by DAergic agents. In the studies presented here it is suggested that TRH receptor activation with MK771 results in DA D1 stimulation which induces FPT and FPL, since the D1 antagonists attenuate FPT and FPL. Because of this facilitation of D1 neurotransmission by MK771 and the potentiation of FPT by the

high dose raclopride it is feasible to hypothesise that there may be a D1:D2 interaction modulating FPT such as has been described for the stimulation of adenylate cyclase by SKF 38393 and its potentiation by haloperidol (Saller and Salama, 1986) *ie.* DA D2 receptors exert an inhibitory tone on D1 receptors and when D2 receptors are inhibited an increase in D1 receptor tone occurs which potentiates TRH receptor stimulated FPT. However, it must be considered that sulpiride and the lower dose of raclopride did not have the same effect as the high dose of raclopride, it must therefore be considered that this is an might be an anomalous result.

The results presented in this chapter suggest that DA D1 or D2 receptors do not modulate blinking induced by the TRH analogue MK771. FPT and FPL are attenuated by DA D1 receptors suggesting a permissive role for this DA receptor sub-type on TRH induced FPT and FPL. The role of DA D2 receptors is a little more confusing; they have either no effect or, as has been suggested, may interplay with D1 receptors in the modulation of FPT. However, this latter hypothesis needs further clarification.

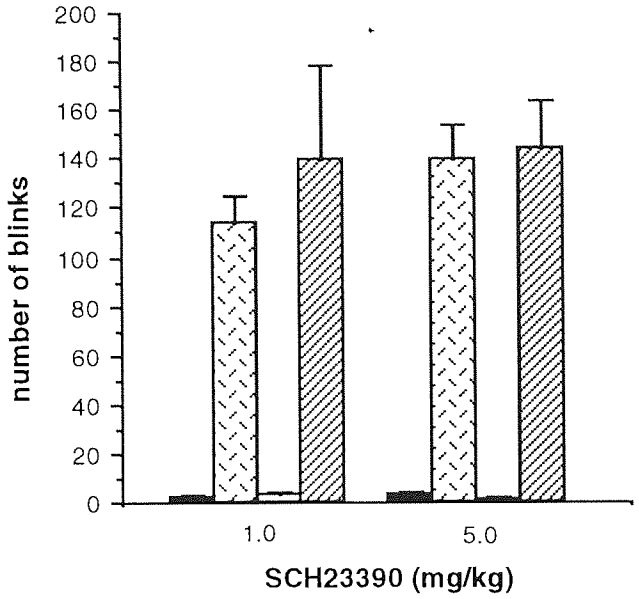


Figure 2.1. Lack of affect of SCH23390 on MK771-induced blinking. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - SCH23390 + Saline and hatched bar - SCH23390 + MK771.

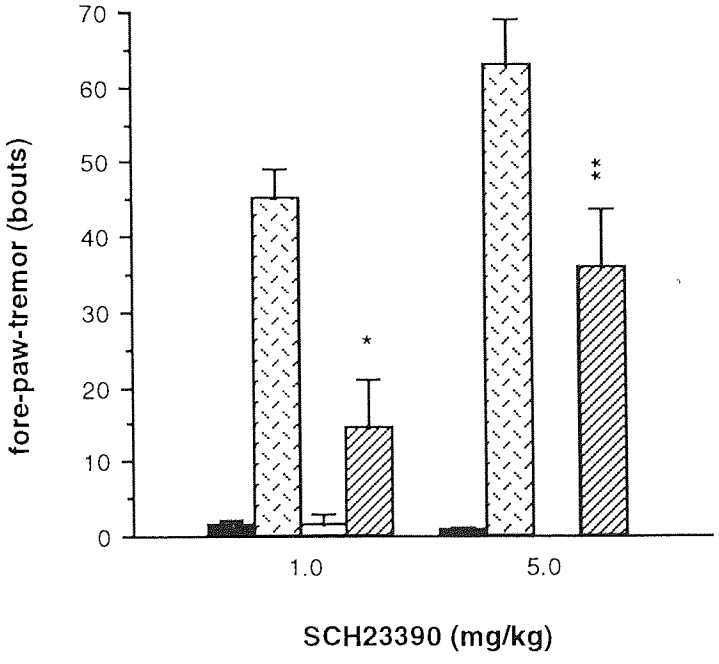


Figure 2.2. SCH23390 attenuates MK771-induced FPT. **, $p<0.01$ and *, $p<0.05$ significantly different from control. Bars as *figure* 2.1.

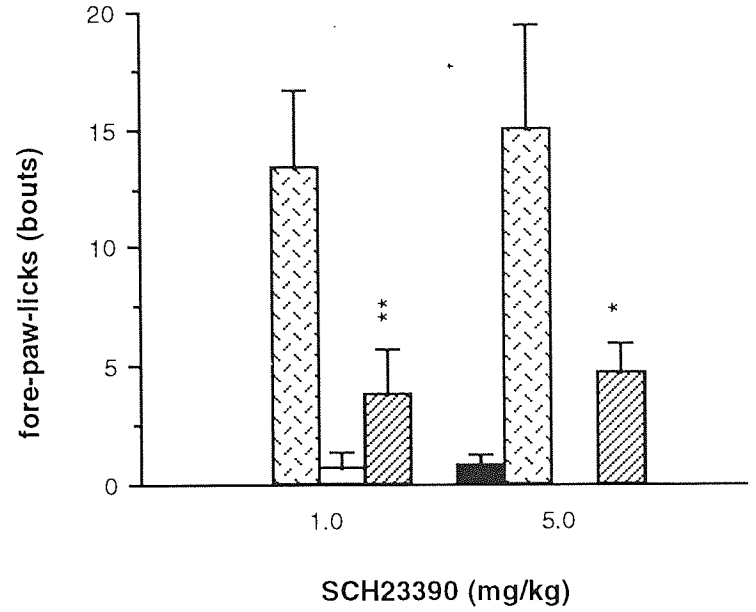


Figure 2.3. SCH23390 attenuates MK771-induced FPL. **, $p<0.025$ and *, $p<0.05$ significantly different from control. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - SCH23390 + Saline and hatched bar - SCH23390 + MK771.

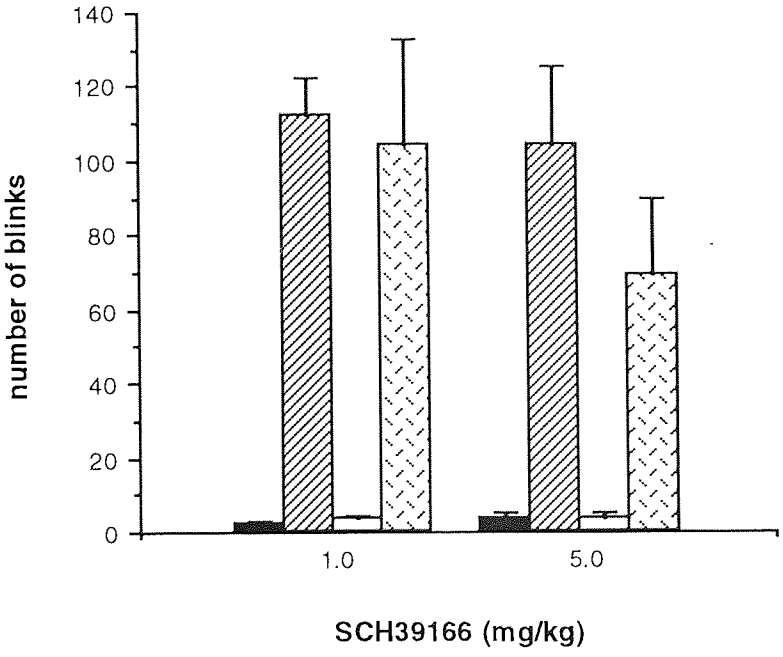


Figure 2.4. Lack of effect of SCH39166 on MK771-induced blinking. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - SCH39166 + Saline and hatched bar - SCH39166 + MK771.

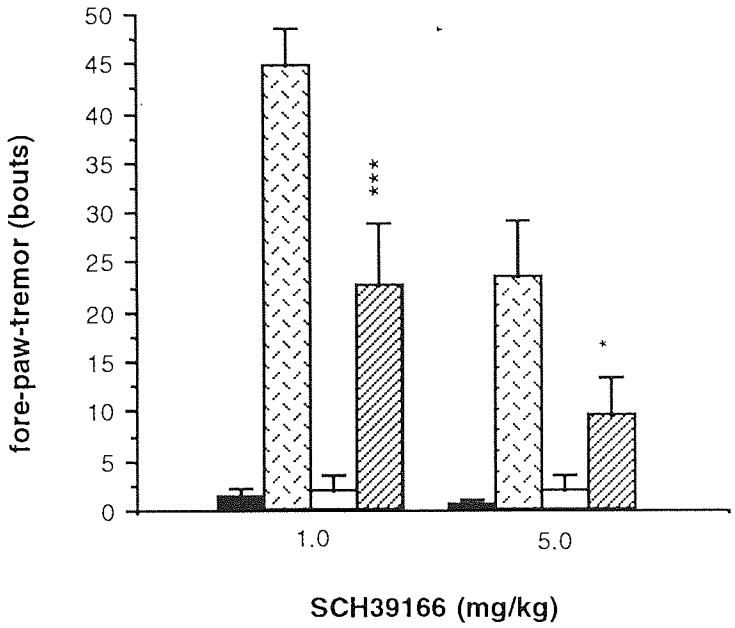


Figure 2.5. SCH39166 attenuated MK771-induced FPT. ***, $p<0.005$ and *, $p<0.05$ significantly different from control. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - SCH39166 + Saline and hatched bar - SCH39166 + MK771.

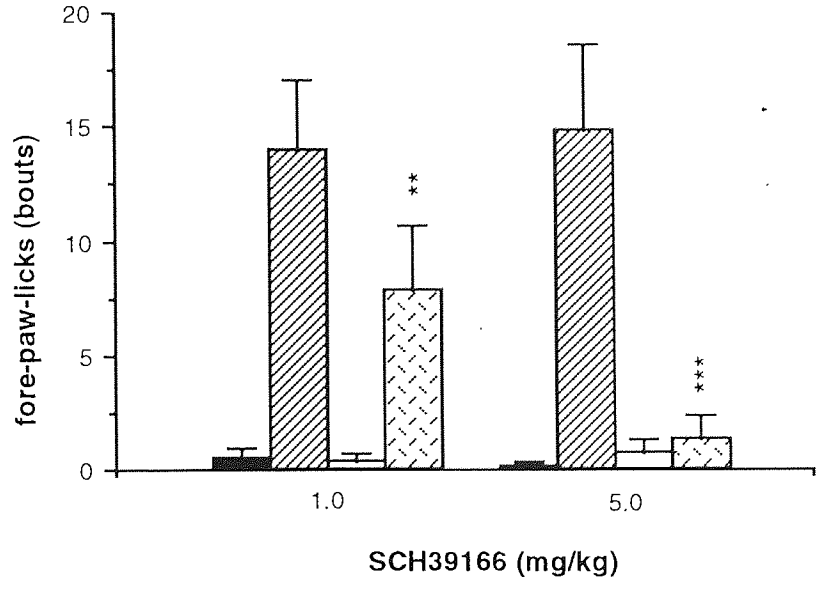


Figure 6. SCH39166 attenuates MK771-induced FPL. ***, $p<0.005$ and **, $p<0.025$ significantly different from control. Bars - as *figure* 2.4.

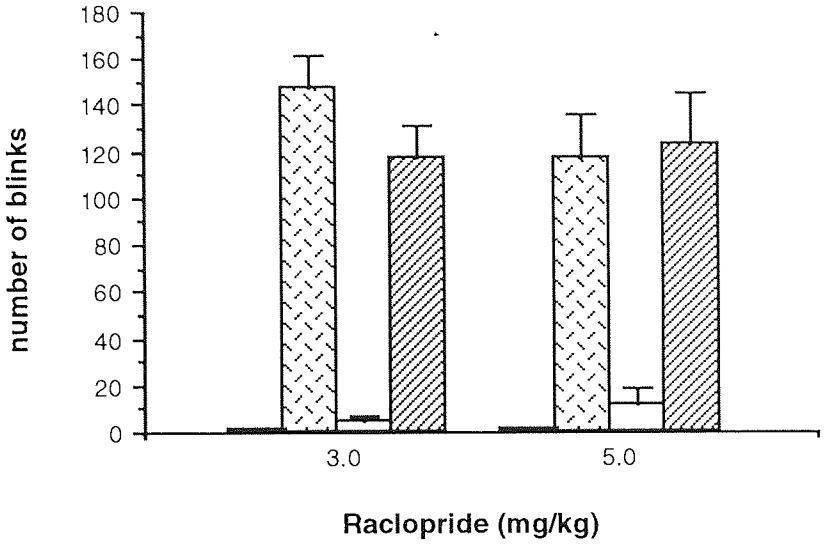


Figure 7. Lack of effect of raclopride on MK771-induced blinking. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - raclopride + Saline and hatched bar - raclopride + MK771.

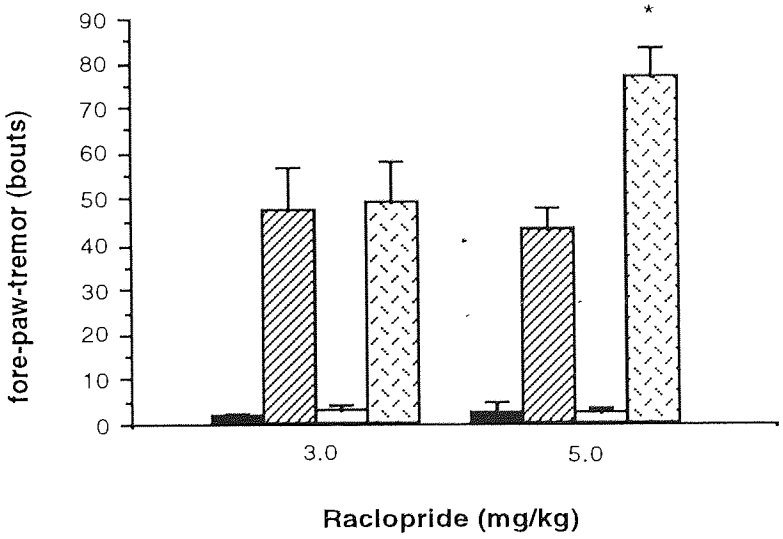


Figure 2.8. Raclopride (5.0mg/kg) potentiates MK771-induced FPT. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - raclopride + Saline and hatched bar - raclopride + MK771. *, $p<0.05$ *cf.* Saline + MK771 treated mice.

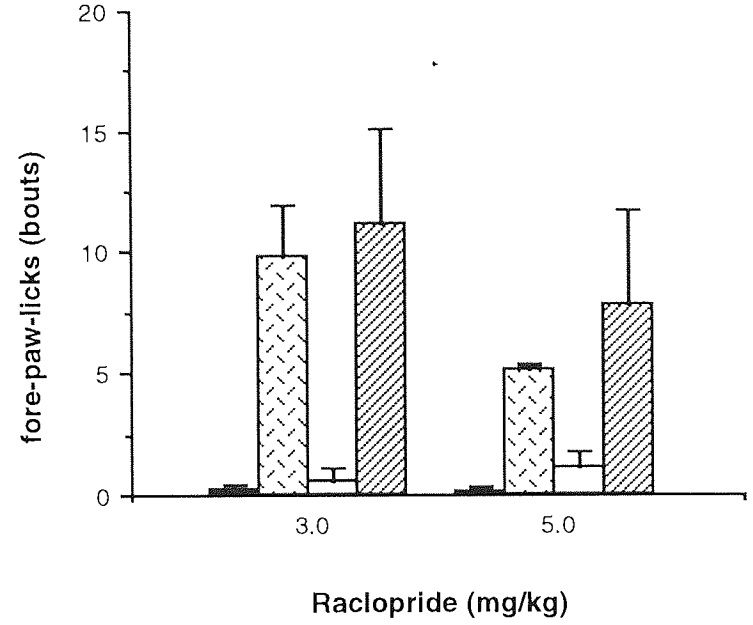


Figure 2.9. Lack of effect of raclopride on MK771-induced FPL. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - raclopride + Saline and hatched bar - raclopride + MK771.

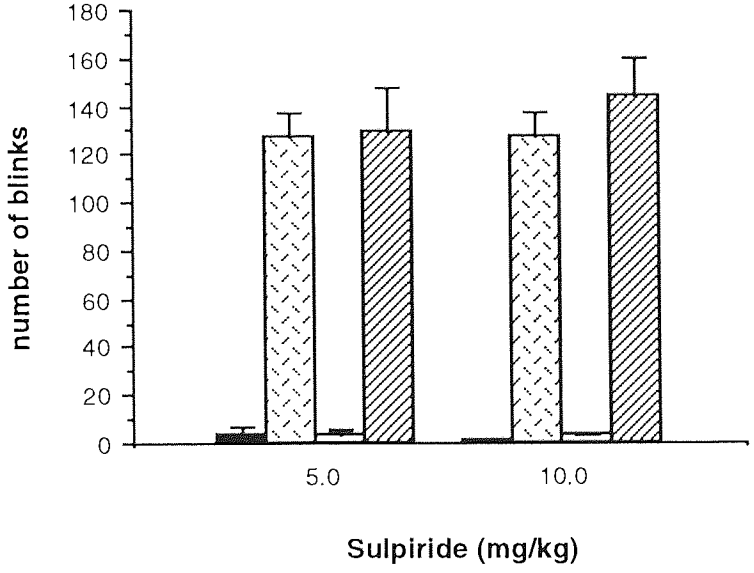


Figure 2.10. Lack of effect of sulpiride on MK771-induced blinking. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - sulpiride + Saline and hatched bar - sulpiride + MK771.

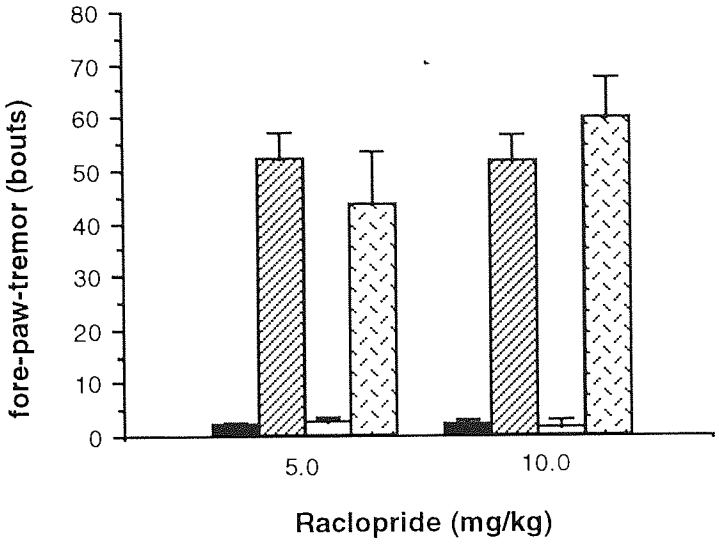


Figure 2.11. Lack of effect of sulpiride on MK771-induced FPT. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - sulpiride + Saline and hatched bar - sulpiride + MK771.

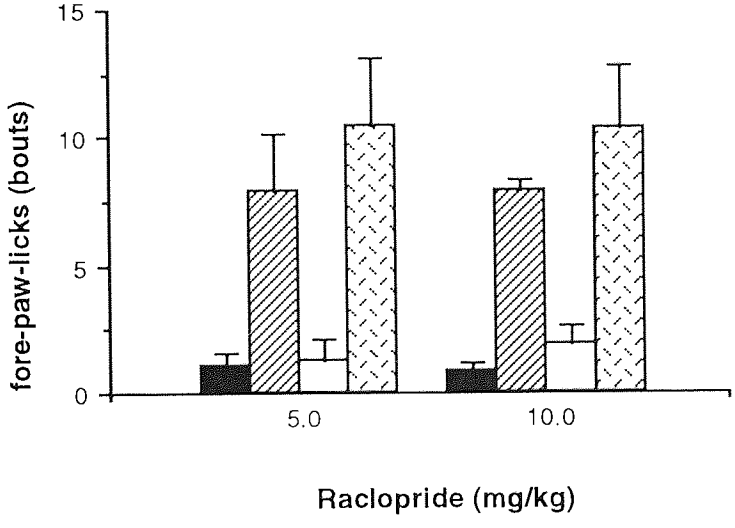


Figure 2.12. Lack of effect of sulpiride on MK771-induced FPL. Filled bar - Saline +Saline; stippled bar - Saline + MK771; open bar - sulpiride + Saline and hatched bar - sulpiride + MK771.

CHAPTER 3.

THE EFFECT OF 5-HT ANTAGONISTS ON MK771-INDUCED BEHAVIOURS.

CHAPTER 3.

Introduction

Head shakes (HS) and WDS have long been known to be a behavioural consequence of the administration of agents which increase serotonergic neurotransmission, and are believed to be a consequence of 5-HT2A receptor activation (see thesis introduction for full details; Kennett and Curzon, 1991; Kennett *et al.*, 1994a).

Behavioural studies have indicated that central administration of TRH can induce HS and WDS in rats and mice (please refer to General Introduction). Administration of the TRH and its analogues CG3509, CG3703, RX77368 and MK771 have been shown to induce WDS and HS behaviours (see General Introduction) and the 5-HT2A/2C receptor antagonist ritanserin antagonised WDS induced by the TRH analogue CG3509 in rats (Fone *et al.*, 1989a). In mice, ritanserin and the 5-HT2A/2C antagonist ICI 169,369 (Blackburn *et al.*, 1988) attenuated HS induced by intra-cerebro-ventricular (icv) TRH (Dursun and Handley, 1991; Dursun, 1992). However, ritanserin (0.5mg/kg i.p) did not antagonise WDS induced by MK771 (Pranzatelli, 1988), but the same dose of ritanserin significantly attenuated DOI induced HS in mice (Heaton, 1992). TRH and its analogues have also been shown to induce other rapid-phasic-movements such as FPT and FPL (see General Introduction). FPL induced by CG3509 was antagonised by prior intrathecal administration of ritanserin (Fone *et al.*, 1989a). FPT and FPL induced by icv administered TRH was also antagonised by ritanserin (1.0mg/kg i.p.) and ICI 169,369 (2.0mg/kg) (Dursun and Handley, 1991; Dursun, 1992). In the same study, spontaneous blinking was reported and was similarly antagonised by ritanserin and ICI 169,369 but not by haloperidol (Dursun and Handley, 1991; Dursun, 1992). This latter finding was somewhat surprising given the suggested role of DA D2 receptors in the control of spontaneous blinking (see General Introduction).

The aims of this study were to investigate the role of 5-HT2A and 5-HT2C receptors in modulating TRH receptor mediated FPL, FPT and blinking, using the specific antagonists ritanserin, ketanserin, mianserin and SB200646A as pharmacological probes. The effects of prazosin were analysed given the affinity of ketanserin at the alpha-1 adrenoreceptor (Blackburn *et al.*, 1988).

The drugs in this study were chosen on the basis of their affinities for the 5-HT2A and 5-HT2C receptor subtypes (see Dursun, 1992) and the doses and routes of administration based on previous studies conducted in this laboratory by other workers and by reference to previously published work: the dose of MK771 (2.5mg/kg i.p.) was determined from preliminary studies (Chapter 1); 1.0mg/kg i.p. ritanserin attenuated TRH-induced behaviours following TRH (Dursun and

Handley, 1991); pilot experiments were conducted with the rather high dose of 1.0 mg/kg i.p. of ketanserin, but following the lack of efficacy at this dose the dose was increased to 5.0mg/kg i.p.; mianserin displays affinity for the 5-HT2C and 5-HT2A receptor (Hoyer *et al.*, 1988) and a dose of 2.0mg/kg was chosen because the ID_{50} for the inhibition of mCPP-induced hypophagia was 2.11mg/kg i.p. (Kennet and Curzon, 1991); 2.0 mg/kg i.p. prazosin administered to rats attenuated TRH analogue induced WDS and the time spent fore-paw-licking (Fone *et al.*, 1987) and SB200646A (20.0 and 40.0 mg/kg p.o.) reduced mCPP-induced hypolocomotion and hypophagia, but was without effect on the DOI head shake frequency (Kennett *et al.*, 1994a), implying lack of behavioural effects at the 5-HT2A receptor at these doses.

Additional methods

Vehicle (saline, 10.0ml/kg i.p.), ritanserin (1.0mg/kg i.p., 3.0mg/kg i.p. and 5.0mg/kg i.p.; 20-30g, n=6-12 mice/treatment group), ketanserin tartrate (1.0mg/kg s.c.; 20-30g, n=5-11 mice/treatment group), mianserin (2.0mg/kg s.c.; 20-30g, 6-11 mice/treatment group) and prazosin (2.0mg/kg i.p.; 20-30g in weight, n=7-9 mice/treatment group) were administered 22.5 minutes prior to MK771 (2.5mg/kg i.p.) or saline. Vehicle (1% methyl cellulose in 0.9% saline with a drop of BRIJ 35, 10.0ml/kg p.o.) or SB200646A (20.0mg/kg p.o. and 40.0mg/kg p.o.), was administered 52.5 minutes prior to MK771. Blinking, FPL and FPT behaviours were counted 7.5 minutes later for a 5 min. period.

Statistical analyses: unpaired t-tests were carried out between Saline+MK771 and Drug+MK771 groups following a non-significant effect of drug alone (Drug+Saline *cf.* vehicle treated groups). Group differences in the SB200646A experiment were analysed using a two-way ANOVA model and potentiation of MK771's effects on FPT in this experiment were analysed using a linear regression ANOVA model. All significance values are displayed on the relevant figures.

Results

Effects of drugs on MK771-induced behaviour:

Ritanserin

Ritanserin (1.0 and 5.0mg/kg) + Saline was without effect on blinking, FPT and FPL compared with saline treated mice (*figure* 3.1). Ritanserin (1.0mg/kg) significantly attenuated MK771-induced blinking (32.13% reduction (-32.13%)), which was not apparent at the higher doses (3.0 and 5.0mg/kg; *figure* 3.1). In the same mice ritanserin pre-treatment significantly attenuated FPT (1.0mg/kg, -42.17%; 3.0mg/kg, -27.84%; 5.0mg/kg, -35.19%) and FPL (1.0mg/kg,

-73.58%; 3.0mg/kg, -63.71%; 5.0mg/kg, -65.32 %) at all doses used (*figures* 3.2 and 3.3).

Ketanserin

Ketanserin (1.0 and 5.0mg/kg) was without effect on blinking, FPT and FPL in saline treated rats and significantly attenuated MK771-induced blinking at both doses tested (1.0mg/kg, -59.39%; 5.0mg/kg, -51.59%; *figure* 3.4). However, the lower dose (1.0mg/kg) was ineffective at producing a significant reduction in FPT (-49.88%) and FPL (-49.28%) behaviours (*figures* 3.5 and 3.6). At the higher dose (5.0mg/kg) significance was reached (FPT, -51.85%; FPL, -97.79%; *figures* 3.5 and 3.6, respectively).

Prazosin

Prazosin (2.0mg/kg i.p.) had no effect on blinking, FPT and FPL in saline pre-treated mice and was without effect on MK771-induced blinking (-14.2%, *figure* 3.7). However, the number of FPT and FPL bouts were significantly reduced by prior administration of prazosin (FPT, -47.35; FPL, -86.35%; *figures* 3.8 and 3.9 respectively).

Mianserin

Mianserin (2.0 mg/kg s.c.) alone had no effect on blinking, FPT and FPL in saline treated mice. FPT and FPL were significantly attenuated by mianserin (-63.77% and -77.6%; *figures* 3.11 and 3.12, respectively), but no significant effect was observed on blinking behaviour (*figure* 3.10).

SB200646A

At both of the doses tested SB200646A (20.0 and 40.0mg/kg) had no effect on MK771-induced blinking (two-way interaction term, $F(2,50)= 0.03$, $p<0.987$) and FPL (two-way-interaction term $F(2,50)=1.46$, $p<0.242$) or the effects of saline alone (*figures* 3.13 and 3.16), but potentiated MK771-induced FPT (*figures* 3.14 and 3.16; two-way interaction term, $F(2,50)=2.56$,$p<0.087$ *ie.* non-significant trend; significance was revealed using a 1way-regression ANOVA of SB200646A treated mice, $F(1,28)=5.24$, $p<0.03$).

Discussion

The experiments presented here suggest that some of the rapid-phasic-movements were modulated by 5-HT2A receptors, as might be hypothesised from previous studies (see General Introduction).

Prior administration of the 5-HT2A/2C antagonist ritanserin antagonised both WDS and FPL behaviours in rats following intrathecal administration of CG3509 (Fone *et al.*, 1989a). Icv administration of TRH induced HS, scratching, tail-tremor, Straub-tail and FPT which were attenuated by prior administration of ritanserin and ICI 169,369 (Dursun and Handley, 1991; Dursun, 1992). Similarly in the

experiments presented here ritanserin and the relatively high doses of ketanserin which do not discriminate between 5-HT2A and 5-HT2C receptor blockade attenuated MK771-induced blinking, FPT and FPL. However, at the higher doses of ritanserin (3.0 and 5.0mg/kg) no change in blinking behaviour was observed and even the effects at the lower dose (1.0mg/kg) showed a relatively small inhibition (*figure* 3.1). The reason for this is somewhat unclear, given the significant reduction in blinking seen with ketanserin at both of the doses tested (1.0 and 5.0mg/kg) (*figure* 3.4) and previous findings in this laboratory (Dursun and Handley, 1991; Dursun, 1992). Similarly the attenuation of FPT by ritanserin was not very large, but the attenuation of FPL was greater, which is paralleled by the effect of ketanserin (5.0mg/kg) which caused a 51.85% reduction in FPT but almost abolished FPL (-97.79%). The lower dose of ketanserin (1.0mg/kg), however, did not significantly attenuate FPT and FPL (*figures* 3.5 and 3.6). It is thought that this may represent a dose related effect since the higher (5.0mg/kg) dose reached significance (*figures* 3.5 and 3.6) (indeed, the MK771-induced FPL response was almost totally abolished) and a non-significant trend was indicated for the 1.0mg/kg FPT data. The high dose of ketanserin (5.0mg/kg) is on the high side *cf.* other behavioural studies *eg.* those conducted on HS (Kennett and Curzon, 1991; Dursun, 1992), but abolition of MK771-induced blinking and FPT has not been observed in the studies presented here. Ritanserin is poorly soluble in water and this fact may be related to the rather poor efficacy of the drug against the behaviours tested, even though the drug was first dissolved in acid pH, with sonication and mild heating (see methods). Ketanserin displays affinity at the alpha-1 site (Blackburn *et al.*, 1988) and the alpha-1 adrenoceptor antagonist prazosin attenuated FPT (*figure* 3.8) and FPL (*figure* 3.9) but not blinking (*figure* 3.7). This attenuation of FPT and FPL is consistent with previous reports indicating that antagonism of the alpha-1 adrenoeceptor attenuates FPL and WDS behaviours in rats (Fone *et al.*, 1987 and 1989b). Given this failure of prazosin to attenuate blinking it appears that the effects of ketanserin on blinking may be due to antagonism at the 5-HT2A receptor, as is the case with the low dose of ritanserin (see later). However, the attenuation of FPL and FPT by ketanserin could be due to additive effects on the 5-HT2A and alpha-1 receptors. The lack of effect of prazosin on blinking is consistent with the lack of effect of the alpha-1 agonist phenylephrine and the mixed agonist phentolamine on blinking (Karson, 1983).

Mianserin (2.0m/kg) reduced MK771-induced blinking but this did not reach significance (*figure* 3.10), but FPT and FPL were significantly attenuated (*figures* 3.11 and 3.12). The ID_{50} for the inhibition of mCPP (a mixed 5-HT agonist, with high affinity for the 5-HT2C receptor) induced hypophagia by mianserin was 2.11mg/kg i.p., but the ID_{50} of mianserin for the inhibition of 5-HTP (with

carbidopa) HS was 0.12mg/kg i.p. (Kennett and Curzon, 1991) demonstrating that, at the dose used here, mianserin is not selective between 5-HT2A or 5-HT2C receptors, and thus would have been expected to significantly attenuate MK771-induced blinking.

SB200646A is an antagonist at the 5-HT2C and 5-HT2B receptors with a lack of affinity and behavioural effect at the 5-HT2A receptor (Forbes *et al.*, 1993; Kennett *et al.*, 1994a). The drug was without effect on FPL and blinking behaviours induced by MK771 (*figures* 3.13 and 3.16) and, somewhat surprisingly, significantly potentiated FPT (*figures* 3.14 and 3.15). This may indicate that endogenous 5-HT may reduce FPT by an action at the 5-HT2C/2B sites following TRH receptor stimulation, but further studies are necessary to confirm this interaction. It is thought that the 5-HT2C effects of ritanserin do not have an effect on the reduction of FPL and blinking (low dose only), since SB200646A did not attenuate these behaviours. However, it is possible that the relatively small attenuation of FPT by ritanserin could be explained by opposing 5-HT2A : 5-HT2C antagonism with the predominant effect lying on the side of the 5-HT2A receptor.

It appears that 5-HT2A receptor antagonism modulates MK771-induced blinking, FPT and FPL, and that 5-HT2C receptors probably do not modulate blinking and FPL, but antagonism at this receptor sub-type potentiates FPT, indicating that endogenous 5-HT may serve to attenuate this type of behaviour. However, the evidence for blinking must be viewed with some conservatism, since the results are not confirmatory. Alpha-1 adrenoceptors do not appear to modulate blinking, but their effects on FPT and FPL are consistent with previous reports indicating that this receptor-type is facilitatory for FPL and WDS induced by TRH analogues.

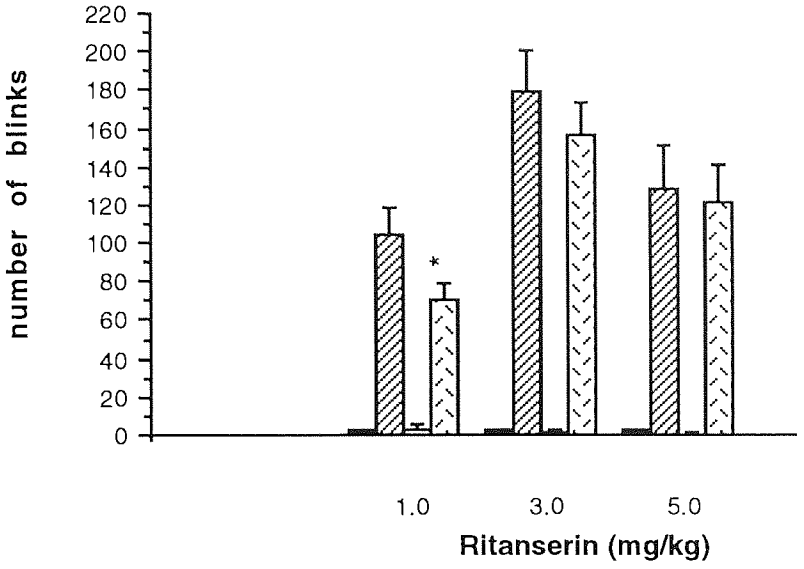


Figure 3.1. Ritanserin attenuated MK771-induced blinking at the lower dose tested (1.0mg/kg i.p). *, $p<0.05$ *cf.* Saline + MK771 treated control mice. From left to right, filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Ritanserin + Saline and stippled bar , Ritanserin + MK771.

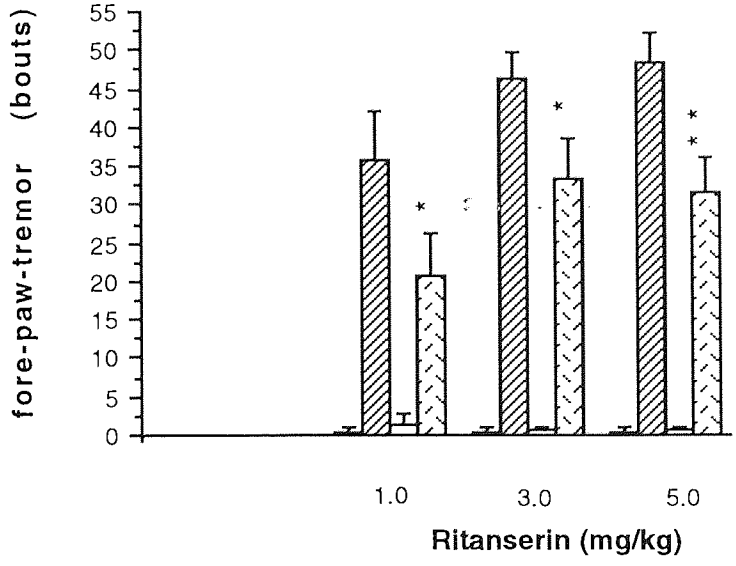


Figure 3.2. Ritanserin attenuated MK771-induced FPT. **, $p<0.01$ and *, $p<0.05$ *cf.* Saline + MK771. Bars as *figure* 3.1.

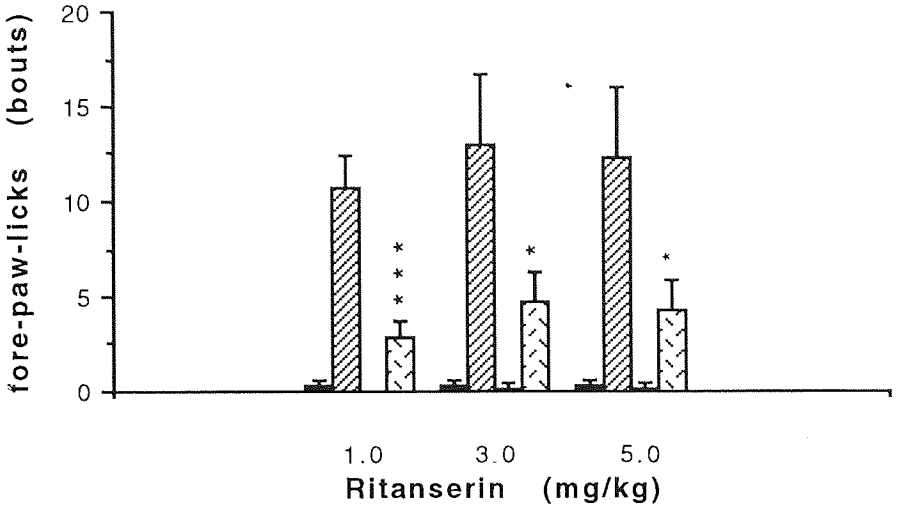


Figure 3.3. Ritanserin attenuated MK771-induced FPL. ***, p<0.001 and *, p<0.05 *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Ritanserin + Saline and stippled bar , ritanserin + MK771.

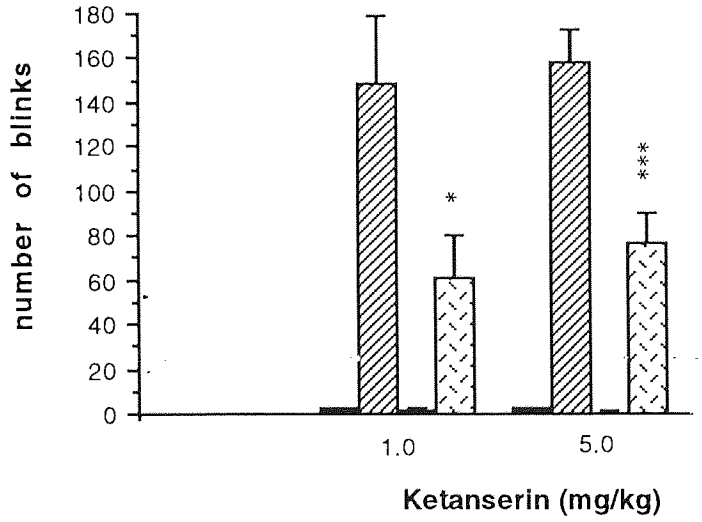


Figure 3.4. Ketanserin attenuated MK771-induced blinking. ***, p<0.001 and *, p<0.05 *cf.* Saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Ketanserin + Saline and stippled bar, Ketanserin + MK771.

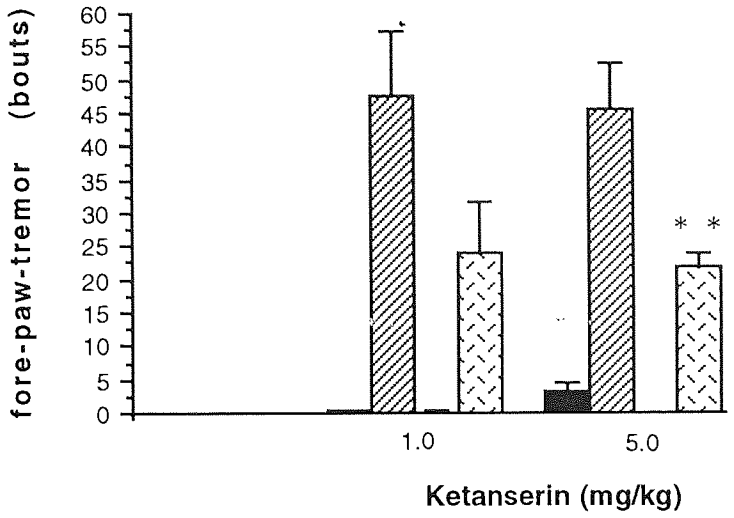


Figure 3.5. Ketanserin attenuated MK771-induced FPT. **, p<0.01*cf.* Saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Ketanserin + Saline and stippled bar, Ketanserin + MK771.

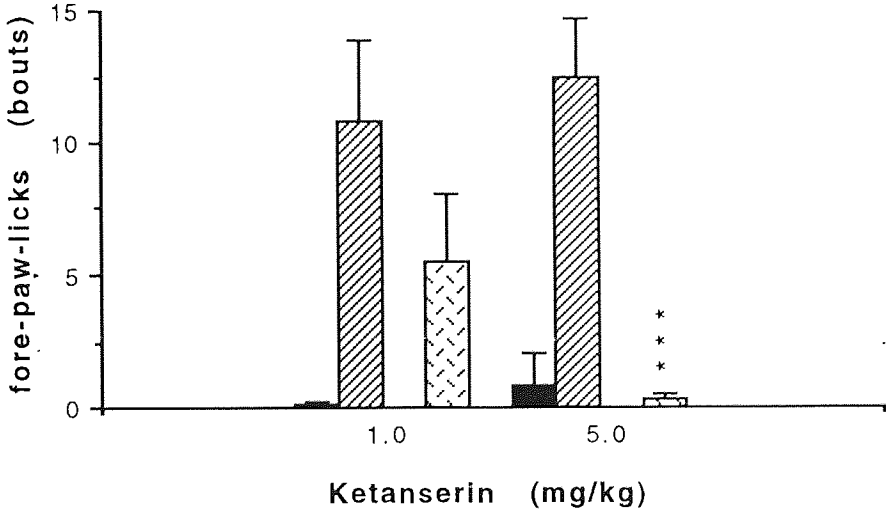


Figure 3.6. Ketanserin attenuated MK771-induced FPL. ***, p<0.001 *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Ketanserin + Saline and stippled bar, ketanserin + MK771.

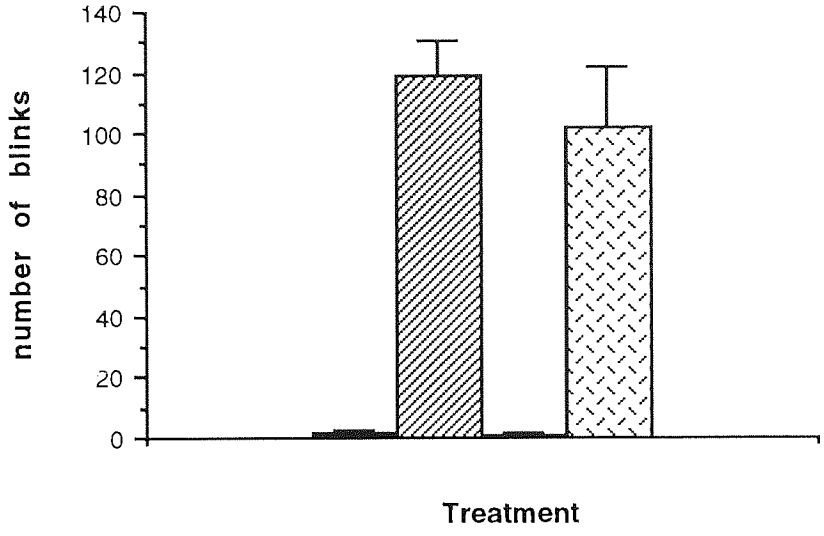


Figure 3.7. Prazosin (2.0mg/kg i.p.) lacked effect on MK771-induced blinking. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Prazosin + Saline and stippled bar, Prazosin + MK771.

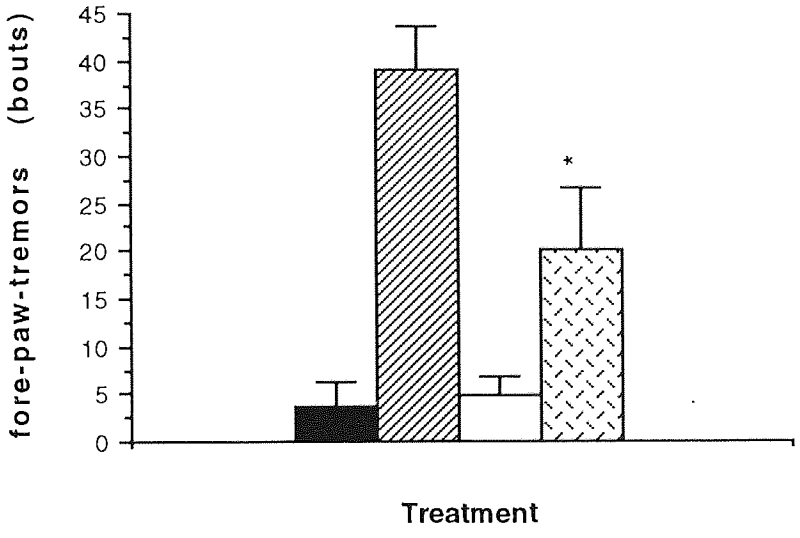


Figure 3.8. Prazosin (2.0mg/kg i.p.) attenuated MK771-induced FPT. *, $p<0.05$ *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Prazosin + Saline and stippled bar , Prazosin + MK771.

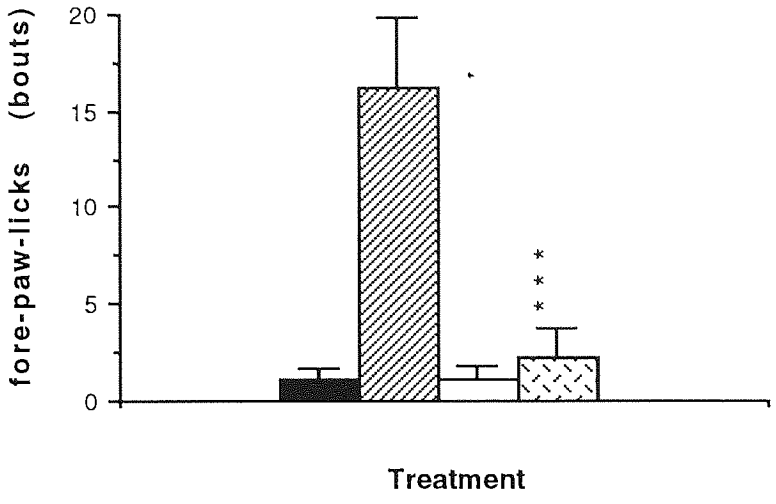


Figure 3.9. Prazosin (2.0mg/kg i.p.) attenuated MK771-induced FPL. **, p<0.01 *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Prazosin + Saline and stippled bar , Prazosin + MK771.

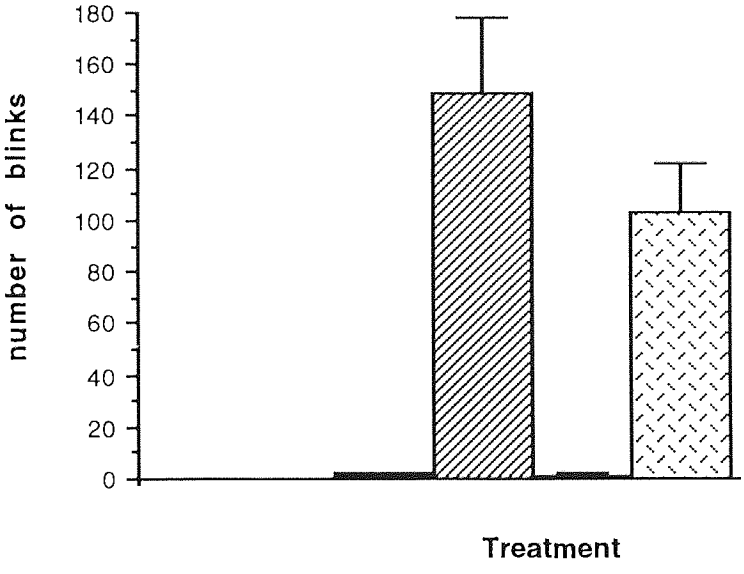


Figure 3.10. Mianserin (2.0mg/kg s.c.) was without significant effect on MK771-induced blinking. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Mianserin + Saline and stippled bar, Mianserin + MK771.

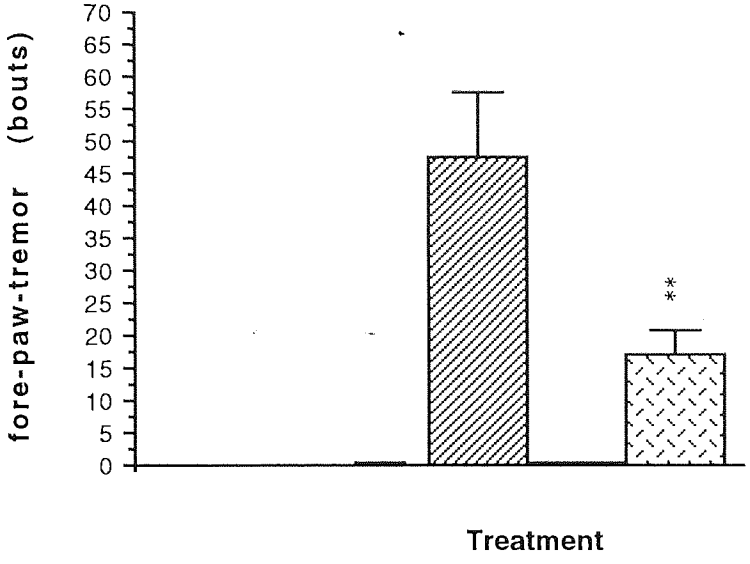


Figure 3.11. Mianserin (2.0mg/kg s.c.) attenuated MK771-induced FPT. **, $p<0.01$ *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Mianserin + Saline and stippled bar, Mianserin + MK771.

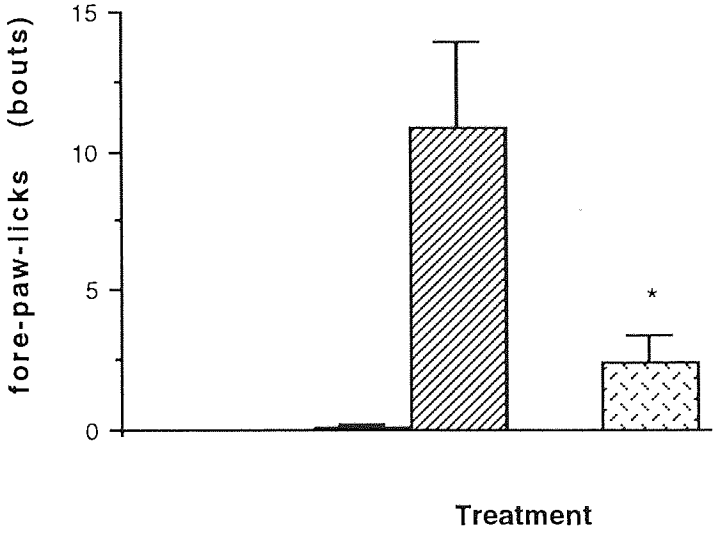


Figure 3.12. Mianserin (2.0mg/kg s.c.) attenuated MK771-induced FPL. *, $p<0.025$ *cf.* saline + MK771. Filled bar, Saline + Saline; hatched bar, Saline + MK771; open bar, Mianserin + Saline and stippled bar, Mianserin + MK771.

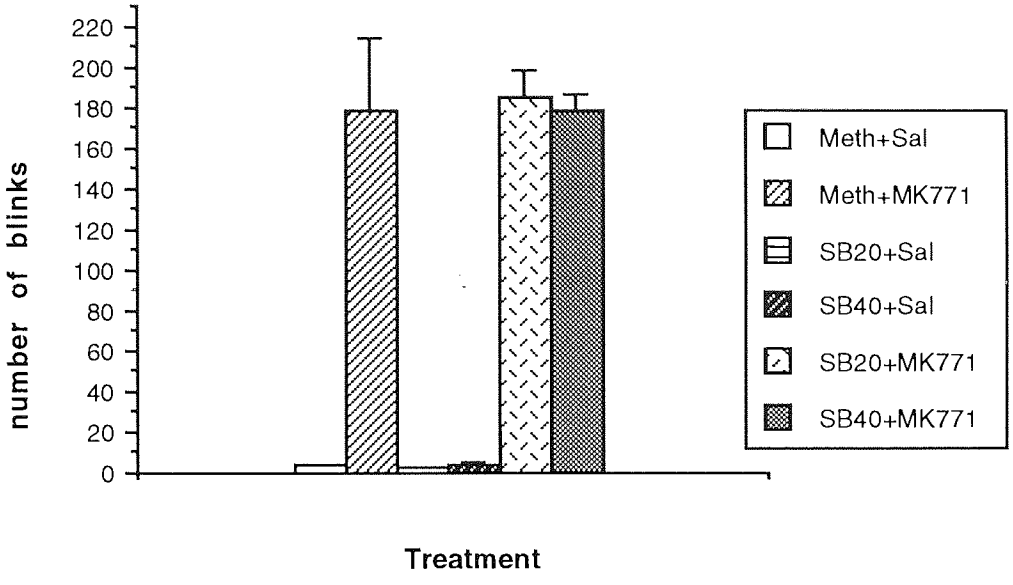


Figure 3.13. SB200646A was without effect on MK771-induced blinking.

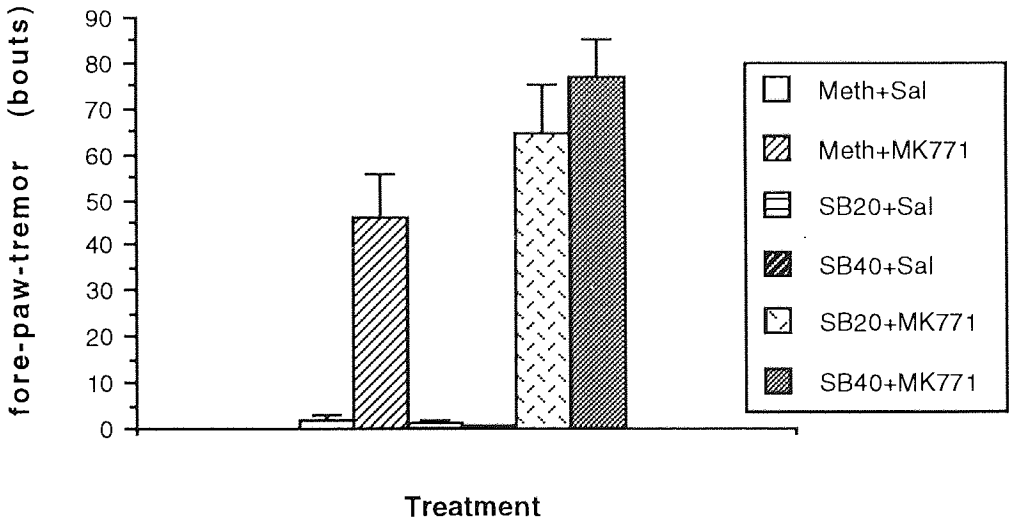


Figure 3.14. SB200646A potentiated MK771-induced FPT.

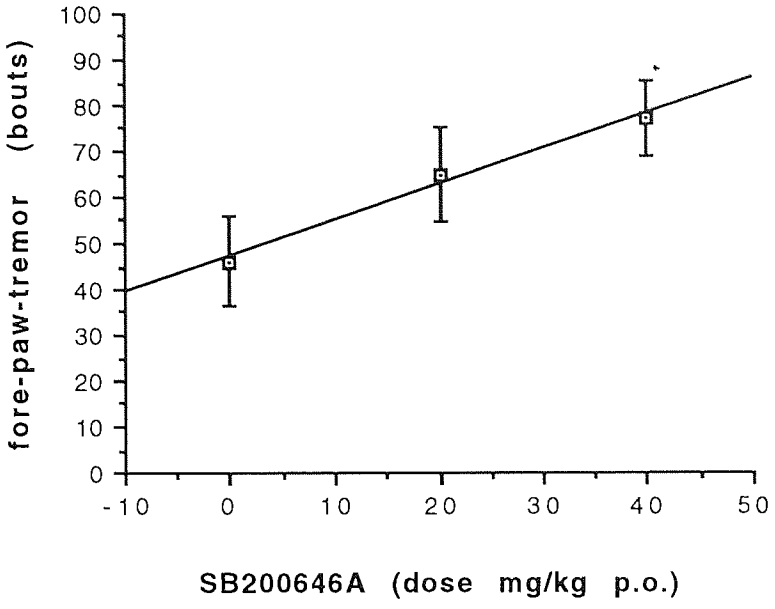


Figure 3.15. SB200646A significantly potentiated MK771-induced FPT.

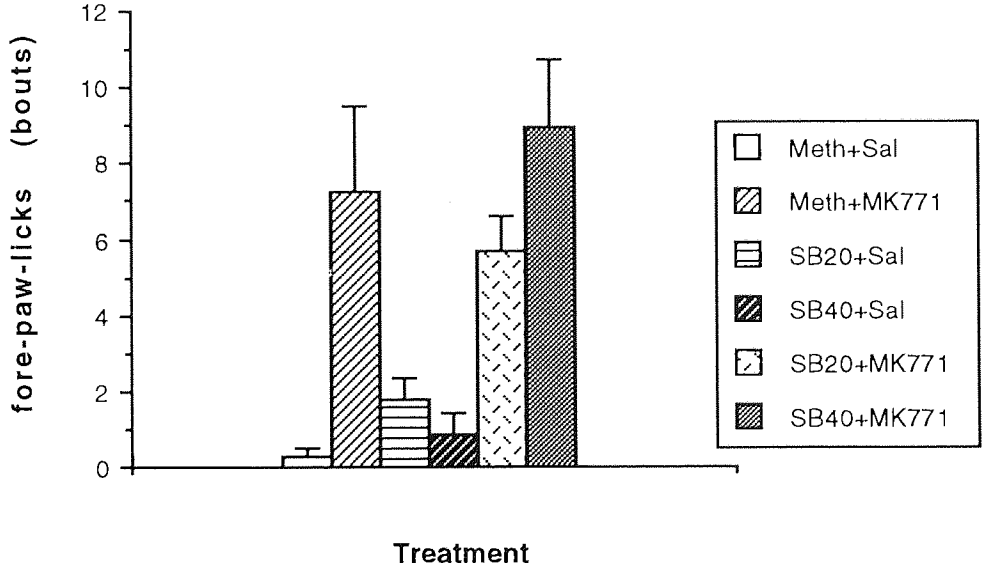


Figure 3.16. SB200646A was without effect on MK771-induced FPL.

CHAPTER 4.

THE EFFECTS OF 5-HT1A AGONISTS ON MK771-INDUCED BEHAVIOURS.

CHAPTER 4.

Introduction

Previous studies conducted both in this laboratory and by other workers have reported a series of intriguing experiments demonstrating the modulation of 5-HT2A receptor responses by a different population of receptors, namely 5-HT1A receptors. In rats the 5-HT2A/2C antagonist ritanserin potentiated locomotor activity in rats treated with the 5-HT1A agonist RU24968 (Goodwin and Green, 1985). Similarly, the 8-OH DPAT and 5-MeODMT behavioural syndrome was enhanced by prior treatment with ritanserin, ketanserin and ICI170,809 (Backus *et al.*, 1990). However, the 5-HT2A/2C agonist DOI potentiated the 8-OH DPAT induced 5-HT behavioural syndrome in the Mongolian gerbil, which was reversed by ritanserin (Eison and Wright, 1992). In the same study (Eison and Wright, 1992) the authors described a reciprocal hindlimb body scratch following DOI (which they considered to be analogous to the ear scratch seen in mice) which was potentiated by 8-OH DPAT and gepirone, consistent with the potentiation of the DOI-ear scratch response by 8-OH DPAT (Heaton and Handley, 1989). The DOI-induced HS has been inhibited by 8-OH DPAT (Arnt and Hyttel, 1989; Heaton and Handley, 1989; Darmani *et al.*, 1990; Dursun and Handley, 1993; Berendsen, 1991; Schreiber *et al.*, 1995; Kitamura *et al.*, 1994), MDL 73005 EF (Dursun and Handley, 1993), ipsapirone (Schreiber *et al.*, 1995), S14671 (Schreiber *et al.*, 1995), S14506 (Schreiber *et al.*, 1995), flesinoxan (Schreiber *et al.*, 1995) and buspirone (Dursun and Handley, 1993; Schreiber *et al.*, 1995). The mescaline-induced HS was antagonised by the 5-HT1A agonists and partial agonists 8-OH DPAT, gepirone, buspirone and BMY 14802 (Bristow *et al.*, 1991). The 5-HTP but not the 5-MeODMT-induced HS was inhibited by 8-OH DPAT (Goodwin and Green, 1985). The low doses of 5-HT1A agonists required to inhibit the quipazine-induced HS (*cf.* those required to induce the 5-HT syndrome) led to the suggestion that the population of 5-HT1A receptors responsible for the inhibition of the HS response were located pre-synaptically (Yocca *et al.*, 1990), but this hypothesis remains equivocal since administration of the 5-HT synthesis inhibitor para-chlorophenylalanine (pCPA) reverses 8-OH DPAT and buspirone inhibition of the DOI HS (Dursun and Handley, 1993) but failed to reverse the inhibition by ipsapirone (Schreiber *et al.*, 1995). It is, however, possible that two different mechanisms of 5-HT1A inhibition of the HS may occur both at the pre-synaptic and post-synaptic sites. In contrast, ipsapirone potentiated the 5-MeODMT-induced HS in rats, but this was not the case with 5-HTP (Goodwin *et al.*, 1986). Potentiation of the 5-HTP-induced HS has been noted following buspirone administration, effects were considered to be at the postsynaptic site (Kitamura *et al.*, 1994).

Results presented in Chapter 3 outlined a possible role for 5-HT2A receptor modulation of TRH analogue (MK771) induced behaviours. In the present set of experiments the 5-HT1A agonists buspirone, 8-OH DPAT and the 5-HT1A antagonist (S)-WAY-100135 were tested for their ability to modulate MK771-induced blinking, FPT and FPL. The population of 5-HT1A receptors responsible for this modulation by 5-HT1A agonists was assessed using pCPA to deplete endogenous 5-HT. The effects of (S)-WAY-100135 were assessed on the spontaneous and 5-HT2A/2C agonist (DOI) induced HS response in mice.

Additional Methods

Vehicle (saline, 10.0ml/kg i.p.), buspirone (0.5mg/kg i.p., 1.0mg/kg i.p., 5.0mg/kg i.p., 10.0mg/kg i.p. and 20.0mg/kg i.p.; 20-30g, n=6-10 mice/treatment group), 8-OH DPAT (0.05mg/kg i.p., 0.1mg/kg i.p., 1.0mg/kg i.p. and 5.0mg/kg i.p.; 20-30g, n=6-11 mice/treatment group) or (S)-WAY-100135 (3.0 and 5.0mg/kg s.c.) were administered 15 min. prior (or 30min prior-(S)-WAY-100135 experiment) to MK771 (2.5mg/kg i.p.) or saline vehicle. Blinking, FPL and FPT behaviours were counted 7.5 min. after saline or MK771 administration for a 5 min. period.

pCPA depletion studies

pCPA pre-treatment time evaluation

A suitable duration of pre-treatment with pCPA to induce maximal depletion of brain 5-HT was assessed. Mice were injected with either vehicle (0.9% saline, 10.0ml/kg i.p.) or pCPA (methyl ester) (150mg/kg i.p., dose calculated as free base) each evening (16:00 - 18:00) for 3, 7 or 13 days (20-30g; n=10 per treatment group). Brains were removed the day after, the cerebellum was removed and the brains snap frozen in liquid nitrogen and stored on dry ice for transportation to Boots Pharmaceuticals for determination of brain monoamine concentration (see later).

Experimental sessions

Mice (20-30g in weight, n=10 per treatment group) were pre-treated with saline vehicle or 150mg/kg pCPA (dose calculated as the free base) for 7 days prior to experimental sessions (16:00 - 18:00). MK771 (2.5mg/kg i.p.) treated mice were pre-treated (7.5 minutes earlier) with buspirone (1.0 or 20.0mg/kg i.p.), 8-OH DPAT (0.1 or 5.0mg/kg i.p.) or saline vehicle and blinking, FPL and FPT behaviours were counted 7.5 min. after MK771 for a 5 min. period. Immediately after each experimental session the mice were killed by cervical dislocation, the brain removed from the skull and the cerebellum dissected out. The brain (-cerebellum) was then snap frozen in liquid nitrogen, stored on dry ice (for the

duration of the experiment), transferred to a -70°C freezer until transport to Boots (on dry ice) for monoamine determination.

Each experiment (8-OH DPAT or buspirone) was split into half and conducted over a 2 day period for logistical reasons.

Measurement of the brain monoamine concentrations

Measurement of the brain monoamines NA, 5-HT and DA was performed by high-performance-liquid-chromatography with electrochemical detection (HPLC-ECD) at Boots Research Nottingham (now Knoll Pharmaceuticals) in order to confirm the extent of the pCPA depletion procedure. Tissues were homogenised in 10 volumes (w/v) of 0.1M percloric acid containing 0.4mM sodium metabisulphite (antioxidant) and 0.8μM isoprenaline (internal standard) using a polytron PT 10-35 disruptor (setting 5-6). After centrifugation at 1100g for 15min. at 4°C and 15000g for 5min. at 4°C, 50μl of the resulting supernatant was injected onto the HPLC system. The HPLC system consisted of a Spectra Physics 8810 pump (flow rate 1.0 ml/min) connected via a WISP 712 refrigerated autoinjector to a 5μm Hypersil ODS-1 reversed-phase column (length 250mm x 4.6mm internal diameter [i.d.]) maintained at 45°C and protected by a Brownie Aquapore RP-300 precolumn (length 30 x 4.6mm i.d.). The mobile phase was 0.1M sodium dihydrogen orthophosphate-orthophosphoric acid buffer (pH 3.3) containing 16% (v/v) methanol, 2.8mM 1-octanesulphonic acid sodium salt and 0.7 mM EDTA. NA, 5-HT and DA were detected with a BAS LC-4 amperometric detector connected to a TL-5 flow cell set at a potential of +0.75V versus a silver/silver chloride electrode. Concentrations of monoamines (ng/g wet tissue) were quantified by reference to the recovery of the internal standard isoprenaline.

The effects of (S)-WAY-100135 on spontaneous or DOI head shakes.

Saline vehicle (10.0ml/kg s.c.) or (S)-WAY-100135 (3.0, 5.0, or 10.0mg/kg s.c.) were administered 30min. prior to counting spontaneous HSs for 30min., with the experimenter blind to the treatment each mouse had received.

Saline vehicle (10.0ml/kg s.c.) or (S)-WAY-100135 (5.0 and 10.0mg/kg s.c.) were administered 30min. prior to a submaximal dose of DOI (1.0mg/kg i.p.). DOI-induced HSs were counted 5min. later for a 6min. period, with the experimenter blind to the treatment each mouse had received.

Statistical analyses: in the preliminary set of experiments unpaired t-tests were carried out between [Saline + MK771] and [Drug + MK771] groups following a non-significant effect of drug ([Drug + Saline] *cf.* [Saline + Saline]). Group differences in the pCPA depletion experiment were analysed using a 3-Way ANOVA (with agonist, pCPA and day acting as factors) model and following significant effects *post-hoc* tests were performed (Student's t-test with Bonferoni

correction as required (two comparisons were performed and the new probability value for significance was $p<0.025$). Following a significant effect of day of experimentation or day interaction each study was split into two separate experiments (day 1 and day 2) and 2-way ANOVA carried out, where necessary *post-hoc* tests were performed as above. Tukey's test was used to compare the effects of pCPA on monoamine concentrations in the 5-HT1A agonist studies. 1-Way ANOVA was performed on the effects of (S)-WAY-100135 on MK771-induced blinking, FPL and FPT and spontaneous and DOI HSs.

Results

8-OH DPAT

8-OH DPAT attenuated MK771-induced blinking at the 0.1mg/kg dose tested ($p<0.005$), but was ineffective at all other doses tested (0.05, 1.0 and 5.0mg/kg), *figure* 4.1. FPT was attenuated by 8-OH DPAT at the higher dose tested (5.0mg/kg) ($p<0.01$), *figure* 4.2 and 8-OH DPAT attenuated FPL at all but the lowest dose of 8-OH DPAT used (*ie.* 0.1mg/kg, $p<0.05$; 1.0mg/kg, $p<0.005$ and 5.0mg/kg, $p<0.025$), *figure* 4.3).

Buspirone

Buspirone significantly attenuated blinking at a dose of 1.0mg/kg ($p<0.025$) and a non-significant trend was noted at the lowest dose tested (0.5mg/kg, $p<0.10$), the other doses tested (5.0, 10.0 and 20.0mg/kg) were without effect on MK771-induced blinking, *figure* 4.4). FPT was dose dependently attenuated by buspirone (0.5mg/kg, not significant; 1.0mg/kg, $p<0.005$; 5.0mg/kg, $p<0.0005$; 10.0mg/kg, $p<0.0005$ and 20.0mg/kg, $p<0.0005$), *figure* 4.5 and had similar effects on MK771 induced FPL (0.5mg/kg, not significant; 1.0mg/kg, $p<0.025$; 5.0mg/kg, $p<0.005$; 10.0mg/kg, $p<0.05$ and 20.0mg/kg, $p<0.01$), *figure* 4.6.

(S)-WAY-100135

(S)-WAY-100135 was without effect on MK771-induced blinking, FPT and FPL (*figure* 4.7).

pCPA pre-treatment time evaluation

pCPA depletion of 5-HT in mouse brain was highest when pCPA (150mg/kg) was administered daily over a 7 day time period (*table* 4.1). This treatment regimen was used in all further studies with pCPA.

pCPA-behavioural sessions

These were split into two equal sessions, conducted over two days. For this reason the day of experiment was also taken into account in the final statistical analyses of the experiments.

8-OH DPAT

Blinking. Significant main effects of 8-OH DPAT ($F(2,45)=3.44$, $P<0.041$) were noted on blinking, but pCPA was without effect ($F(1,45)=0.27$, $p<0.605$) following 3-Way ANOVA . The lower dose of 8-OH DPAT tested (0.1mg/kg) significantly attenuated blinking in saline ($p<0.025$, *figure* 4.8) but not pCPA pre-treated mice ($p<0.10$, *figure* 4.8). *Post-hoc* analyses of the main effect of day ($F(1,45)=7.09$, $p<0.011$) revealed significant differences between day 1 and day 2 in 8-OH DPAT (0.1 ($p<0.05$) and 5.0mg/kg ($p<0.01$)) pCPA pre-treated mice, *figure* 4.9. 2-way ANOVA of day 1 and day 2 effects revealed a significant main effect of 8-OH DPAT on day 1 ($F(2,23)=4.73$, $p<0.019$) but not on day 2. However, in *post-hoc* tests no significant effects were observed.

Fore-paw-tremor. 8-OH DPAT significantly affected MK771-induced FPT ($F(2,47)=7.88$, $p<0.001$), *post-hoc* tests revealed attenuation by the high dose of 8-OH DPAT tested (5.0 mg/kg) in saline pre-treated mice ($p<0.005$, *figure* 4.9) but not pCPA pre-treated mice ($p<0.10$, *figure* 4.10). No effect of day or other interactions were noted for this behaviour.

Fore-paw-licking. Significant effects of 8-OH DPAT ($F(2,44)=11.34$, $p<0.0001$) and significant 8-OH DPAT x day interaction ($F(2,44)=4.26$, $p<0.02$) were recorded. *Post-hoc* tests revealed a significant attenuation of MK771-induced FPL by 8-OH DPAT only at the higher dose tested (5.0mg/kg) in saline pre-treated ($p<0.005$) and pCPA pre-treated mice ($p<0.005$), but attenuation by the lower dose did not reach significance, *figure* 4.11. Given the significant 8-OH DPAT x day interaction the effects of day 1 and day 2 were separated and treated as two separate experiments (*figure* 4.12). 2-Way ANOVA was carried out on each day of experimentation and significant effects of 8-OH DPAT on day 2 only ($F(2,24)=0.63$, $p<0.002$) were observed. *Post-hoc* analyses revealed significant attenuation of FPL in 8-OH DPAT (0.1 and 5.0mg/kg, $p<0.01$) treated mice pre-treated with saline, significant attenuation was also demonstrated in the pCPA pre-treated mice given the higher dose of 8-OH DPAT (5.0mg/kg, $p<0.01$), *figure* 4.12.

Biochemical analyses. pCPA had no effect on whole brain (- cerebellum) concentration of DA ($F(1,47)=1.03$, $p<0.226$) or NA ($F(1,47)=0.42$, $p<0.520$) but 5-HT was attenuated in the same mice ($F(1,47)=943.61$, $p<0.0001$). *Post-hoc* tests revealed significant depletion of 5-HT concentration in saline or 8-OH DPAT (0.1 and 5.0mg/kg) mice pre-treated with pCPA *cf.* saline pre-treated mice ($p<0.005$, *table* 4.3). Significant effects of day ($F(1,47)=43.99$, $p<0.0001$) and day x pCPA ($F(1,47)=0.014$, $p<0.014$) were observed on DA concentrations. It was found that DA concentrations were significantly attenuated on day 2 *cf.* day 1 in the saline and 8-OH DPAT (0.1 and 5.0mg/kg) groups in the saline pre-treated but not the pCPA pre-treated groups ($p<0.01$, *figure* 4.13). Splitting the results into day of

experiment revealed significant effects of pCPA on day 1 ($F(1,28)=1035.25$, $p<0.0001$), but not day 2. *Post-hoc* tests revealed that there was a significant difference between saline and pCPA pre-treated mice given 8-OH DPAT (5.0mg/kg, $p<0.05$), *figure* 4.13. Similarly NA ($F(1,47)=43.99$, $p<0.0001$) and 5-HT ($F(1,47)=18.18$, $p<0.0001$) concentrations were reduced on day 2 *cf.* day 1 in saline but not pCPA pre-treated mice given 8-OH DPAT (0.1 and 5.0mg/kg) (NA; $p<0.01$ and $p<0.005$, (*figure* 4.14) and 5-HT; $p<0.01$ and $p<0.01$ (*figure* 4.15), respectively). 2-Way ANOVA of 5-HT concentrations on days 1 and 2 revealed a significant effect of pCPA ($F(1,28)=8.84$, $p<0.007$; day 1 and $F(1,24)=189.23$. $p<0.0001$; day 2) and significant *post-hoc* tests were found in each of the agonist or vehicle treated groups. 2-way ANOVA of NA concentrations on day 1 or day 2 failed to reveal any significant effects.

Buspirone

Blinking. Significant effects of buspirone ($F(2,46)=3.28$, $p<0.047$), pCPA x buspirone ($F(2,46)=4.94$, $p<0.011$) and pCPA x buspirone x day ($F(2,46)=3.27$, $p<0.047$) were observed on blinking following 3-Way ANOVA. The lower dose of buspirone tested (1.0mg/kg) significantly attenuated blinking in saline ($p<0.005$, *figure* 4.16) but not pCPA pre-treated mice. The higher dose of buspirone (20.0mg/kg) failed to alter MK771-induced blinking in saline pre-treated mice, but significantly increased blinking in pCPA pre-treated animals *cf.* pCPA + saline control animals ($p<0.025$), *figure* 4.16. No significant effects of pCPA were found in *post-hoc* tests. *Post-hoc* analyses of the effects of the day of experimentation revealed a significant difference between day 1 and day 2 in control animals [saline+saline+MK771] ($p<0.01$, *figure* 4.17). On day 1 significant pCPA x buspirone interactions were found ($F(2,24)=3,94$, $p<0.037$), but no effects were seen on day 2. Significance from saline vehicle group was found in buspirone (1.0 and 20.0mg/kg; $p<0.0002$ and $p<0.0019$, respectively), no differences were found between pCPA and saline pre-treated animals in each of the drug groups tested, *figure* 4.17.

Fore-paw-tremor. Buspirone significantly affected MK771-induced FPT ($F(2,43)=65.13$, $p<0.0001$) but no effects of pCPA, day or interactions involving these factors were observed, *post-hoc* tests revealed a significant attenuation of MK771-induced FPT in saline pre-treated mice (1.0mg/kg, $p<0.025$; 5.0mg/kg, $p<0.0005$) and pCPA pre-treated mice (5.0mg/kg of buspirone only, $p<0.0005$), *figure* 4.18.

Fore-paw-licking. Significant effects of buspirone alone were noted ($F(2,44)=4.71$, $p<0.014$) but not pCPA or day of experiment. *Post-hoc* tests revealed that buspirone (20.0mg/kg) significantly attenuated FPL in saline but not

pCPA pre-treated mice ($p<0.01$), but no effects of the lower dose of buspirone (1.0mg/kg) were observed, *figure* 4.19.

Biochemical analyses. pCPA had no effect on whole brain (- cerebellum) concentration of DA ($F(1,48)=0.36$, $p<0.554$) or NA ($F(1,48)=0.02$, $p<0.896$) but 5-HT concentrations were altered in the same mice ($F(1,48)=910.17$, $p<0.0001$), no significant effects of day of experimentation were observed. *Post-hoc* tests revealed significant depletion of 5-HT concentration in saline or buspirone (1.0 and 20.0mg/kg) mice pre-treated with pCPA *cf.* saline pre-treated mice ($p<0.005$, *table* 4.4). However, significant effects of buspirone on NA ($F(1,48)=4.02$, $p<0.024$) and DA ($F(1,48)=10.81$, $p<0.0001$) concentrations were observed. *Post-hoc* analyses revealed a significant attenuation in DA concentrations in buspirone (20.0mg/kg) treated mice given prior saline and pCPA ($p<0.005$, *table* 4.4). NA concentrations were reduced in saline pre-treated mice by the higher dose of buspirone (20.0mg/kg) only ($p<0.025$), but a non-significant suggestion of attenuation was indicated for the same group pre-treated with pCPA ($p<0.05$, N.B. significance was re-defined as $p<0.025$ as a Bonferoni correction term was used), *table* 4.4.

The effects of (S)-WAY-100135 on Spontaneous and DOI HSs.

(S)-WAY-100135 had no effect on spontaneous ($F(2,29)=1.71$, $p<0.202$) (*figure* 4.20) and DOI-induced ($F(2,34)=1.67$, $p<0.206$) HS (*figure* 4.21).

Discussion

Data presented here suggest that low doses of 5-HT1A agonists attenuate MK771-induced blinking in mice, conversely higher doses of the same agonists attenuated FPL and FPT. Depletion studies with pCPA were performed over three different treatment durations 3, 7 and 13 days to obtain the highest level of 5-HT depletion, this was achieved following the 7 day treatment protocol (*tables* 4.1 and 4.2). Subsequent experiments were performed using this protocol. Other investigators have found that a 3 day pre-treatment regimen depleted 5-HT to 71.5% (using 300mg/kg) (Dursun and Handley, 1993), which reversed the ability of 8-OH DPAT and buspirone to inhibit the DOI HS response in mice. In the studies with the 5-HT1A agonists 8-OH DPAT and buspirone presented here significant 5-HT depletions of 70.10 - 76.65% ($p<0.005$, 8-OH DPAT, *table* 4.3) and 72.7 - 79.0% ($p<0.005$, buspirone, *table* 4.4) were achieved. Therefore, the levels of 5-HT depletion achieved here should elucidate whether pre- or post-synaptic actions of the agonists inhibit MK771-induced blinking, FPT and FPL. The dose used by Dursun and Handley (1993) was tested in preliminary studies but poor depletion of 5-HT was found, the decision to use 150mg/kg (free base dose) was taken in collaboration with Mr. Mike Prow at Boots Pharmaceuticals (Knoll) and in the

preliminary duration of administration studies good depletion was achieved using the 7day treatment regimen (mentioned above and refer to *tables* 4.1 and 4.2).

In the depletion studies day variations in the monoamines were noted (see later discussion). Buspirone (20.0mg/kg) significantly decreased DA concentrations in both saline and pCPA pre-treated mice, *table* 4.4. Similarly NA was decreased in the same treatment group in saline pre-treated mice, but this did not reach significance (non-significant trend) in pCPA pre-treated mice, *table* 4.4. These reductions in DA and NA concentrations are consistent with the reduction of these monoamines following buspirone administration in the rat in which an increase in turnover of DA and NA was recorded (Fuller and Perry, 1989).

It might be expected that 5-HT1A agonists would modulate tic-like behaviours induced by MK771 given the effects of 5-HT2A antagonists on blinking, FPT and FPL (Chapter 3) and the effects of 5-HT1A agonists on another tic like behaviour, the 5-HT2A mediated HS. For example, the DOI-induced HS was inhibited by 8-OH DPAT (Arnt and Hyttel, 1989; Heaton and Handley, 1989; Darmani *et al.*, 1990; Dursun and Handley, 1993; Berendsen, 1991; Schreiber *et al.*, 1995; Kitamura *et al.*, 1994), buspirone (Dursun and Handley, 1993; Schreiber *et al.*, 1995) and other 5-HT1A agonists (see above). Indeed this inhibition of the HS response parallels the inhibition of MK771-induced blinking seen following administration of low doses of 8-OH DPAT (0.1mg/kg) and buspirone (0.5mg/kg (non-significant trend) and 1.0mg/kg) (*figures* 4.1 and 4.4, respectively) in the preliminary studies. However, studies have demonstrated that the inhibition of HSs by 5-HT1A agonists is dose dependent (Schreiber *et al.*, 1995; Yocca *et al.*, 1990; Dursun and Handley, 1993). This dose related attenuation is consistent with the attenuation of FPT and FPL seen with increasing doses of 5-HT1A agonists. We initially believed that the ability of only lower doses to antagonise MK771-induced blinking might reflect a balance between pre- and post-synaptic effects in so far as an action of the low dose of agonist at the pre-synaptic site attenuated MK771-induced blinking, owing to the greater receptor reserve of this population of receptors (Meller *et al.*, 1990), and that these effects were overcome by the high doses due to an action at post-synaptic 5-HT1A sites reversing the inhibition.

In the serotonergic depletion studies 8-OH DPAT (at 0.1mg/kg but not 5.0mg/kg) significantly attenuated MK771-induced blinking in saline pre-treated mice. In pCPA pre-treated mice this effect failed to reach significance but this non-significant attenuation was of similar magnitude to that seen in saline pre-treated mice so it is likely that the lack of significance may reflect experimental noise, especially since pCPA itself had no effect on blinking, *figure* 4.8. Furthermore, a similar pattern of results was observed in saline and pCPA treated animals on day 1. On day 2 pCPA might have partially antagonised the 8-OH DPAT (0.1mg/kg) response, *figure* 4.9.

Significant effect of day of experiment were observed in the pCPA + 8-OH DPAT (0.1 and 5.0mg/kg) experimental groups (*figure* 4.9). Significant main effects of 8-OH DPAT were observed on day 1 alone, but no *post-hoc* differences were found. The most probable reason for this, and the lack of effects on day 2 is that the sample sizes (n=5/group/day) are too small to reveal any changes. Buspirone (1.0 and 20.0mg/kg) had similar effects to 8-OH DPAT on MK771-induced blinking in saline pre-treated mice *ie.* significant attenuation at the lower dose but not at the higher dose, *figure* 4.16. However, in pCPA pre-treated mice 20.0mg/kg of buspirone increased blinking *cf.* pCPA + saline control group ($p<0.025$), *figure* 4.16. There were no significant differences between saline and pCPA pre-treated mice administered with saline vehicle, indicating that the anomalous results may be due to experimental variability. Splitting the results into day of experiment revealed attenuation of blinking by both doses of buspirone tested, but no effect of pCPA was seen (*figure* 4.17). Ideally both of the 5-HT1A agonist experiments would need to be repeated, preferably performing each experiment over one day. In summary it is not possible to conclude, categorically, that the effect of 8-OH DPAT is either at postsynaptic site(s) or at pre-synaptic site(s). It might be possible that multiple sites were involved in the effects of the agonists and this is the reason for the 'cloudy' results.

In the preliminary set of experiments FPT was only attenuated by the highest dose of 8-OH DPAT tested (5.0mg/kg, *figure* 4.2), which was paralleled in the pCPA study in saline pre-treated mice (*figure* 4.9). In pCPA pre-treated mice the response was blunted considerably and failed to reach significance (*figure* 4.10), indicating either a partial antagonism by pCPA or, since pCPA was not a significant factor in the 3-way ANOVA term and antagonism was still noted in buspirone pre-treated mice (see later), it is possible that this is due to experimental noise. Buspirone attenuated FPT (1.0, 5.0, 10.0 and 20.mg/kg, *figure* 4.5) in the preliminary experiments and also in pCPA and saline pre-treated mice (*figure* 4.18).The failure of pCPA to effect the inhibition of MK771-induced FPT by buspirone (5.0mg/kg) suggests a post-synaptic location for the 5-HT1A receptor mediating these effects. However, it is difficult to make any firm conclusions (based on the 8-OH DPAT findings), so the results remain inconclusive. Buspirone and 8-OH DPAT attenuated MK771-induced FPL (*figures* 4.3 and 4.5, respectively). In the pCPA studies this also seemed apparent for 8-OH DPAT in saline pre-treated mice and pCPA pre-treated mice (*figure* 4.11). Similar effects were observed for buspirone, but the attenuation of FPL in mice treated with the high dose of buspirone (20.0mg/kg) failed to reach significance (*figure* 4.19), but the attenuation is marked and the lack of significance is probably due to experimental noise. The suggestion is therefore that these effects of the 5-HT1A agonists tested are mediated through

their actions at a post-synaptic 5-HT1A site, especially given the null-effect of pCPA (or any pCPA interactions) in the mediation of FPL in the 8-OH DPAT or buspirone experiments. Effects of day were suggested by a significant 8-OH DPAT x day interaction, but no differences were found with *post-hoc* testing (*figure* 4.12). However, a different pattern of results was displayed, insofar as there was a dose dependent reduction in FPL on day 2 but on day 1 the saline control value was essentially the same as that in mice receiving 0.1mg/kg 8-OH DPAT(*figure* 4.12), significant attenuation of FPL was noted in both saline and pCPA pre-treated mice. The reasons for the variations displayed in day of testing are unclear. DA concentrations are significantly lower on day 2 (*figure* 4.13), but it is not thought that this would alter MK771-induced FPL or blinking since no effect of DA antagonists was seen (Chapter 2). NA and 5-HT concentrations were attenuated on day 2 in both of the 8-OH DPAT treated groups, but these changes were only minor and may reflect experimental noise, given the low sample number (n=5). The most probable cause is due to behavioural variation due to the day of testing due to some un-controllable extraneous factor. The attenuation of MK771-induced FPL by buspirone and 8-OH DPAT in this study presented here is consistent with the attenuation of FPL behaviour by 8-OH DPAT administration (25nM) directly into the caudal periaqueductal grey supporting a role for post-synaptic agonist action at 5-HT1A receptors in the behavioural inhibition of FPL behaviour (Beckett *et al.*, 1992). However, the population of the 5-HT1A receptors which modulates MK771-induced blinking, FPT and FPL remains unclear, as does the population of TRH receptors mediating these effects.

It is possible that the depletion of 5-HT achieved in this study is not sufficiently high enough to elucidate whether the MK771-induced behaviours are pre- or post-synaptic. Such that the remaining endogenous 5-HT will cloud the results. In order to overcome this one possibility is to use 5,7-DHT which would cause a 90% or greater depletion.

In summary the attenuation of MK771-induced FPL seen here appears to be due to an action of the 5-HT1A agonists at post-synaptic receptors. However, data presented here is inconclusive as to the site of action(s) of 5-HT1A agonists which inhibit MK771-induced blinking and FPT. It is unclear whether this interaction is via a direct interaction between 5-HT1A receptors and TRH receptors, since 5-HT2A receptors clearly modulate these behaviours (Chapter 3) and post-synaptic interactions between 5-HT2A receptors and 5-HT1A receptors have been described in HS studies (see above). Schreiber and colleagues (1995) suggested that this may be due to opposing effects on secondary messenger systems. 5-HT1A receptors are positively coupled to K^+ channels, negatively coupled to adenylate cyclase and directly inhibit phosphoinositide turnover, whereas 5-HT2A receptors demonstrate

opposite effects on signal transduction mechanisms (for review see Schreiber *et al.*, 1995). Thus by administering a 5-HT1A agonist a functional inhibition of 5-HT2A receptors may occur. Similarly TRH receptors exert their mechanism of action via phosphoinositide formation (Gershengorn, 1986), so a functional inhibition of TRH receptors may occur following 5-HT1A receptor stimulation.

(S)-WAY-100135 lacked efficacy in the 3 paradigms studied. No effect was seen on MK771-induced blinking, FPT and FPL. One reason for the lack of effect on blinking may be due to the doses of (S)-WAY-100135 used, insofar as it appears that there is a narrow window where the effects of 5-HT1A agonists inhibit blinking (*figures* 4.1 and 4.4). The lack of effect on FPL and FPT is, however, more difficult to reconcile. It would be expected that (S)-WAY-100135 would potentiate blinking induced by MK771. It is possible that stimulation of TRH receptors with the analogue MK771 does not increase 5-HT1A receptor tone and therefore (S)-WAY-100135 is without effect. The lack of tone in the serotonergic system is probably the reason for the lack of effect of (S)-WAY-100135 on the spontaneous HS in mice and is supported by the lack of effect of the non-selective 5-HT1A antagonist (-)-tertatolol (Schreiber *et al.*, 1995). In the DOI-induced HS studies it would be expected that (S)-WAY-100135 would increase the HS frequency, since 5-HT1A agonists attenuate this response (*eg.* Arnt and Hyttel, 1989; Heaton and Handley, 1989; Darmani *et al.*, 1990; Bristow *et al.*, 1991; Dursun and Handley, 1993; Berendsen, 1991; Schreiber *et al.*, 1995; Kitamura *et al.*, 1994), however studies in this laboratory have indicated that this response is mediated by the somatodendritic autoreceptor, thus even though there is serotonergic tone (via DOI acting at 5-HT2A receptors) this is postsynaptic and pre-synaptic 5-HT1A tone would theoretically remain unchanged and (S)-WAY-100135 would be without effect. In support of the findings presented here the 5-HT1A antagonists (-)-UH301 and WAY-100635 were without effect on DOI-induced WDS in rats (Renyi and Jiménez, 1994) and the non-selective 5-HT1A antagonist (-)-tertatolol lacked effect on the DOI-induced HS (Schreiber *et al.*, 1995).

In conclusion it appears that 5-HT1A agonists may attenuate MK771-induced blinking at lower doses only, FPT and FPL appear to be inhibited at higher doses of these drugs and it is suggested that the actions of these drugs to inhibit FPL are located post-synaptically. The locus/loci of the inhibition of blinking and FPT are inconclusive. Whether this is via a direct interaction of TRH and 5-HT1A receptors or some other pharmacological scenario remains to be established. (S)-WAY-100135 lacks effect on spontaneous and DOI head shaking and on MK771-induced blinking, FPT and FPL.

DURATION	TREATMENT	NA	DA	5-HT
3 days	Saline	354.08±8.84	887.29±14.18	664.35±27.5
	pCPA	401.16±11.21	857.74±16.90	192.30±10.35
7 days	Saline	452.06±11.58	1007.5±26.59	788.78±39.632
	pCPA	423.47±25.29	1104.26±44.56	140.27±10.57
13 days	Saline	470.48±24.63	1049.90±42.50	618.90±20.35
	pCPA	428.69±14.37	1092.07±19.55	242.27±14.789

Table 4.1. Showing the concentration (ng/g wet weight tissue, mean±SEM, n=10 per treatment group) of nor-adrenaline (NA), dopamine (DA) or 5-hydroxytryptamine (5-HT) in cerebellectomized mouse brain following pCPA or saline treatment for 3, 7 or 13 days.

TREATMENT	NA	DA	5-HT
3 days	+13	3	71
7 days	+7	9	82
13 days	9	+4	61

Table 4.2. Showing the percentage depletion (*cf.* saline controls) of monoamine concentrations following 3, 7 or 13 days administration of vehicle or pCPA.

TREATMENT	Sal+ Sal	Sal+ DPAT 0.1	Sal+ DPAT 5.0	pCPA+ Sal	pCPA+ DPAT 0.1	pCPA+ DPAT
NA	288.2±19.5	283.5±17.5	261.2±16.2	272.9±11.5 (**5.31 %**)	268.6±14.9 (**5.25 %**)	265.6±19.3 (**+1.68 %**)
DA	974.2±66.5	1020.2±63.3	984.2±60.2	950.4±38.5 (**2.44 %**)	990.6±49.4 (**2.90 %**)	911.2±43.4 (**7.42 %**)
5-HT	918.6±36.5	880.5±37.5	956.9±47.7	221.9±16.7 (**75.84 %**) **	209.7±16.0 (**70.10 %**) **	223.4±24.0 (**76.65 %**) *

Table 4.3 Monoamine levels in mouse brain (ng/g wet tissue), measured by HPLC-ECD following behavioural measurements of blinking, FPT and FPL in MK771-treated mice in saline or pCPA pre-treated mice given saline or 8-OH DPAT (0.1 or 5.0mg/kg i.p.) prior to MK771. Values in bold brackets indicate percentage depletion. **, $p<0.005$ significant depletion of 5-HT *cf.* with relative controls. Data expressed in ng/g of monoamine in wet tissue.

TREAT	Sal+ Sal	Sal+ Busp 1.0	Sal+ Busp 20.0	pCPA+ Sal	pCPA+ Busp 1.0	pCPA+ Busp 20.0
NA	278.1±10.6	267.1±9.8	236.5±15.30 +	268.2±12.2 **(3.55 %)**	265.4±11.6 **(0.41 %)**	234.7±13.7 **(0.76 %)** §
DA	1122.3±28.1	1098.1±28.8	901.2±18.1 ++	1120.7±28.1 **(0.14 %)**	1060±106 **(3.46 %)**	903.6±20.1 **(+0.26 %)**++
5-HT	916.7±35.3	932.1±24.6	962.9±42.9	244.0±21.2 **(73.38 %)** **	254.3±16.8 **(72.7 %)** **	201.5±11.6 **(79.0 %)** **

Table 4.4. Monoamine levels in mouse brain (ng/g wet tissue), measured by HPLC-ECD following behavioural measurements of blinking, FPT and FPL in MK771-treated mice in saline or pCPA pre-treated mice given saline or buspirone (1.0 or 20.0mg/kg i.p.) prior to MK771. Values in bold brackets indicate percentage depletion. +, $p<0.025$; ++, $p<0.0005$, significantly different from saline + saline or pCPA + saline controls or §, $p<0.05$ not significantly different from saline. **, $p<0.005$ significant depletion of 5-HT *cf.* with controls. Data expressed in ng/g of monoamine in wet tissue.

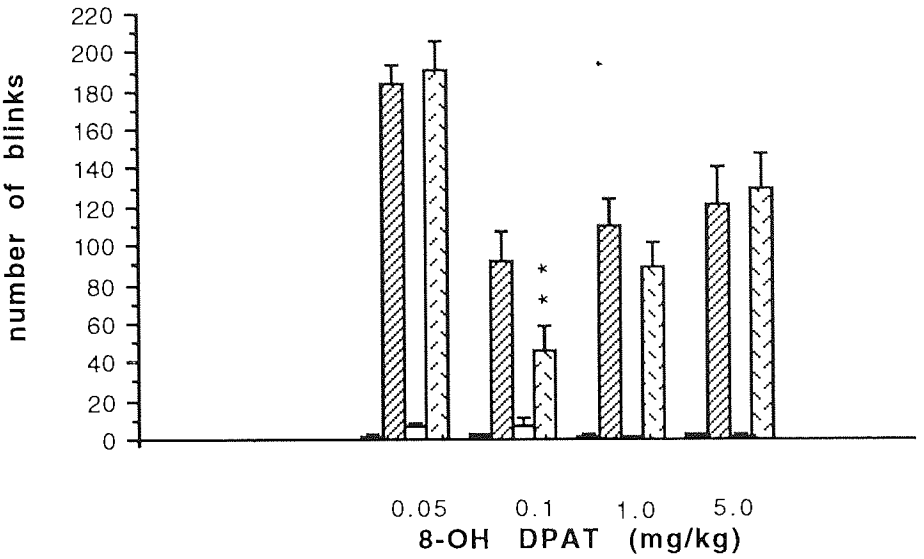


Figure 4.1. 8-OH DPAT attenuated MK771-induced blinking at a dose of 0.1mg/kg only, no significant effect was observed at the other doses tested. Filled bars, Saline + Saline; hatched bars, saline + MK771; open bars, 8-OH DPAT + Saline and stippled bars, 8-OH DPAT + MK771. **, $p<0.005$ *cf.* saline + MK771 treated mice.

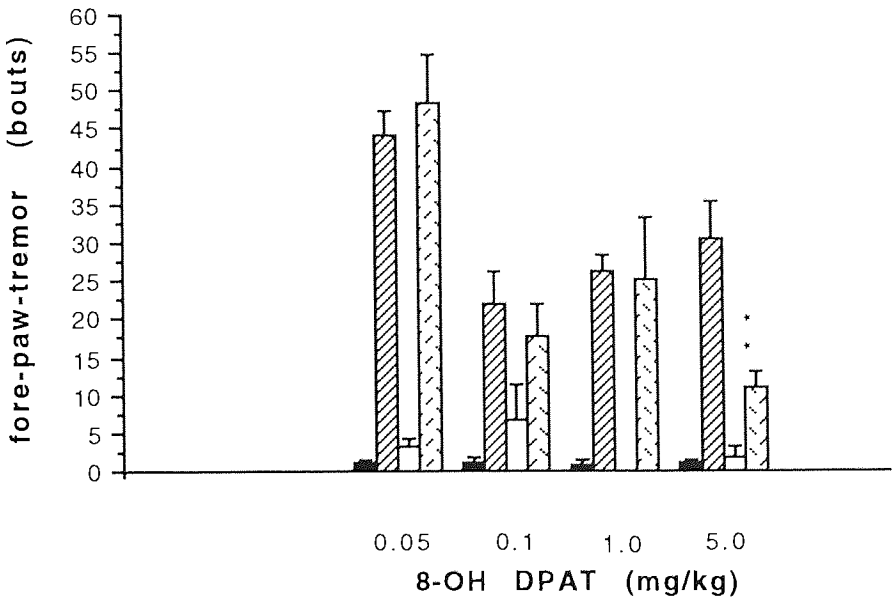
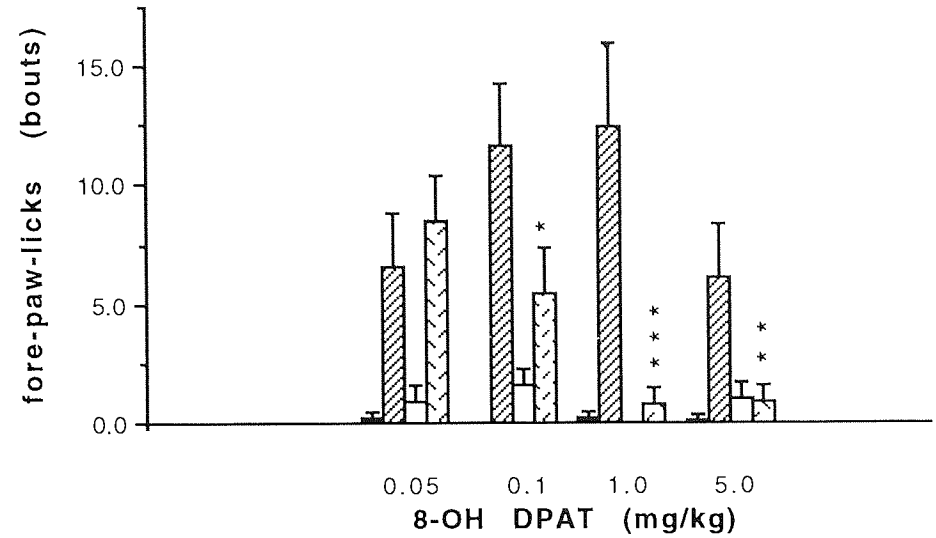


Figure 4.2. 8-OH DPAT attenuated MK771-induced FPT at the high dose tested (5.0mg/kg) , no significant effect was observed at the other doses. Key: bars as *figure* 4.1.



Figuree 4.3. 8-OH DPAT attenuated MK771-induced FPL at all but the lowest doses tested, no significant effect was observed at the other doses tested.Key: bars as *figure* 4.1. ***, p<0.005, **, p<0.025 and *, p<0.05 *cf.* saline + MK771 treated mice.

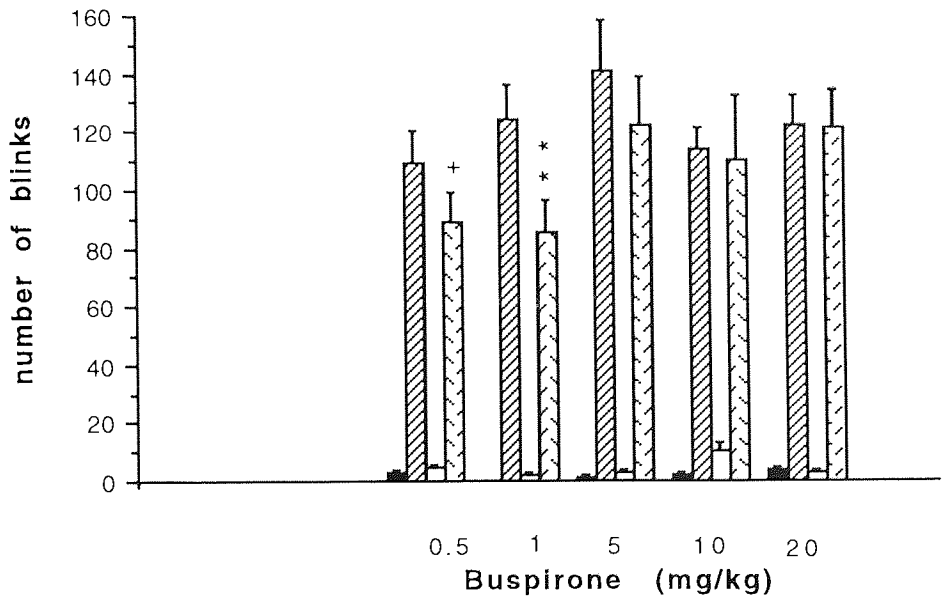


Figure 4.4. Buspirone attenuated MK771-induced blinking at a dose of 1.0mg/kg only, no significant effect was observed at the other doses tested. Filled bars, Saline + Saline; hatched bars, Saline + MK771; open bars, Saline + Buspirone and stippled bars, Buspirone + MK771. **, p<0.025 and +, p<0.10 *cf.* Saline + MK771 treated mice.

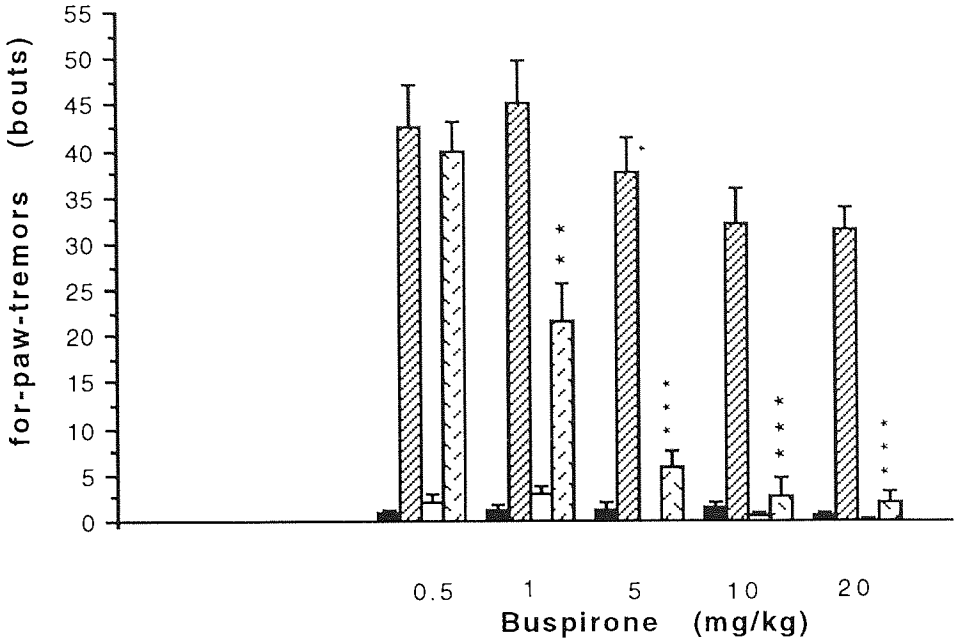


Figure 5.5. Buspirone attenuated MK771-induced FPT at a all but the lowest dose tested. Filled bars, Saline + Saline; hatched bars, Saline + MK771; open bars, Saline + Buspirone and stippled bars, Buspirone + MK771. ***, $p<0.0005$ and **, $p<0.005$ *cf.* Saline + MK771 treated mice.

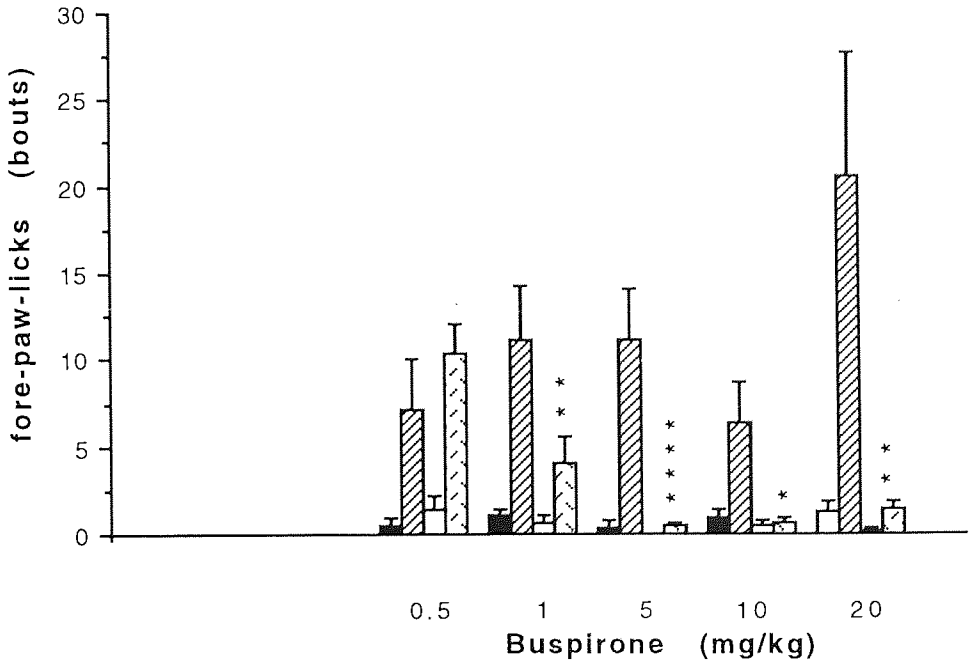


Figure 4.6. Buspirone attenuated MK771-induced FPL at all but the lowest dose tested. Key: bars as *figure* 4.4. ****, $p<0.005$;***, $p<0.01$; **, $p<0.025$ and *, $p<0.05$ *cf.* saline + MK771 treated mice.

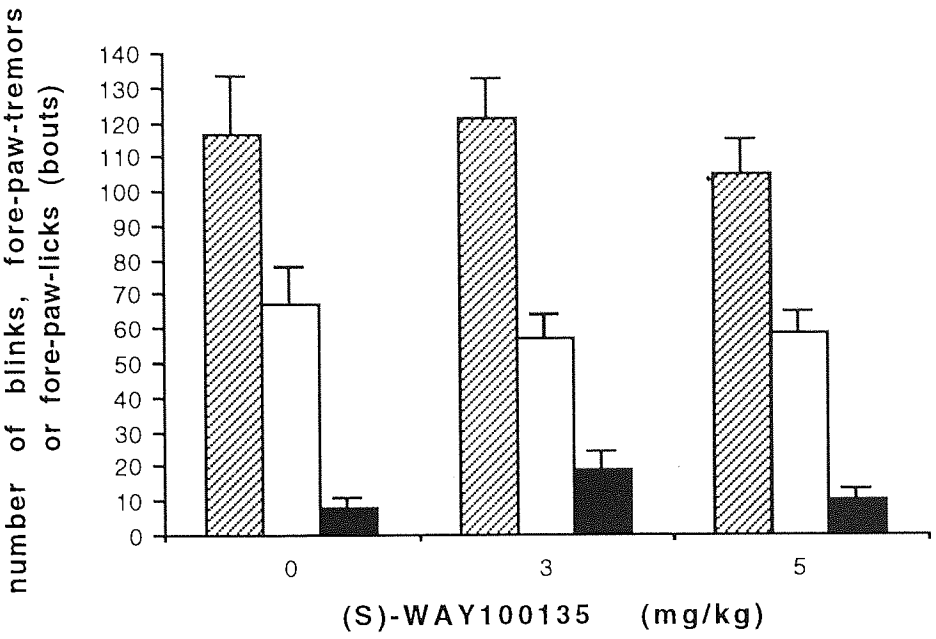


Figure 4.7. (S)-WAY-100135 (3.0 and 5.0mg/kg) failed to alter MK771-induced blinking, FPT or FPL. Hatched bar, blinking; open bar , FPT and closed bar, FPL.

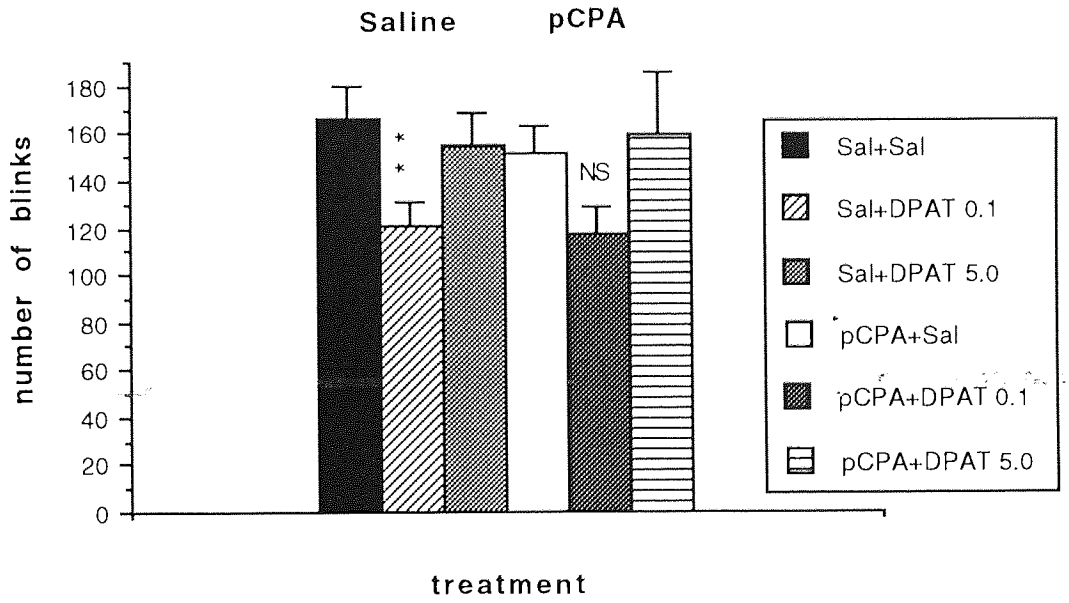


Figure 4.8. The low dose of 8-OH DPAT (0.1mg/kg) attenuated blinking in saline pre-treated mice given MK771, this effect did not reach significance in pCPA treated mice. The high dose (5.0mg/kg) was without effect. **, $p<0.025$ and NS, non-significant ($p<0.10$) *cf.* relative controls.

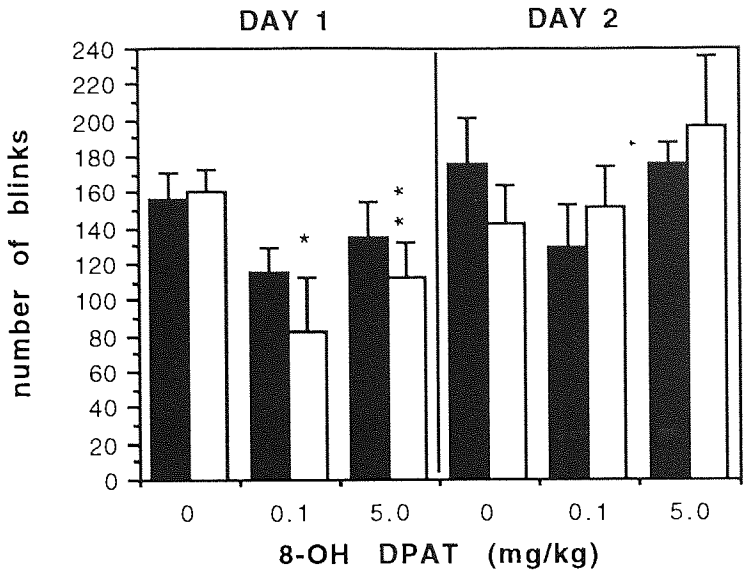


Figure 4.9. Data representing the differential effects of day of expriment and the effects of 8-OH DPAT on MK771-induced blinking in mice. **, $p<0.01$ and *$p<0.05$ *cf.* day 2. Closed bars, saline and open bars pCPA.

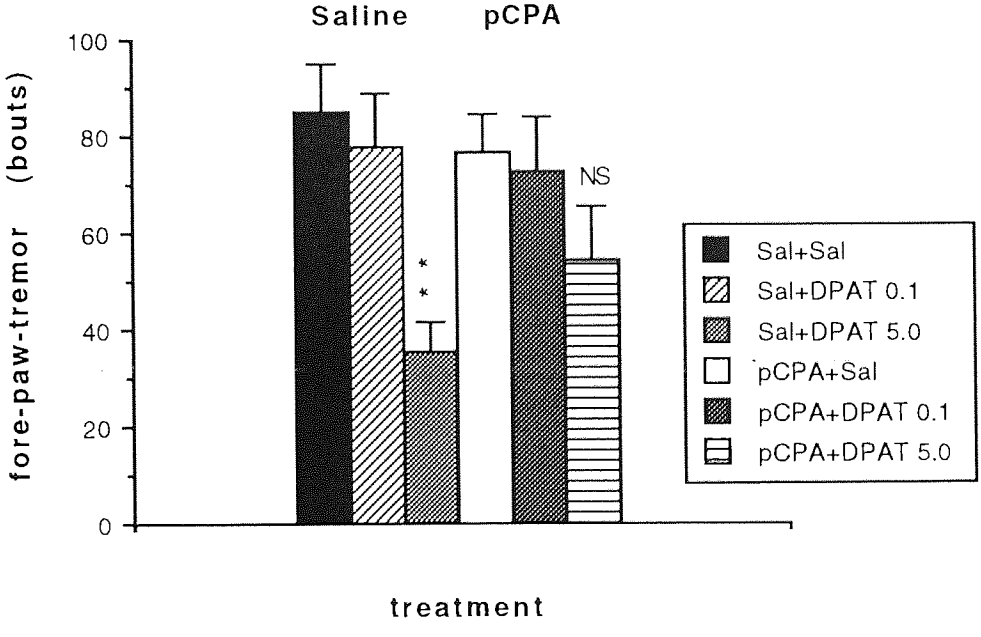


Figure 4.10. 8-OH DPAT (5.0mg/kg only) attenuated FPT in saline pre-treated mice given MK771, this effect did not reach significnce in pCPA treated mice. **, $p<0.005$ and NS, non-significant ($p<0.10$) *cf.* relative controls.

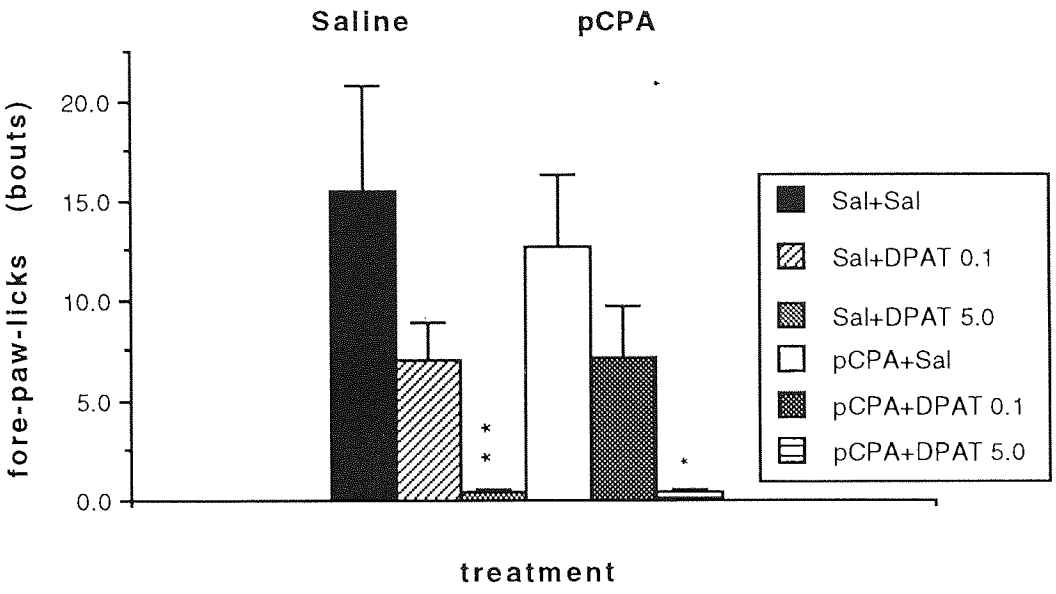


Figure 4.11. 8-OH DPAT (5.0mg/kg only) attenuated FPL in saline pre-treated mice given MK771. Significance from control was not observed at the low dose (0.1mg/kg). **, $p<0.005$ and *, $p<0.01$) *cf.* relative controls.

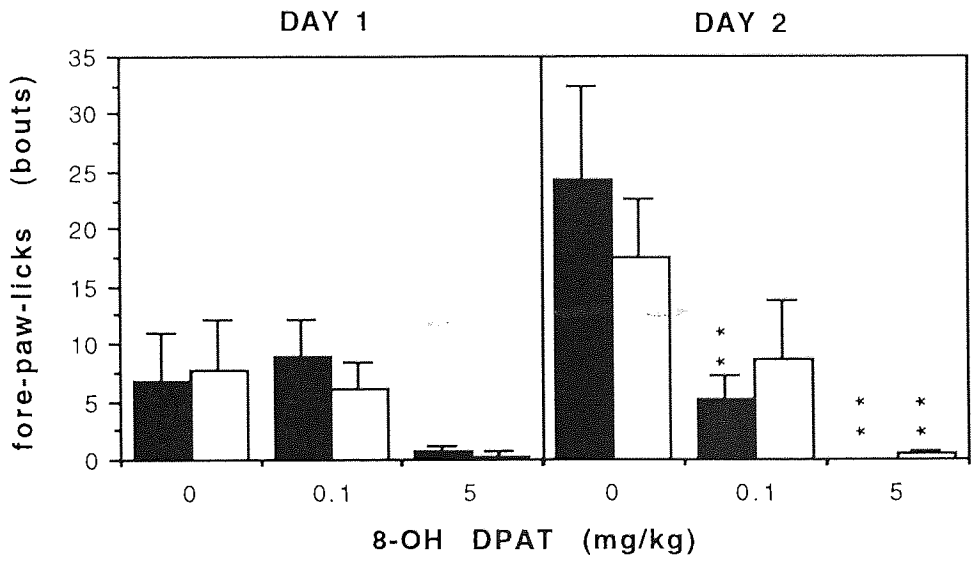


Figure 4.12. Data representing the differential effects of day of expriment and the effects of 8-OH DPAT on MK771-induced FPL in mice. **, $p<0.01$ *cf.* saline control. Closed bars, saline and open bars pCPA pretreatment.

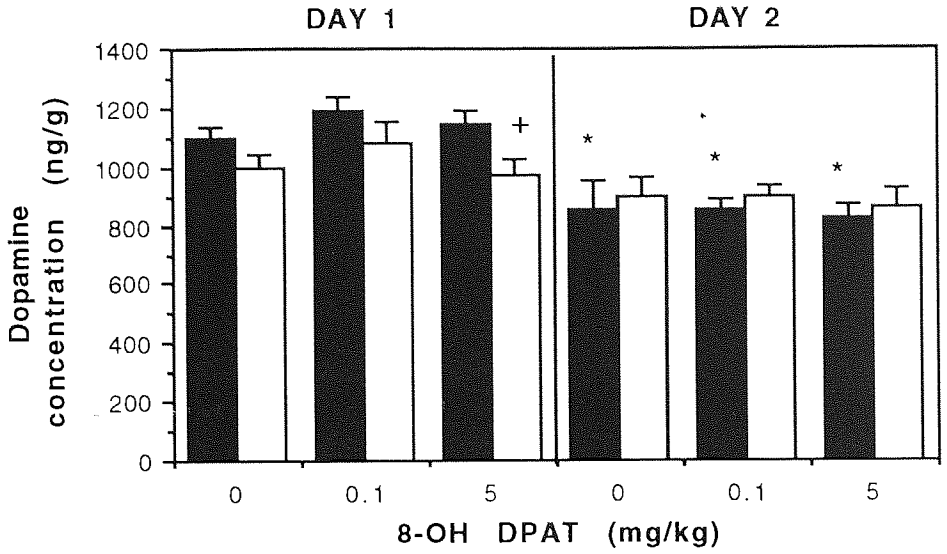


Figure 4.13. Data representing the differential effects of day of expriment and the effects of 8-OH DPAT on DA concentrations in whole brain (-cerebellum) in MK771 treared mice. **, $p<0.01$ *cf.* day 1and *, $p<0.05$ *cf.* saline control (day 2). Closed bars, saline and open bars pCPA pretreatment.

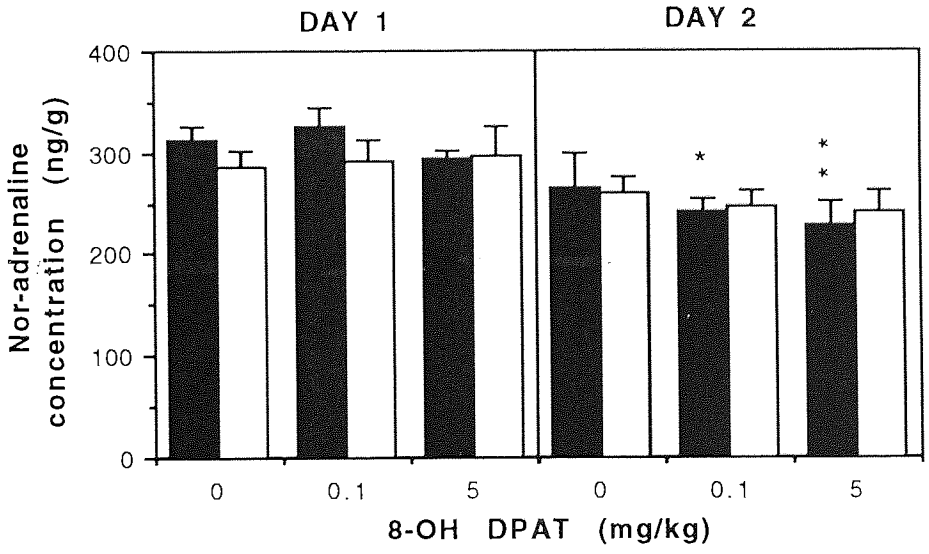


Figure 4.14. Data representing the differential effects of day of expriment and the effects of 8-OH DPAT on NA concentrations in whole brain (-cerebellum) in MK771 treated mice. ***, $p<0.0005$ and *, $p<0.01$*cf.* day 1. Closed bars, saline and open bars pCPA pretreatment.

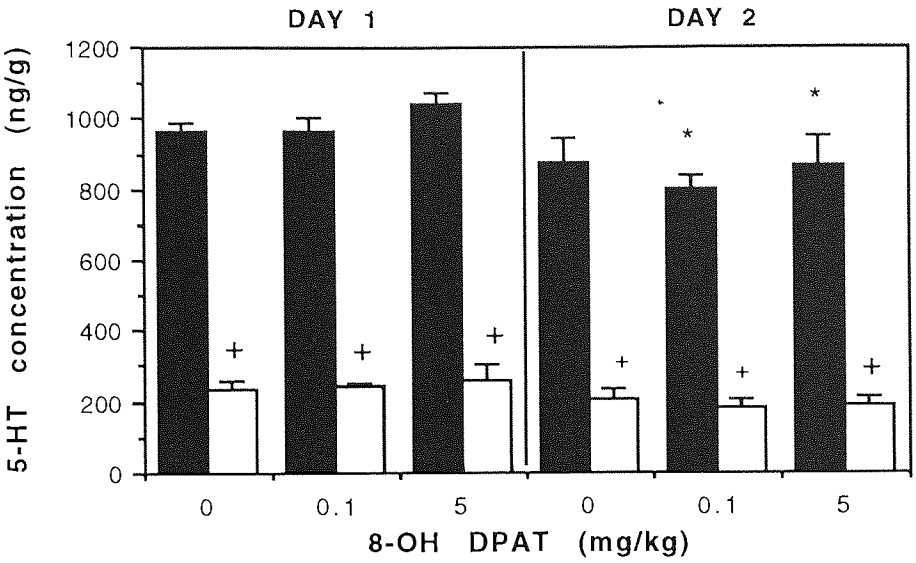


Figure 4.15. Data representing the differential effects of day of experiment and the effects of 8-OH DPAT on 5-HT concentrations in whole brain (-cerebellum) in MK771 treated mice. **, p<0.01 *cf.* day 1 and ++, p<0.01*cf.* saline control. Closed bars, saline and open bars pCPA pretreatment.

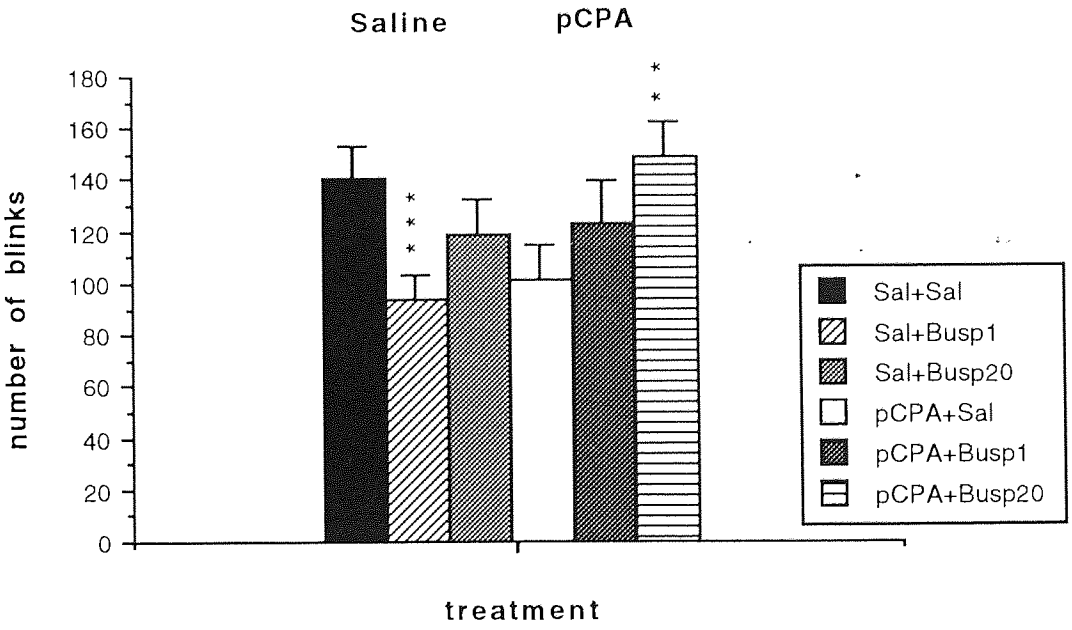


Figure 4.16. Attenuation of MK771-induced blinking in saline pre-treated given buspirone (1.0mg/kg) mice and potentiation in pCPA treated mice. ***, p<0.005 and **, p<0.025 *cf.* saline and pCPA pretreated control mice, respectively.

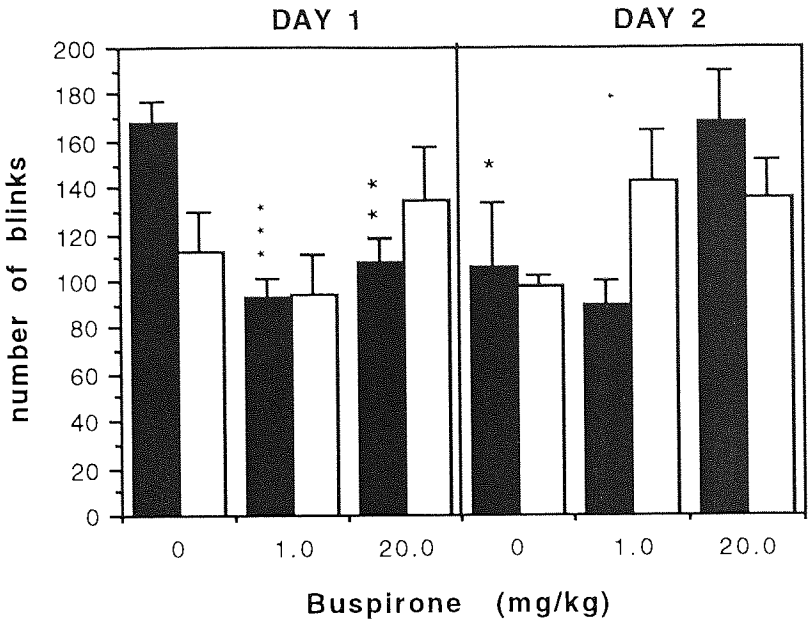


Figure 4.17. Buspirone (1.0 and 20.0mg/kg) attenuated blinking in saline pre-treated mice only on day 1. Blinking was significantly attenuated on day 2 in Saline + Saline controls. ***, $p<0.0002$ and **$p<0.001$ *cf.* saline control; *, $p<0.01$ *cf.* day 1.

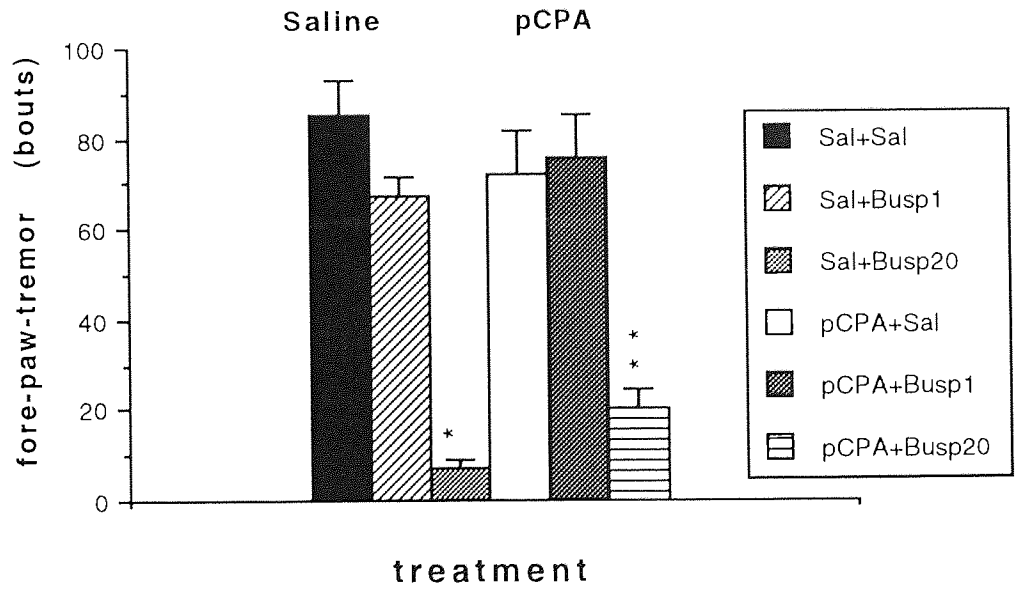


Figure 4.18. Buspirone attenuated FPT in saline and pCPA pre-treated mice given MK771. **, $p<0.0005$ and *, $p<0.025$) *cf.* controls.

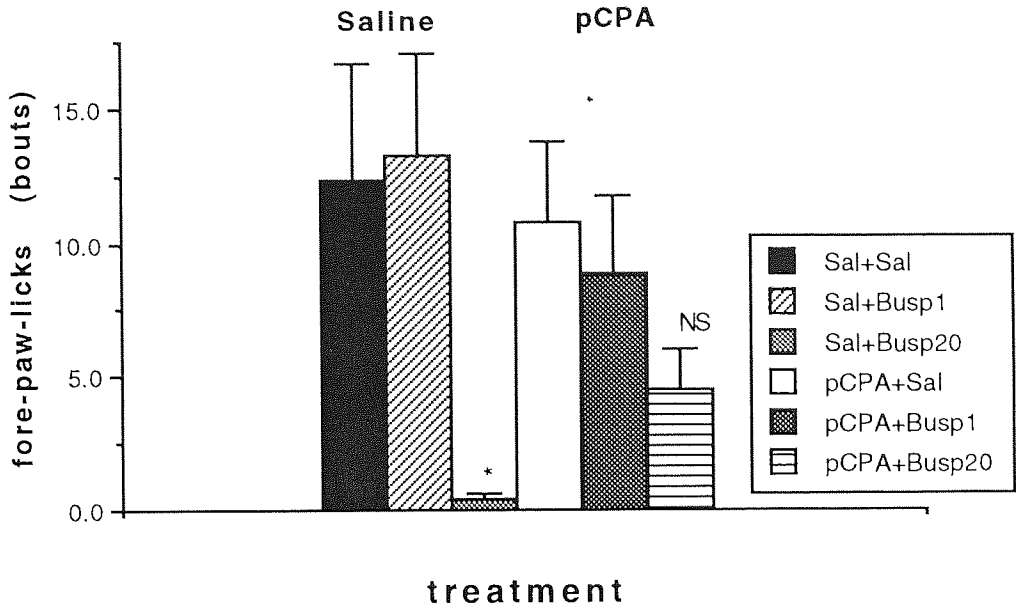


Figure 4.19. Buspirone attenuated FPL in saline pre-treated mice given MK771, the attenuation in thepCPA group did not reach significance. *, $p<0.025$ and NS, not significant *cf.* controls. Closed bars, saline and open bars pCPA pretreatment.

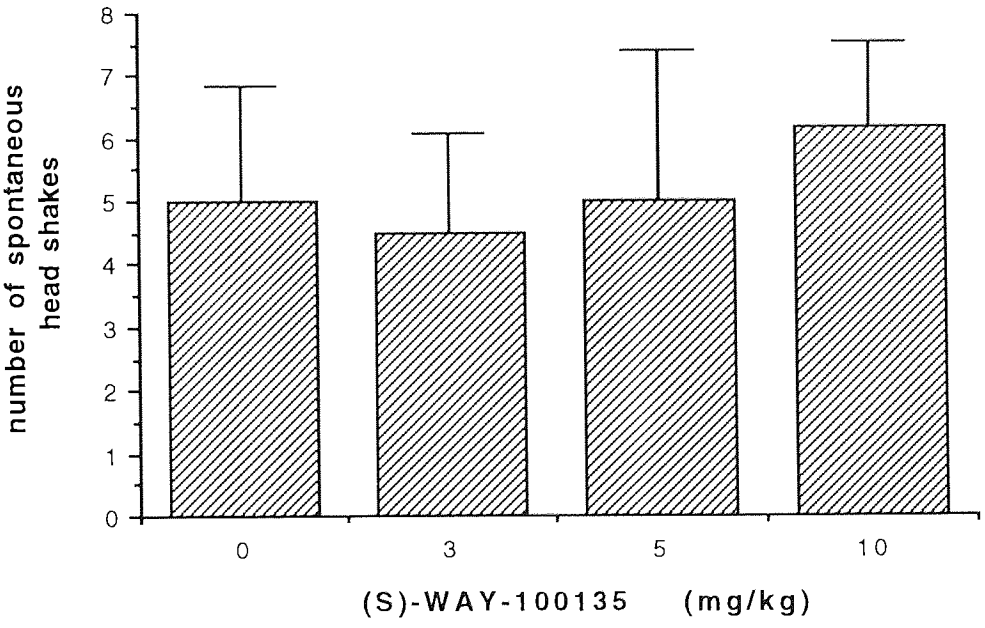


Figure 4.20. (S)-WAY100135 was without effect on the spontaneous HS in mice.

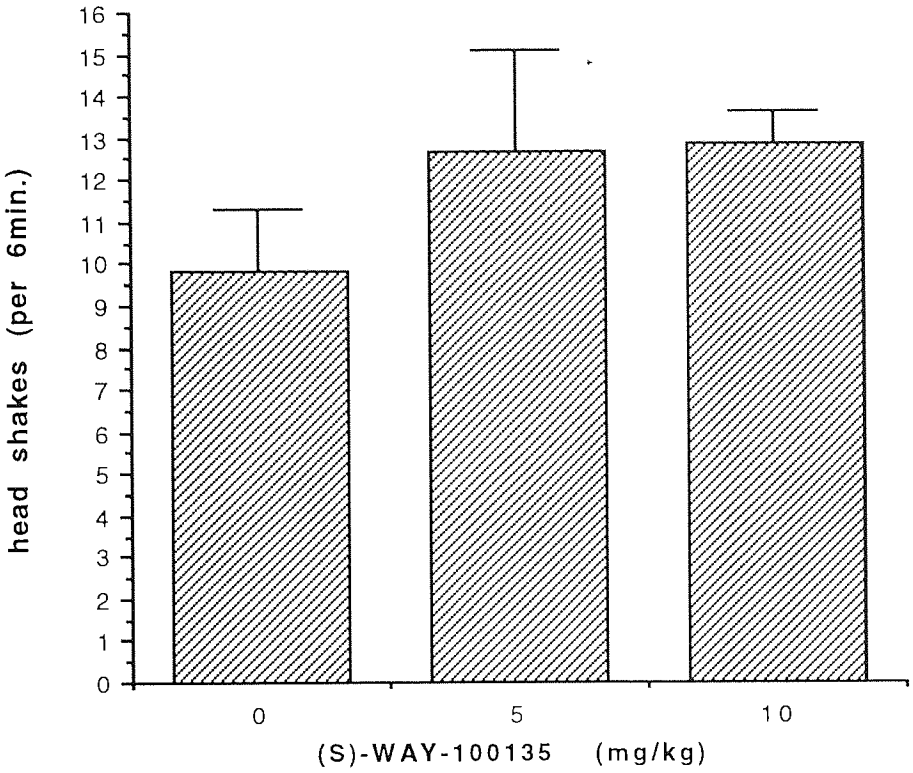


Figure 4.21. (S)-WAY-100135 was without significant effect on the DOI HS in mice.

CHAPTER 5.

ELECTROPHYSIOLOGICAL CHARACTERISATION OF THE NOVEL 5-HT1A ANTAGONISTS (S)-WAY-100135 AND WAY-100635.

CHAPTER 5.

Introduction

The initial aims of this part of the study were to analyse serotonergic unit activity in the DRN during tic like movements in the behaving cat at Prof. Barry Jacobs' laboratory at Princeton University. However, methodological considerations and on-going research in Prof. Jacobs' laboratory meant that this was not feasible so the effects of the 5-HT1A antagonists (S)-WAY-100135 and WAY-100635 on DRN firing were studied.

The specific 5-HT1A agonist 8-OH DPAT has been available as a pharmacological tool for some time now (Gozlan *et al.*, 1983) but in order to be confident about receptor characterisation "one must have operational data with selective agonists and antagonists" (Hoyer *et al.*, 1995). The search for a selective 5-HT1A antagonist has remained elusive with compounds displaying high affinities for other receptors or partial agonist effects (for review see Fletcher *et al.*, 1993a) until the introduction of WAY-100135 and WAY-100635 (Cliffe *et al.*, 1993; Fletcher *et al.*, 1993b and 1994). WAY-100135 displays high affinity at the 5-HT1A receptor (IC_{50}=15, 34 and 437nM for displacement of tritiated 8-OH DPAT; positive enantiomer, racemate and negative enantiomer, respectively) and considerably less affinity for the alpha-1 adrenoceptor (IC_{50}=1491, 1878 and 2781nM; racemate, positive enantiomer, negative enantiomer, respectively) (Cliffe *et al.*, 1993; Fletcher *et al.*, 1993b). In models of post-synaptic 5-HT1A receptor function the racemate and positive enantiomer of WAY-100135 antagonised the 8-OH DPAT behavioural syndrome in the rat (consisting of flattened-body-posture, spontaneous tail-flicks, hypothermia, locomotion and fore-paw-treading) and lacked intrinsic activity (Fletcher *et al.*, 1993b; Millan *et al.*, 1994). Many studies have addressed the effects of WAY-100135 at the somatodendritic autoreceptor. Neurochemical studies indicate that WAY-100135 had no effect on hippocampal 5-HT release alone but the racemate and the positive enantiomer antagonised the reduction of 5-HT concentration in the dialysate caused by the 5-HT1A agonist 8-OH DPAT (Routledge *et al.*, 1993). WAY-100135 lacked intrinsic activity on eating in satiated rats but antagonised 8-OH DPAT-induced hyperphagia (Hartley and Fletcher, 1994). In electrophysiological studies the racemate and the enantiomers of WAY-100135 decreased firing rate of DRN neurones in the anaesthetised rat and the racemate and positive enantiomer antagonised the reduction in neuronal firing caused by 8-OH DPAT (Fletcher *et al.*, 1993b). WAY-100135 was without affect alone in the anaesthetised guinea-pig (Mundey *et al.*, 1994) or rat (Gobert *et al.*, 1995) DRN preparations, but antagonised the suppression of firing caused by 8-OH DPAT.

WAY-100635 is an analogue of WAY-100135 which offers 7.5 times the potency at the 5-HT1A receptor than (S)-(+)-WAY-100135 (Fletcher *et al.*, 1994; Cliffe *et al.*, 1993; Fletcher *et al.*, 1993b). WAY-100635 was without intrinsic effect on the 5-HT syndrome but caused a rightward shift in the dose response of 8-OH DPAT to induce flattened-body-posture, hyperlocomotion and fore-paw-treading (*ie.*, increased the ED_{50}) and antagonised 8-OH DPAT-induced hypothermia in the rat (Fletcher *et al.*, 1994). In models of somatodendritic auto-receptor function WAY-100635 antagonised the hypothermic effects of 8-OH DPAT in the mouse (Fletcher *et al.*, 1994) and antagonised the reduction in 5-HT release induced by 8-OH DPAT (Gurling *et al.*, 1994a and b). In the light phase WAY-100635 was without effect on 5-HT release, but in the dark phase increased 5-HT release (Gurling *et al.*, 1994a and b). In the anaesthetised guinea pig WAY-100635 caused an increase in the firing rate of DRN neurones and antagonised the effects of 8-OH DPAT, if WAY-100635 was administered iontophoretically the increase in firing was not consistent (Mundey *et al.*, 1994b and 1995). Serotonergic unit activity is thought to be behaviourally state dependent (Jacobs and Fornal, 1993) so it may be hypothesised that the anaesthetised rodent models may not offer such a powerful model as a model employing serotonergic unit recording in freely moving animals, such as that employed by Prof. Jacobs' group at Princeton University. However, few studies of the actions of 5-HT1A antagonists have been reported in the behaving animal, for example the non-selective 5-HT1A antagonist spiperone caused an increase in DRN unit activity in the behaving cat model (Fornal *et al.*, 1994a), in this study the actions of spiperone were state dependent, such that increases in firing rate were only recorded in wakefulness and not SWS or REM sleep periods and similar results for WAY-100635 have been published in abstract format (Fornal *et al.*, 1994c and d). Moreover, WAY-100635 lacked intrinsic activity on hippocampal 5-HT release in the light phase (a period of low behavioural arousal in the rat) but increases release in the dark phase (a period of high behavioural arousal) (Gurling *et al.*, 1994b). Thus, it is critically important to address the action of antagonists in a paradigm designed to investigate serotonergic unit activity in the behaving animal.

The aims, therefore, of this study were to assess the effects of (S)-WAY100135 and WAY-100635 on the firing rate of DRN neurones in the freely moving behaving cat model.

Additional Methods

Please refer to General Methods for a fuller description of the methods covered in this chapter.

Each of the cells recorded was verified as being serotonergic according to previously established criteria: 1., slow and highly regular discharge activity (around 1-4 spikes/sec); 2., biphasic action potentials with a relatively long duration (> 2 msec) and 3., complete, or near complete, suppression of activity during REM sleep. Neurones were further characterised by pharmacological challenge with 8-OH DPAT (5-40 μg/kg i.v.), a selective 5-HT1A agonist (which also has high affinity for 5-HT7 receptors) which potently and completely inhibits the discharge of central serotonergic neurones by an action at the somatodendritic autoreceptor (Sprouse and Aghajanian, 1986). The dose of 8-OH DPAT was titrated to suit the unit discharge activity of the neurone tested, since evidence suggests that the higher the tonic activity of DRN neurones the less responsive it is to the actions of 5-HT1A agonists (Fornal *et al.*, 1994a).

Antagonist studies with (S)-WAY100135 and WAY-100635 were carried out following verification of serotonergic cell type. Unit activity was allowed to return to baseline following administration of 8-OH DPAT and an arbitrary time period was allowed for the metabolism/expulsion of 8-OH DPAT (this period of time was usually 2-3hours). Antagonists were administered either alone, in order to analyse their intrinsic activity upon serotonergic unit activity, or in conjunction with 8-OH DPAT in order to verify whether these compounds antagonised the reduction in serotonergic unit activity seen with 8-OH DPAT. 1min. of baseline unit activity was recorded prior to remote administration (see General Methods) of either saline (0.05-0.10ml/kg, n=20), (S)-WAY-100135 (0.1 (n=7), 0.5(n=12) or 1.0mg/kg i.v. (n=6)) or WAY-100635 (0.1 (n=6) or 0.5mg/kg i.v. (n=10)). (S)-WAY-100135 (0.5mg/kg i.v., n=4) or WAY-100635 (0.5mg/kg i.v., n=4) were administered 15min. prior to 8-OH DPAT (5-40μg/kg i.v.) and unit activity collected for 5min. Results were compared to 8-OH DPAT (alone) control.

All drugs were prepared freshly in sterile saline (0.9%) vehicle in injection volumes of 0.05-0.10ml/kg. Mild heat (hot water bath) and sonication were required to dissolve the drugs. N-numbers refer to the n-number of the cells recorded, these cells were recorded from a total of 11 cats.

Statistical analyses. Individual unit activity data for each antagonist dose or saline vehicle tested was analysed using a Repeated Measures ANOVA. Maximal changes (in percentage of baseline unit activity over a 1min. time period) following administration of antagonists were compared with maximal percentage changes in saline vehicle treated cats using a 1-Way ANOVA, with *post-hoc* Newman-Keuls test; *ie.* the maximum percentage decrease in unit activity seen with (S)-WAY-100135 was statistically compared with the maximal percentage decrease in unit activity in saline treated cats and *vice versa* for WAY-100635. Similarly, antagonism of 8-OH DPAT response was compared by comparing maximal

percentage reductions in unit activity in 8-OH DPAT treated cats with the maximal reductions in antagonist + 8-OH DPAT treated cats using 1-Way ANOVA with *post-hoc* Newman-Keuls test.

Data presentation: data are presented as mean firing rate spikes/sec ± S.E.M. unless stated otherwise.

Results

Each of the cells tested was histologically verified as being present within the DRN. Representative polygraphic traces are displayed in *figures* 5.11 and 5.12.

The effects of (S)-WAY-100135 on serotonergic unit activity.

Repeated measures ANOVA revealed significant reduction of unit activity after 0.5 ($F(36,258)=0.56$, $p<0.0001$, *figure* 5.3) and 1.0 mg/kg ($F(36,221)=2.39$, $p<0.0001$, *figure* 5.4) but not 0.1mg/kg ($F(36,258)=0.56$, $p<0.981$, *figure* 5.2) or saline control ($F(36,739)=0.05$, $p<1.0$, *figure* 5.1). Maximal percentage reductions following (S)-WAY-100135 were compared with those seen in saline treated cats and dose dependently reduced serotonergic unit activity (($F(3,44)=14.92$, $p<0.0001$, *figure* 5.5), significant differences were revealed with a *post-hoc* Newman-Keuls test (0.1mg/kg, $p<0.01$; 0.5mg/kg $p<0.01$ and 1.0 mg/kg, $p<0.01$).

The effects of WAY-100635 on serotonergic unit activity.

WAY-100635 caused a dose related increase of serotonergic unit activity of cat DRN neurones (*figures* 5.7, 5.8 and 5.9). Repeated measures ANOVA failed to reveal a significant increase in unit activity in 0.1 ($F(36,219)=0.13$, $p<1.0$, *figure* 5.7) and 0.5 mg/kg ($F(36,369)=0.49$, $p<0.994$, *figure* 5.8) and saline control treated cats ($F(36,739)=0.05$, $p<1.0$, *figure* 5.1). Maximal percentage increases in unit activity following WAY-100635 were compared with those seen in saline treated cats and dose depedently increased serotonergic unit activity (($F(2,35)=17.77$, $p<0.0001$, *figure* 5.9) and significant differences were revealed with a *post-hoc* Newman-Keuls test (0.5mg/kg $p<0.01$ and 1.0 mg/kg, $p<0.01$, *figure* 5.9).

(S)-WAY-100135 and WAY-100635 antagonism of 8-OH DPAT suppression of unit activity.

8-OH DPAT (mean dose=13.75±1.25 µg/kg i.v. ((S)-WAY-100135 experiment) or 13.75±1.25 µg/kg i.v. (WAY-100635 experiment)) caused an almost complete suppression of unit activity (*figures* 5.6 and 5.10, respectively) and these effects were antagonised by the prior administration (15min. earlier) of 0.5 mg/kg i.v. of (S)-WAY-100135 ($F(1,7)=20.64$, $p<0.004$, *figure* 5.6) or WAY-100635 ($F(1,7)=616.05$, $p<0.00001$, *figure* 5.10).

Discussion

(S)-WAY100135 and WAY-100635 both acted as antagonists for the suppression of DRN neuronal firing by the 5-HT1A agonist 8-OH DPAT. However, their effects on unit activity were opposite when administered alone; (S)-WAY100135 caused a suppression of unit activity wheras WAY-100635 increased firing.

The actions of (S)-WAY100135 were surprising and suggest that this compound may be acting as a partial agonist at the 5-HT1A receptor in this paradigm. This finding is not consistent with behavioural and neurochemical studies indicating that WAY-100135 has no intrinsic activity on feeding and 5-HT release in the hippocampus, but antagonised the 8-OH DPAT response (Hartley and Fletcher, 1994; Routledge *et al.*, 1993). WAY-100135 also lacked intrinsic activity on single unit activity in the anaesthetised guinea pig and rat DRN (Mundey *et al.*, 1994a; Gobert *et al.*, 1995). Supporting the findings presented here, WAY-100135 suppressed unit activity when administered alone, but also displayed antagonism of 8-OH DPAT reduction in firing in the anaesthetised rat (Fletcher *et al.*, 1993b). The authors believed that these effects of WAY-100135 were not due to its action at the 5-HT1A receptor, since this response was weak when compared to other partial agonists and the suppression of neuronal firing did not reach anymore than 31% and did not differ between the racemate and the two enantiomers tested. In the anaesthetised cat WAY-100135 potently suppressed unit activity in the DRN, but was without effect on the inhibition of firing induced by 8-OH DPAT (Escandon *et al.*, 1994). *In-vitro* (S)-WAY-100135 antagonised the suppression of firing in a DRN slice preparation in a concentration dependent manner but at higher concentrations (>1μM) decreased tonic firing rates consistent with data presented here (Lanfumey *et al.*, 1993). However, these effects persisted in the presence of the non-selective ß-blocker (-)-tertatolol, which antagonises 5-HT1A receptors, but were abolished when the concentration of phenylephrine in the perfusion bath was increased (phenylephrine was present in order to drive the DRN neurones), suggesting that the suppressant actions of (S)-WAY-100135 might be due to a blockade of the alpha-1 adrenoceptor, since tonic stimulation of this receptors is necessary for the discharge of the DRN (Baraban and Aghajanian, 1980; Van der Maelen and Aghajanian, 1983) and prazosin reduces 5-HT release *in-vivo* (Claustre *et al.*, 1991). Lanfumey and colleagues reported that this hypothesis was consistent with the micromolar affinity of (S)-WAY-100135 for the alpha-1 adrenoceptor (IC_{50}=1.88μM) (Fletcher *et al.*, 1993b). Interestingly at a lower dose than that tested here (0.025mg/kg i.v.) (S)-WAY-100135 still antagonised the suppressant effect of 8-OH DPAT but alone failed to suppress unit activity (Personal Communication from Dr. Casey Fornal), so it may be that at higher doses

appreciable alpha-1 adrenergic blockade occurs suppressing serotonergic unit activity in the DRN. Interestingly administration of the partial agonists BMY7378 and NAN190 (to a lesser degree) mirrored almost exactly the effects of 8-OH DPAT (Fornal *et al.*, 1994b), but (S)-WAY-100135 fails to cause such a marked suppression in this study (and see Fletcher *et al.*, 1993b who only found a 31% suppression, see earlier). The antagonism of the 8-OH DPAT inhibition of firing is consistent with previous reports indicating antagonism of the 8-OH DPAT reduction in 5-HT release (Routledge *et al.*, 1993), DRN firing in anaesthetised and *in-vitro* rodent preparations (Fletcher *et al.*, 1993b; Mundey *et al.*, 1994a; Gobert *et al.*, 1995; Lanfumey *et al.*, 1993) and 8-OH DPAT-induced hyperphagia (Hartley *et al.*, 1994). Antagonism of the suppression of DRN firing by the auto-receptor agonists S14671, S14489, S15535 and S15931 has also been demonstrated (Millan *et al.*, 1995; Gobert *et al.*, 1995). Studies with other 5-HT1A antagonists have also indicated the antagonism of agonist effects at the autoreceptor: WAY-100635 (this study; Gartside *et al.*, 1995a and b; Mundey *et al.*, 1994b, 1995); (-)tertatolol (Millan *et al.*, 1994; Gobert *et al.*, 1995), spiperone (Fornal et al, 1994a; Blier *et al.*, 1989 and 1993; Lum and Piercey, 1988; Gobert *et al.*, 1995; Millan *et al.*, 1995) and (S)-UH301 (Nomikos *et al.*, 1992).

The effects of WAY-100635 are consistent with those expected of a competitive antagonist at the 5-HT1A auto-receptor *ie.* an increase in basal firing rate and antagonism of the 8-OH DPAT inhibition of firing, and suggests that, in this paradigm, WAY-100635 acts as a 'silent antagonist' (*ie.* lacks agonist activity in any model of receptor function (Boddeke *et al.*, 1992). The increase in firing is consistent with previous reports indicating that WAY-100635 increased firing in the anaesthetised guinea-pig DRN (Mundey *et al.*, 1994b and 1995) and that spiperone increased firing in the freely moving cat DRN (Fornal *et al.*, 1994a). An increase was found in anaesthetised rat DRN cells tested with WAY-100635 (60% of cells tested) (Gartside *et al.*, 1995a) or (-)-tertatolol (66% of cells tested) (Prisco *et al.*, 1993), neither of which reached significance. WAY-100635 lacked intrinsic activity on feeding in the satiated rat (Hartley *et al.*, 1994) and 5-HT release in the light phase (Gurling *et al.*, 1994a and b) and anaesthetised rats (Gartside *et al.*, 1995a).

Many reports have supported the antagonist action of WAY-100635 at the 5-HT1A autoreceptor. WAY-100635 antagonised the actions of 8-OH DPAT on the reduction of 5-HT release (Gurling *et al.*, 1994a and b), firing in anaesthetised rodents (Mundey *et al.*, 1994a and b; Gartside *et al.*, 1995), hypothermia in the mouse (Fletcher *et al.*, 1994) and hyperphagia (Hartley *et al.*, 1994). Similarly, the reduction in firing and 5-HT release induced by paroxetine and a monoamine-oxidase-inhibitor (tranylcypromine), was antagonised by WAY-100635 (Gartside *et al.*, 1995a and b). The antagonism of the 8-OH DPAT response in this and other

paradigms was consistent with antagonism by other 5-HT1A antagonists of the somatodendritic autoreceptor: such as WAY-100135 (this study; Fletcher *et al.*, 1993b; Routledge *et al.*, 1993; Lanfumey *et al.*, 1993; *etc.*, see above), (-)-tertatolol (Millan *et al.*, 1994; Gobert *et al.*, 1995), spiperone (Fornal et al, 1994a; Blier *et al.*, 1989 and 1993; Lum and Piercey, 1988; Gobert *et al.*, 1995; Millan *et al.*, 1995) and (S)-UH301 (Nomikos *et al.*, 1992). However, of the above studies few authors have analysed the electrophysiological effects of antagonists in a freely moving animal model, apart from the work presented here, and the increase in firing and antagonism of 8-OH DPAT seen with spiperone in the freely moving behaving cat model (Fornal *et al.*, 1994a).

In summary, WAY-100635 acts as a silent antagonist at the 5-HT1A autoreceptor and the effects of (S)-WAY-100135 are consistent with a partial agonist profile of this compound. However, the effects of (S)-WAY-100135 could be due to an antagonist action at the alpha-1 adrenoceptor, but this warrants further investigation.

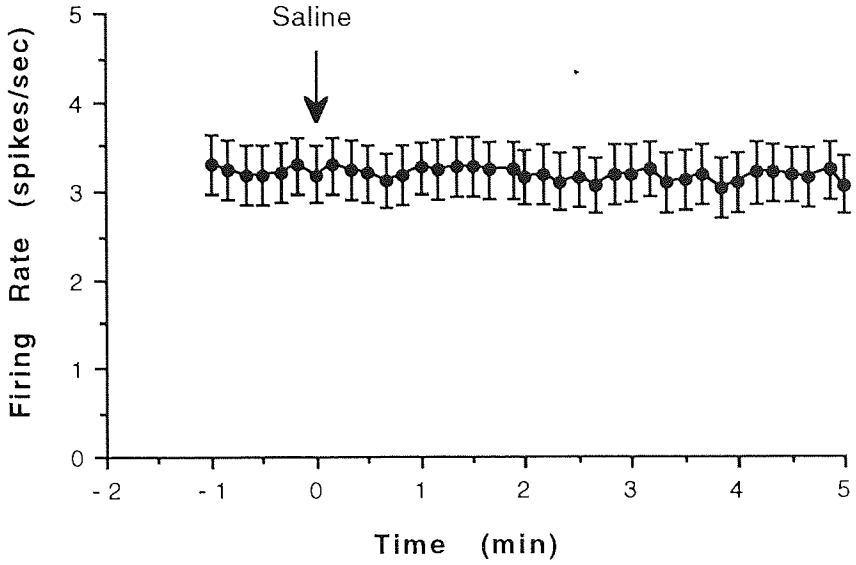


Figure 5.1. Demonstrating the lack of effect of saline on serotonergic unit activity in the DRN (n=20 cells).

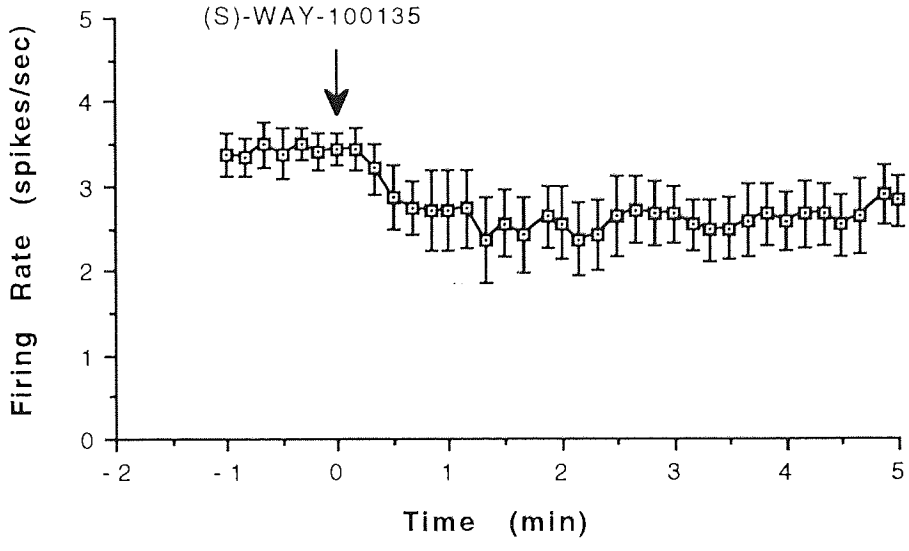


Figure 5.2. The effect of (S)-WAY-100135 (0.1mg/kg i.v.) on firing rate, n=7 cells.

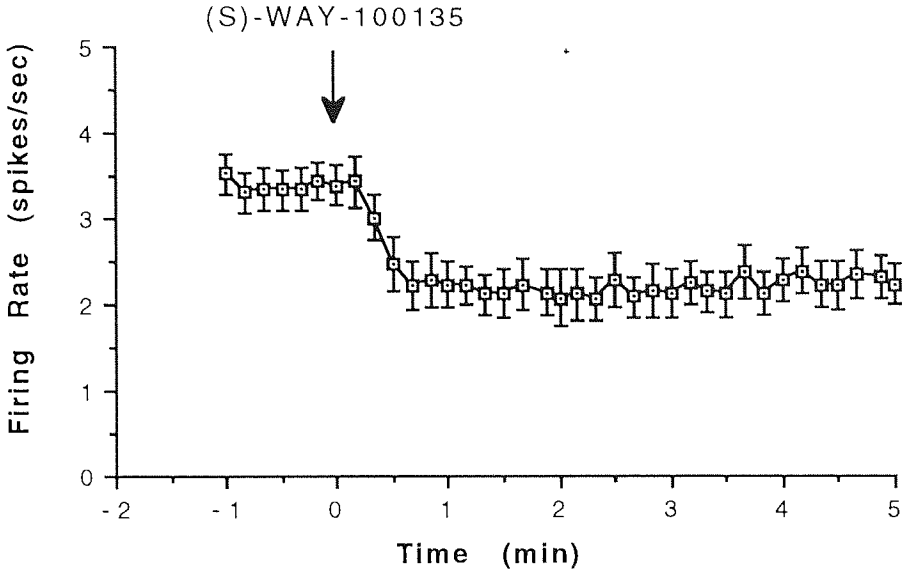


Figure 5.3. (S)-WAY100135 (0.5mg/kg i.v.) reduces firing rate, n=12 cells.

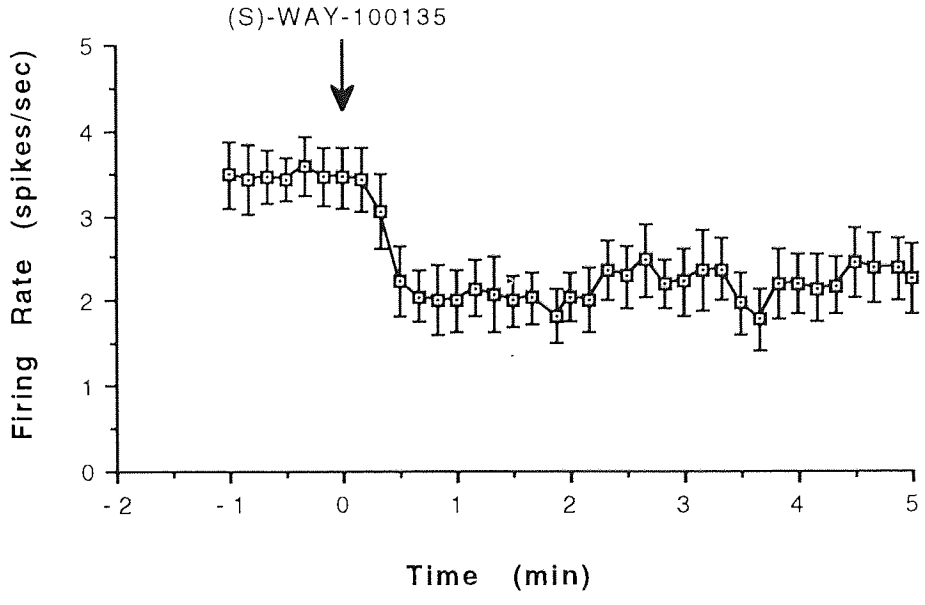


Figure 5.4. (S)-WAY100135 (1.0mg/kg i.v.) reduces firing rate, n=6 cells.

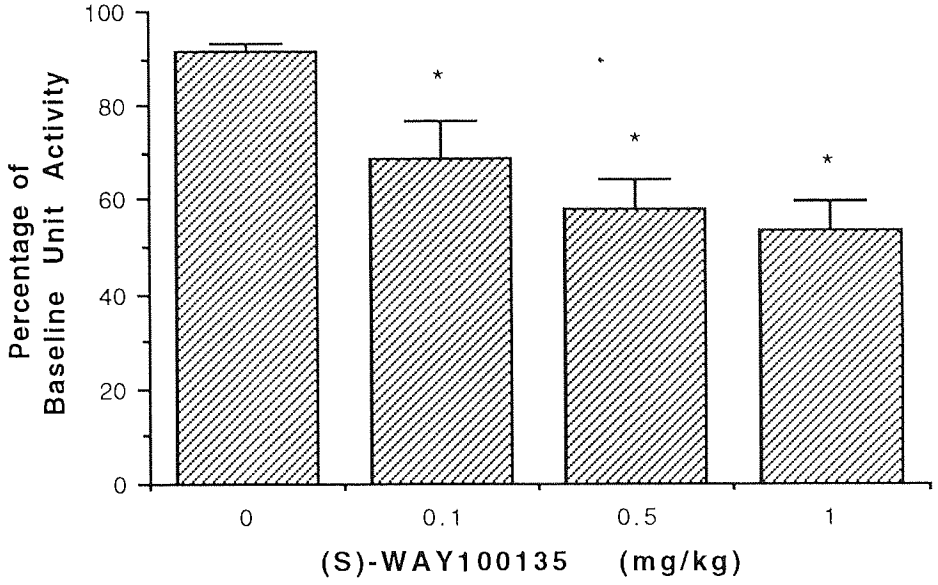


Figure 5.5. Maximum percentage decrease in unit activity following (S)-WAY-100135. *, p<0.01 *cf.* saline control.

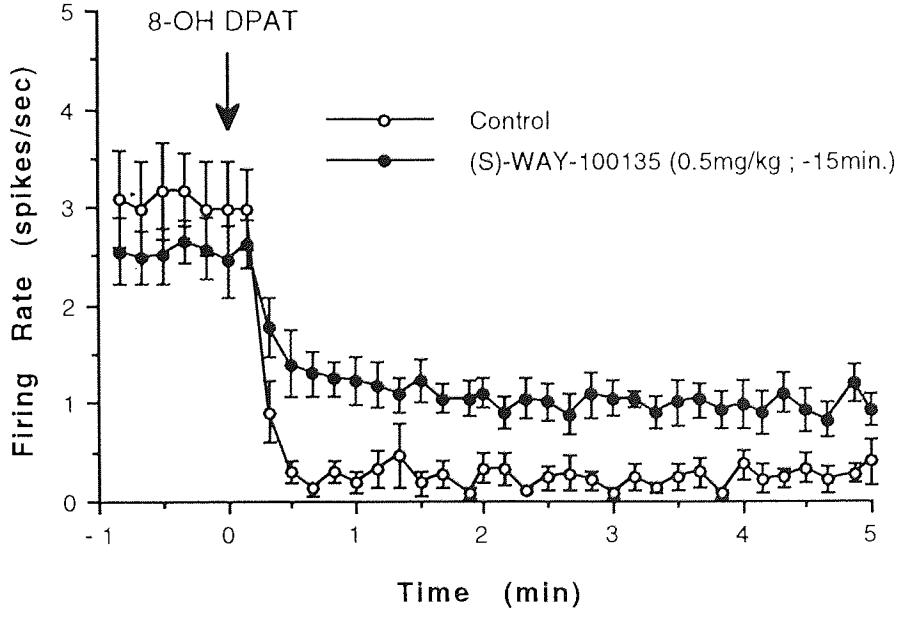


Figure 5.6. (S)-WAY-100135 (0.5mg/kg i.v.) attenuates the 8-OH DPAT inhibition of firing, n=4 cells.

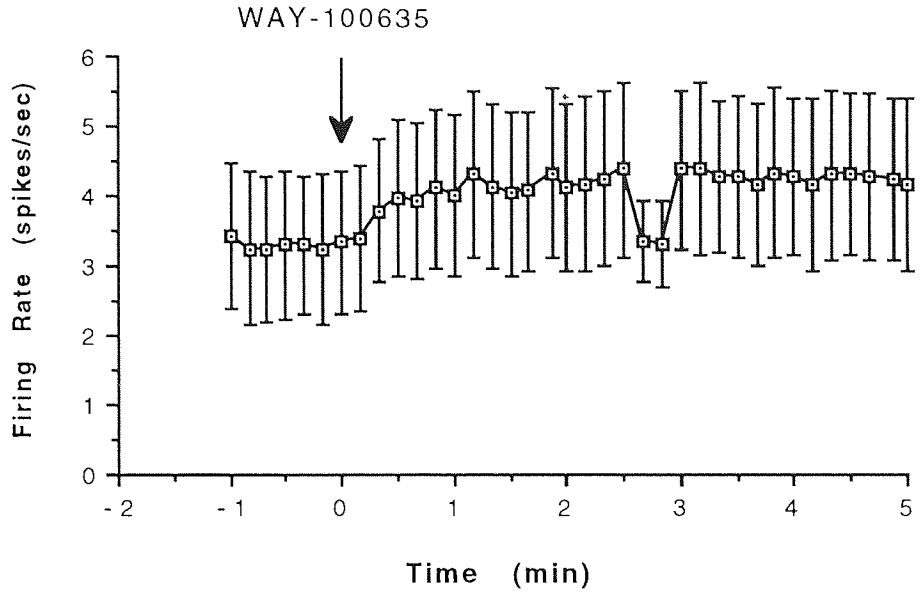


Figure 5.7. The effect of WAY-100635 (0.1mg/kg i.v.) on firing rate, n=6 cells.

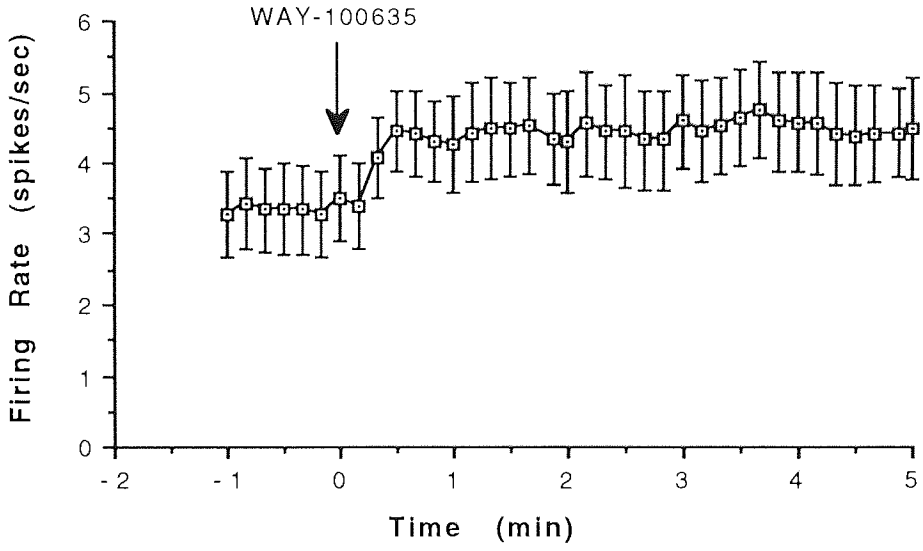


Figure 5.8. The effect of WAY-100635 (0.5mg/kg i.v.) on firing rate, n=10 cells.

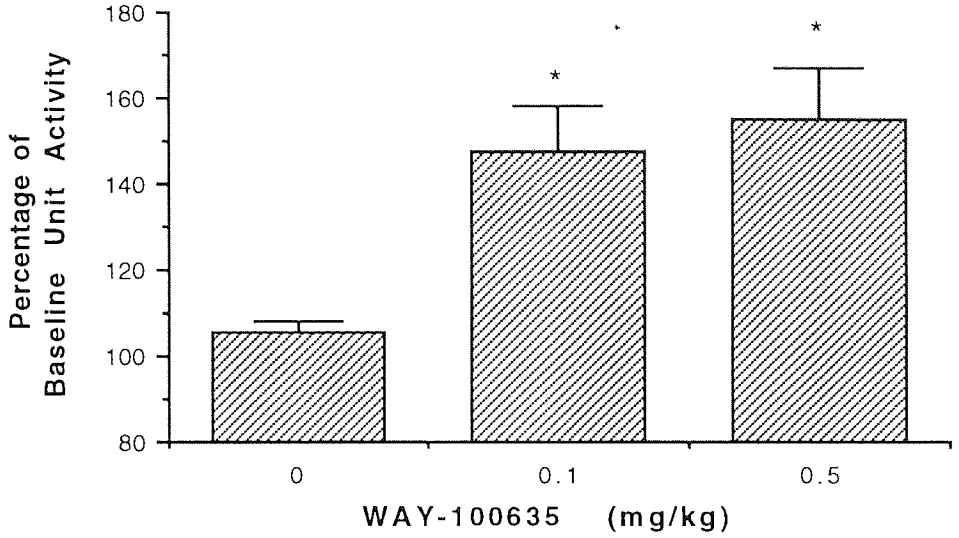


Figure 5.9. Maximum percentage change in unit activity in unit activity following WAY-100635. *, p<0.01 *cf.* saline control.

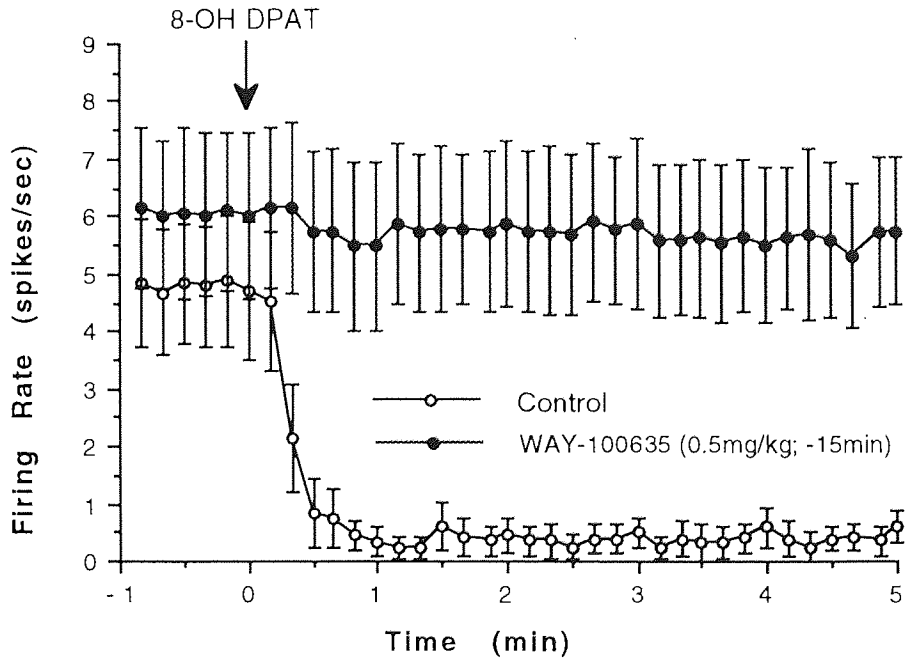


Figure 5.10. WAY-100635 (0.5mg/kg i.v.) attenuates the 8-OH DPAT suppression of firing, n=4 cells.

Figure 5.11. Traces representing the unit activity after various treatments in a single DRN 5-HT neurone. Key: 1. baseline unit activity and unit activity after; 2. saline, 3. 8-OH DPAT (15µg/kg), 4. (S)-WAY100135 (0.5mg/kg), 5. WAY-100635 (0.5mg/kg), 6. 8-OH DPAT + (S)-WAY1000135 and 7. 8-OH DPAT + WAY100635 in a single DRN 5-HT neurone. X denotes the addition of 8-OH DPAT, all other drugs were administered prior to the traces displayed.

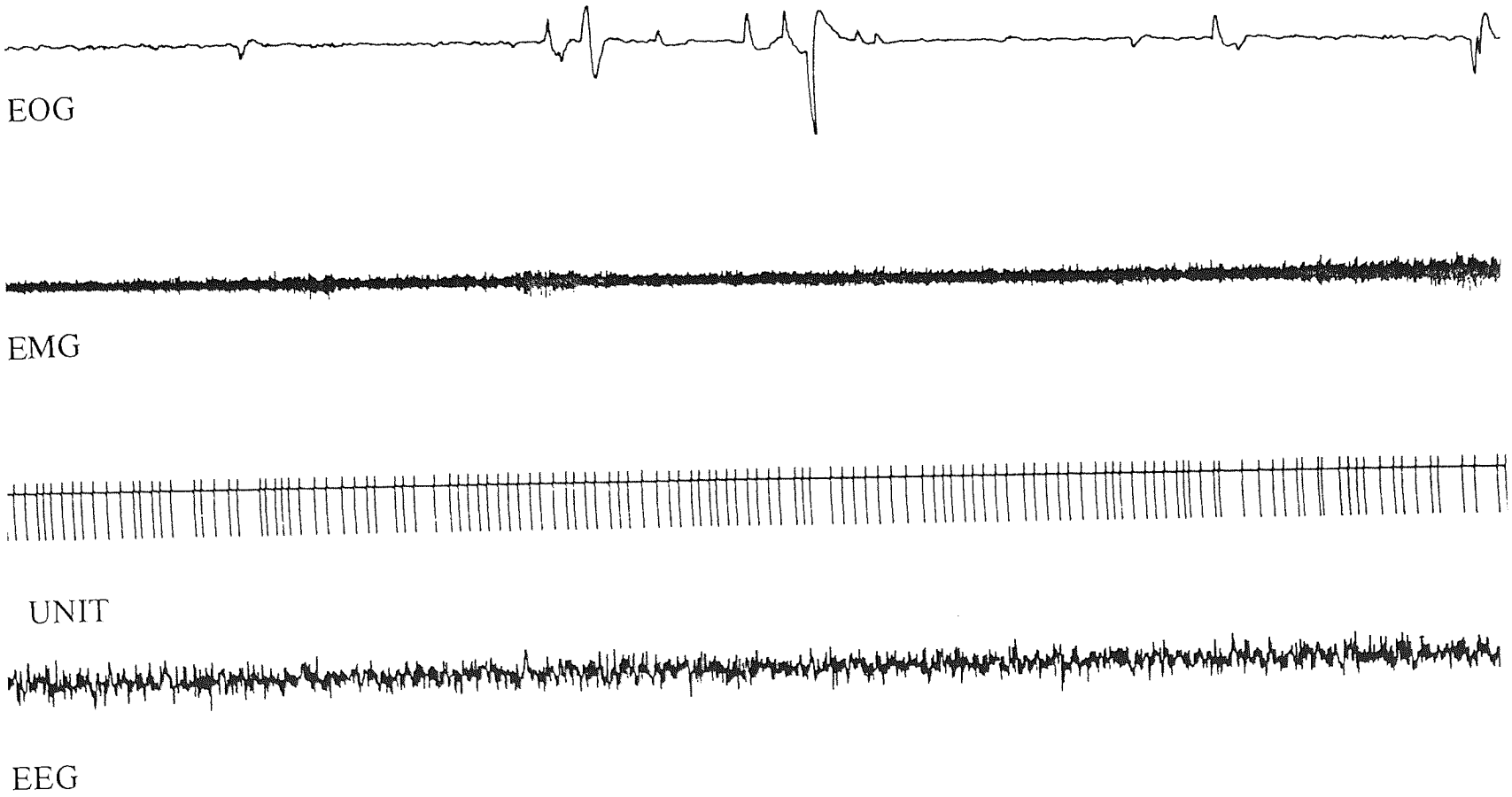


Figure 5.12. Demonstration of representative EOG, EMG, DRN serotonergic unit activity and EEG traces.

CHAPTER 6.

KYNURENINE POTENTIATION OF THE DOI HEAD SHAKE IN MICE.

CHAPTER 6.

Introduction

Kynurenine is the first stable metabolite of the kynurenine pathway (which metabolises over 90% of body tryptophan (Wolf, 1974)). Recent studies have demonstrated that plasma kynurenine concentrations were elevated in all of seven patients suffering from GTS (Dursun *et al.*, 1994). It has been suggested that tic-like movements such as head-shakes, which occur in rodents after a wide variety of pharmacological challenges, may model the tics observed in GTS (Handley and Dursun, 1992; see General Introduction for more information).

In mice, systemic administration of kynurenine or its metabolite 3-hydroxykynurenine potentiated the HS induced by centrally administered 5-HT or systemic 5-HTP (Handley and Miskin, 1977). 5-HT-related HS are thought to be related to stimulation of the 5-HT2A receptor (*eg.* Kennett and Curzon, 1991; Kennett *et al.*, 1994a), but the interaction of kynurenine with specific 5-HT2 receptor agonists has not previously been demonstrated.

The purpose of the study presented here was to examine the effects of kynurenine on the 5-HT2A mediated head-shake response caused by the selective 5-HT2A/2C agonist DOI (Glennon *et al.*, 1986).

Additional Methods

Male MF1 mice (Aston bred; weighing 24-32 g, n=6 per treatment group). Kynurenine (0.5mg/kg s.c.) or vehicle (0.9% saline) was administered to experimentally naive mice 120 min. prior to the determination of HS frequency (Handley and Miskin, 1977) and vehicle (saline) or a submaximal dose of DOI (1.0mg/kg i.p.) (Heaton and Handley, 1989) 5 min. prior to determination. The HS frequency was counted from video recordings for a 10 min. period, with the observer blind to the treatment group.

Results

As shown in *figure* 6.1, DOI increased the head-shake frequency compared to control mice (general linear 2-Way ANOVA, $F(1,20)=22.62$; $p\leq0.0001$: *post-hoc* Tukey's test; $p\leq0.01$) which were significantly potentiated by kynurenine ($p<0.01$), although there was no effect of kynurenine alone.

Discussion

The results presented here demonstrate that kynurenine potentiates the effects of DOI on HS frequency in the mouse. This is consistent with previous findings from our laboratory which indicated that 5-HT and 5-HTP HSs could be potentiated by

kynurenine and 3-hydroxykynurenine (Handley and Miskin, 1977). It is likely that these effects are due to an interaction with the 5-HT2A receptor (either directly or indirectly) since the 5-HT2C receptor is not involved in the HS response (Kennett and Curzon, 1991; Kennett *et al.*, 1994a; Schreiber *et al.*, 1995). Further research is required to elucidate the mechanisms by which kynurenine and/or its further metabolite(s) interact with this effect of 5-HT2A receptor activation.

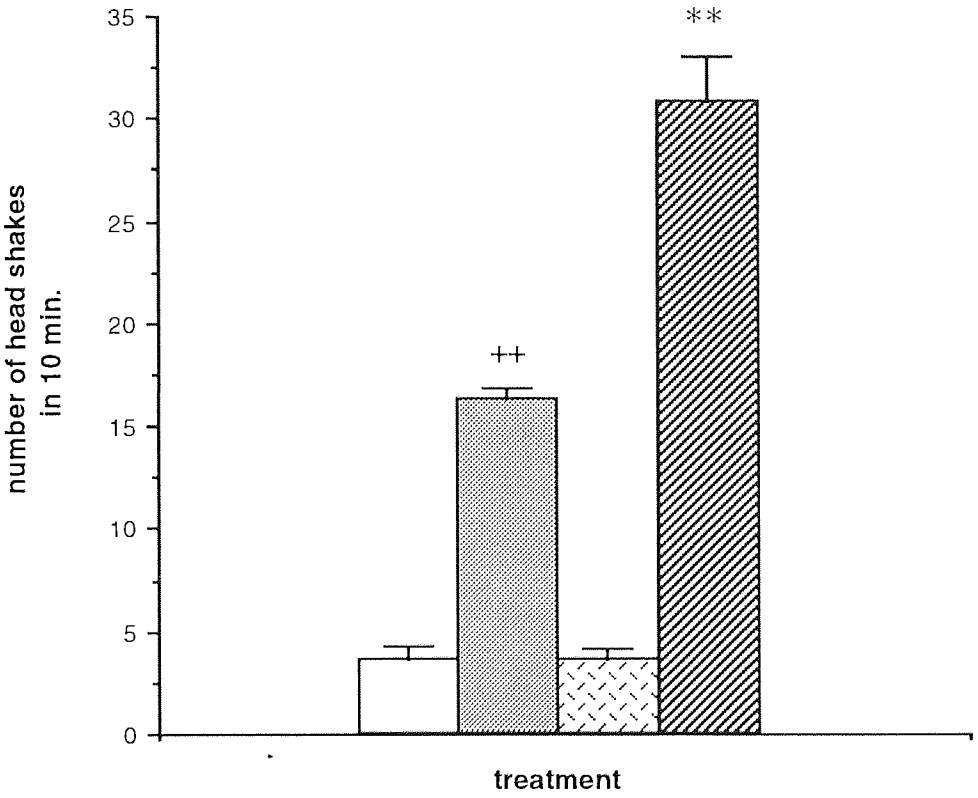


Figure 6.1. Data representing the potentiation of the DOI HS by kynurenine. (**, $p<0.01$ *cf.* Saline+DOI; ++, $p\leq0.01$ *cf.* Saline+Saline). Open bar, Saline+Saline; shaded bar, Saline+DOI; stippled bar, Kynurenine+Saline and hatched bar - Kynurenine+DOI.

GENERAL DISCUSSION.

GENERAL DISCUSSION.

1. Evaluation of the pharmacology of Tic Like Behaviours Measured

The pharmacology of MK771-induced blinking, FPT and FPL were investigated along with the effects of (S)-WAY-100135 on spontaneous and DOI head shakes. The effects of kynurenine were also investigated on the DOI HS.

Results exhibited in Chapter 1 demonstrated that the effects of the drugs used in the MK771 studies were investigated at peak time of behavioural activation and all antagonist studies were conducted at approximately 50% of the maximal response, in order to detect either potentiating or antagonist properties of the drugs tested. No significant differences between acutely isolated and mice tested in pairs were seen for FPL and blinking, in these initial studies FPT was not tested. The effects of MK771 on blinking are consistent with a preliminary report which indicated that TRH could induce blinking in mice (Dursun and Handley, 1991), the induction of FPT by TRH and analogues has been demonstrated in rats before, but not quantified (Simasko and Horita, 1985; Yarborough *et al.*, 1978; Wei *et al.*, 1975; Costall *et al.*, 1979; Fone *et al.*, 1987 and FPL has been demonstrated in rats (Fone *et al.*, 1987; 1988; 1989a and b; Johnson *et al.*, 1989; Jackson, 1990).

Results presented in Chapter 2 demonstrate that DA D1 and D2 antagonists were without effect on MK771-induced blinking. This is in contrast to previous reports which indicated that the selective D1 agonist dihydrexidine induced blinking, an effect which was blocked by D1 antagonists (Taylor *et al.*, 1991; Elsworth *et al.*, 1991) and that D2 agonists induced blinking (Karson, 1983; Casey *et al.*, 1980; Karson *et al.*, 1980; Elsworth *et al.*, 1991; Lawrence *et al.*, 1991). However, haloperidol did not antagonise MK771-induced blinking (Dursun and Handley, 1991). Thus the data presented suggests that MK771-induced blinking is not under the control of dopaminergic systems. Rather it appears that serotonin receptors may modulate this behaviour (Chapter 3 and 4). Thus, 5-HT2A antagonists significantly attenuated MK771-induced blinking, but the selective 5-HT2B/2C antagonist SB200646A was without affect, suggesting that 5-HT2A but not 5-HT2B/2C receptors modulate MK771-induced blinking. Low doses of the 5-HT1A agonists 8-OH DPAT and buspirone significantly attenuated blinking, but this was not observed at higher doses, no conclusive evidence as to the locus of this modulation was obtained (Chapter 4). The alpha-1 adrenoceptor antagonist prazosin had no effect upon blinking (Chapter 3). Previously data on the modulation of blinking by serotonergic agents has been minimal. One report indicated that LSD was without effect (Karson, 1983) and TRH-induced blinking was attenuated by ritanserin and ICI169,369 (Dursun and Handley, 1991;

Dursun, 1992). These results therefore suggest that the pharmacology of MK771-induced blinking is different from the previously characterised pharmacology of spontaneous blinking. Previous studies have indicated that cholinergic mechanisms might be involved in blinking, such that the cholinergic antagonist atropine increases blinking and physostigmine (a choline-esterase inhibitor) decreases blinking (Karson, 1989; Schelkunov, 1986). However, TRH and analogues caused an increase in acetylcholine release (Hutson *et al.*, 1990; Giovanini *et al.*, 1991). It is therefore suggested that the effects of MK771 on blinking are not due to its effects on the cholinergic system, since it would be expected that TRH (or analogues) would decrease blinking due to the actions on the cholinergic system. The serotonergic control of blinking is similar to that of the HS response in that the HS response is related to activation of the 5-HT2A receptor (Green *et al.*, 1983; Green and Heal, 1985; Kennett and Curzon, 1991; Dursun, 1992), 5-HT1A agonists attenuate HS (Arnt and Hyttel, 1989; Heaton and Handley, 1989; Darmani *et al.*, 1990; Dursun and Handley, 1993; Berendsen, 1991; Schreiber *et al.*, 1995; Kitamura *et al.*, 1994) and the 5-HT2C/2B receptor antagonist was without effect on DOI HS in rats (Kennett *et al.*, 1994a).

TRH or TRH-analogue induced FPT and FPL have not previously been demonstrated in the mouse. FPL was not significantly different when acutely isolated and paired mice were compared. Results of the experiments displayed in Chapter 2 indicate that D1 antagonists attenuated MK771-induced FPL and FPT, whereas the D2 antagonists were generally without effect (apart from a significant potentiation of FPT by the higher dose of raclopride). These results suggest a permissive role of D1 receptors on TRH-induced FPT and FPL and that the D2 receptor sub-type might not be involved in the modulation of FPT and FPL. However, an alternative hypothesis was presented suggesting that an interaction between D1 and D2 receptors might occur in the expression of MK771-induced FPT behaviour, but further experiments are indicated to verify this.

5-HT2A antagonist pre-treatment (Chapter 3) attenuated MK771-induced FPT and FPL, consistent with the antagonism of FPL by ritanserin (Fone *et al.*, 1989a), the effects of 5-HT2A antagonists on TRH receptor-induced FPT have not previously been established. Pre-treatment with the novel 5-HT2C/2B, antagonist SB200646A indicated that MK771-induced FPL was not modulated by these receptor sub-types. However, potentiation of the FPT response was found, indicating that MK771-induced FPT may be under the inhibitory control of 5-HT2C or 5-HT2B receptors, consistent with the inhibitory tone of 5-HT2C receptors upon back-muscle-contractions (Fone and Sharma, 1993). Prazosin attenuated FPL, consistent with previous reports indicating that alpha-1 adrenoceptor antagonists attenuated FPL in the rat induced by TRH and

TRH analogues (Fone *et al.*, 1987 and 1989b). Alpha-1 adrenoceptor antagonism has been reported to inhibit HS following administration of 5-MeODMT, spontaneous and DOI HS (Handley and Brown, 1982; Heal *et al.*, 1986; Dursun and Handley, 1992; Dursun, 1992).

Data presented in Chapter 4 demonstrated that MK771-induced FPT and FPL were dose dependently antagonised by the 5-HT1A agonists 8-OH DPAT and buspirone. It appeared that the inhibition of FPL and perhaps FPT was mediated via post-synaptic 5-HT1A sites, (but the latter was inconclusive). Studies in the rat have indicated that FPL was antagonised by 8-OH DPAT administration into the caudal-periaqueductal grey in previously untreated rats (Beckett *et al.*, 1991). Thus, FPT and FPL behaviours appear to display similar pharmacology to the HS response, in that the HS has been shown to be related to blockade of the 5-HT2A receptor (Green *et al.*, 1983; Green and Heal, 1985; Kennett and Curzon, 1991; Dursun, 1992). Prior administration with the 5-HT1A antagonist (S)-WAY-100135 failed to have any effect on MK771-induced behaviour, or spontaneous and DOI HS, this is consistent with the lack of intrinsic activity demonstrated by the compound (see Chapter 5 for full citations) and demonstrates that during the behaviours in question there is probably inefficient transmission through the 5-HT1A receptor so that blockade fails to have any effect. 5-HT1A receptor agonists attenuated the HS response in mice (Arnt and Hyttel, 1989; Heaton and Handley, 1989; Darmani *et al.*, 1990; Dursun and Handley, 1993; Schreiber *et al.*, 1995; Kitamura *et al.*, 1994).

DA D1 antagonist pre-treatment antagonised both DOI and spontaneous HS in mice (Dursun, 1992) as did D2 antagonist pre-treatment with haloperidol and pimozide, an effect which was thought to be due to their action at the 5-HT2A receptor (Dursun, 1992). However, Dursun (1992) demonstrated that raclopride and sulpiride were without effect on spontaneous or DOI HS.

Data presented in Chapter 6 demonstrated that kynurenine potentiated the DOI HS response, consistent with a previous report indicating that this dose potentiated the 5-HT and 5-HTP-induced HS response in mice. It is therefore indicated that kynurenine (or kynurenine metabolites) might interact in some way with 5-HT2A receptors, but whether this is a direct or in-direct interaction remains to be established.

The actions of serotonergic drugs appears to be somewhat variable with respect to the behaviours studied. For example the 5-HT2A antagonists attenuated FPL behaviour to a greater extent than FPT and blinking (blinking and FPT were inhibited to a similar extent) (Chapter 3). Similarly the 5-HT1A agonists appeared to be more potent against MK771-induced FPL than FPT and blinking was only attenuated by the low doses of 5-HT1A agonists (Chapter 4). These results suggest that there may be different

populations of 5-HT receptors or possibly different serotonergic neurones involved in the modulation of each of these behaviours.

2. Evaluation of Tic Like Models as *in vivo* Models of GTS

Validation of an animal model can only take place when the three categories of validity are fulfilled: face validity, construct validity and predictive validity (Abramson and Seligman, 1977; McKinney and Bunney, 1969). However, since the aetiology of GTS is not fully understood (see General Introduction for details) construct validity cannot be assessed, to date only one group have assessed predictive validity. Insofar as the 5-HT1A agonists attenuate HS (it has been proposed that this and other tic like behaviours offer strong face validity as a model for GTS and thus may represent a model for GTS syndrome (Handley and Dursun, 1992)), and case studies have indicated that buspirone was effective in the treatment of GTS (Dursun *et al.*, 1995; Burke *et al.*, 1995). Face validity is the similarity of the characteristics presented in an animal model, together with the pharmacology of those characteristics, and is essential for the initial development of a model.

The MK771-induced behaviours presented here display similar characteristics to those observed in GTS. For example, GTS patients display an increase in spontaneous blink rates and this is often one of the first symptoms of the disease (Cohen *et al.*, 1980; Bonnett, 1982). Tics of the limbs have been indicated, as has hand smelling (please refer to *tables* 1.4.2 and 1.4.3, General Introduction), thus MK771-induced FPT and FPL may model these tics observed in GTS sufferers. Both tics observed in GTS patients and those demonstrated here fulfil the criteria for tics in being, abrupt, rapid and swift movements which agrees with DSM-III-R (1987) criteria.

GTS symptoms are responsive to administration of serotonergic agents, such as chronic treatment with LSD (Smith, 1969), methysergide (Shapiro *et al.*, 1978) and buspirone (Dursun *et al.*, 1995; Burke *et al.*, 1995). The D1 antagonists attenuated FPT and FPL, but the D2 antagonists were without effect, however in another study haloperidol attenuated FPT (Dursun and Handley, 1991), effects which were considered to be due to the actions of haloperidol at the 5-HT2A receptor sub-type (Dursun, 1992). Analysis of a variety of compounds has indicated that the effectiveness of these compounds to inhibit HS in mice is related to their binding profile at the 5-HT2A receptor primarily, but alpha-1 and D1 antagonism may also play a role (Dursun, 1992). Thus, the actions of the alpha-1 antagonist prazosin and the D1 antagonists upon FPL and FPT is not entirely unexpected and furthermore this would explain why the DA D2 antagonists raclopride and sulpiride were without effect on MK771-induced behaviours. The pharmacology of MK771-induced FPT and FPL may therefore display

pharmacological similarities with the pharmacology of the HS response which has been proposed as a possible model for GTS (Handley and Dursun, 1992). Therefore, FPL and FPT may offer face validity as possible models for GTS too.

MK771-induced blinking displayed a different pharmacology to that of FPL and FPT, insofar as the DA D1 antagonists SCH23390 and SCH39166 and the alpha-1 adrenoceptor antagonist prazosin were without effect. It is therefore suggested that D1 and alpha-1 adrenoceptors do not modulate TRH-receptor mediated blinking at all, in contrast to the HS response (Dursun, 1992). In this respect TRH-receptor mediated blinking may offer face validity as a possible model for GTS, but more accurately may model the serotonergic perturbation displayed in the disease (see General Introduction). However, it would be pertinent to investigate the pharmacology of this behaviour to a greater extent.

Plasma kynurenine concentrations are elevated in GTS patients (Dursun *et al.*, 1994) and have potentiated the DOI HS response in mice (Chapter 6). Thus, further evidence is provided, which elaborates on that already obtained (Dursun, 1992; Handley and Dursun, 1992), to suggest that the DOI HS response provides face validity as a model for GTS.

The tic like behaviours presented here and the HS response (Dursun and Handley, 1992; Dursun, 1992; data presented here) are almost certainly related to 5-HT2A receptors and it has been proposed that 5-HT2A agonists are hallucinogens (Glennon *et al.*, 1987; Glennon and Lucki, 1989). However, it is unlikely that global changes in 5-HT2A receptors occur in GTS (see Dursun, 1992). One report has suggested that hallucinations occur in GTS, but this is not a common feature of the disease (Comings 1990b) which would tend to argue against a general 5-HT dysfunction but more likely is the possibility of a local dysfunction occurring in the disease (Dursun, 1992). Handley (1995) proposed that local control may be important in the control of serotonergic function based on the hypotheses of Jacobs and Fornal (1993). It appears that the message carried by serotonergic neurones may be modulated locally, for example glutamate may modulate the actions of 5-HT (see Jacobs and Fornal, 1993 for review). Therefore in order to establish whether there was a change in global 5-HT neurotransmission during a tic like behaviour we decided to analyse the effects of a tic like movement upon serotonergic unit activity in the cat (*ie.* we wanted to record whether serotonergic neurone was activated above baseline during a tic like movement). In order to perform these experiments it was necessary to visit Prof. Barry Jacobs' laboratory at Princeton University. However, upon arrival it became apparent that this line of investigation was not possible, owing to methodological considerations, such as the presence of electrical noise during a HS and the possibility of finding a neurone

which fired under such circumstances was minimal, especially in the allotted time. Therefore the pharmacological effects of the novel 5-HT1A antagonists (S)-WAY-100135 and WAY-100635 in the DRN were analysed.

3. The Effects of 5-HT1A Antagonists on Neuronal Discharge in the DRN

(S)-WAY-100135 appeared to act as a partial agonist at the 5-HT1A autoreceptor *ie.* it had an agonist profile when administered alone but antagonised the suppressant action of 8-OH DPAT (Chapter 5). Such findings are consistent with the profile of other partial agonists at the somatodendritic autoreceptor (Millan *et al.*, 1994; Claustre *et al.*, 1991; Sharp *et al.*, 1993; Greuel and Glasser, 1992; Sharp *et al.*, 1990; Gurling *et al.*, 1993; Hjorth and Sharp, 1990; Williams and Dourish, 1992; Mundey *et al.*, 1994a; Fletcher *et al.*, 1993b; Fornal *et al.*, 1994b). However studies have indicated that (S)-WAY-100135-induced reduction of firing rate in the DRN may be due to its alpha-1 antagonist effects (Lanfumey *et al.*, 1993), see Chapter 5 for full discussion. However, (S)-WAY100135 will still be of considerable value as a pharmacological tool for probing 5-HT1A autoreceptor function (*eg.* Routledge *et al.*, 1993; Hartley and Fletcher, 1994; Fletcher *et al.*, 1993b; Mundey *et al.*, 1994a; Gobert *et al.*, 1995).

WAY-100635, as expected of a 5-HT1A antagonist augmented the unit discharge activity of DRN serotonergic neurones and antagonised the suppressant actions of 8-OH DPAT (Chapter 5). Such a profile is consistent with an antagonist action of WAY-100635 at the somatodendritic autoreceptor and the effects of the non-selective 5-HT1A antagonist spiperone in the same paradigm (Fornal *et al.*, 1994a). This suggests that WAY-100635 will offer an extremely valuable tool for the psychopharmacologist to probe the actions of the 5-HT1A autoreceptor. It has been suggested that it may be useful for the treatment of a variety of neurological and psychiatric conditions, such as anxiety, dementia, phobias, schizophrenia and depression (for review see Fletcher *et al.*, 1993a),. However, these authors stipulate that a great deal more pre-clinical work is needed. In a preliminary study the 5-HT1A receptor antagonist pindolol has proven useful in the treatment of depression when co-administered with serotonin specific re-uptake inhibitors or monoamine oxidase inhibitors (Artigas *et al.*, 1994). Pindolol reduced the latency of effective treatment by the serotonin-specific reuptake inhibitors and monoamine reuptake inhibitors. In pre-clinical studies penbutalol and (S)-UH301 potentiated the increase in extracellular 5-HT following citalopram pre-treatment (Hjorth, 1993). Similarly, administration of WAY-100635 antagonised the suppression of firing induced by paroxetine and tranylcypromine and potentiated the effects of these compounds on the reduction of extracellular 5-HT concentrations (Gartside *et al.*,

1995a and b). Thus, WAY-100635 may be a useful adjunct to current depression therapy if it is found to be safe for clinical use.

Positron-emission tomography (PET) studies have demonstrated that 5-HT1A receptors in the human brain may be visualised with [^{11}C]-WAY-100635 *in-vivo* (Bench *et al.*, 1995), interestingly 5-HT1A receptors were visualised in the brain stem, and probably represented binding to 5-HT1A receptors in the raphe nuclei. Thus, WAY-100635 may represent a powerful tool for the elucidation of 5-HT1A function both in the human and animal brain, and will be useful for probing 5-HT1A receptor function in psychiatric disorders.

In conclusion spontaneous blinking induced by MK771 displays different pharmacology to that previously established for spontaneous blinking, the pharmacology of FPT and FPL are also demonstrated. The pharmacology of these latter behaviours appear to offer close similarity with that of the HS response, which has been proposed as a model for GTS. The pharmacology and phenomenological similarity of these behaviours with those observed in GTS suggests that these behaviours might offer face validity as a possible model of the disease. The antagonist actions of the 5-HT1A antagonist WAY-100635 at the somatodendritic autoreceptor suggest that this will be a useful tool for the elucidation of 5-HT1A receptor function. (S)-WAY-100135, lacked intrinsic activity in behavioural paradigms but its action at the somatodendritic autoreceptor suggested a partial agonist profile, but further work will be necessary to establish the role of alpha-1 adrenoceptor antagonism in this effect.

4. Suggestions for Future Studies:

1. Further studies are indicated to investigate the potentiation of MK771-induced FPT by the 5-HT2C/2B antagonist SB200646A.

2. Clinical trials with the 5-HT1A agonists and 5-HT2A receptor antagonists in GTS are suggested.

3. Further studies of the effects of kynurenine pathway metabolites upon HS behaviour are suggested.

4. The interaction of TRH receptor analogues (or TRH) with kynurenine and kynurenine pathway metabolites is suggested.

5. Further studies are indicated to investigate the actions of alpha-1 adrenoceptor actions of (S)-WAY100135 on DRN firing.

6. An investigation of the interaction of WAY-1000635 with serotonin-specific-reuptake inhibitors in the behaving cat paradigm would be of considerable interest.

REFERENCES.

Abramson LY and Seligman MEP (1977) Modelling psychopathology in the laboratory: History and rationale. In Psychopathology: Animal Models (Eds. J. D. Maser and M. E. P. Seligman), Vol. 16, pp209-253, Plenum Press, New York.

Adams RD and Victor M (1981) Principles of neurology. McGraw-Hill, New York, 2nd ed.

Adham N, Borden LA, Schechter LE, Gustafson EL, Cochran TL, Vaysse PJ-J, Weinshank RL and Branchek TA (1993) Cell-specific coupling of the cloned human 5-HT1F receptor to multiple signal transduction pathways. N. S. Arch. Pharmacol. 348:566-575.

Agarwal RA, Rastogi RB abd Singhal RL (1977) Enhancement of locomotor activity and catecholamine and 5-hydroxytryptamine metabolism by thyrotropin releasing hormone. Neuroendocrinology. 23:236-247.

Agren H, Mefford IN, Rudorfer MV, Linnoila M and Potter WZ (1986) Interacting neurotransmitter systems. A non-experimental approach to 5-HIAA-HVA correlation in human CSF. J. Psych. Res. 20:175-193.

Akbari HM, Anderson GM, Pollak ES and Cohen DJ (1993) Serotonin receptor binding and tissue indoles in postmortem cortex of Tourette's syndrome individuals. Soc. Neurosci. Abstr. 19:340.8.

Amlaiky N, Ramboz S, Boschert U, Plassat JL and Hen R (1992) Isolation of a mouse 5-HT1E-like serotonin receptor expressed predominantly in hippocampus. J. Biol. Chem. 267:19761-19764.

Ananth J, Pecknold JC and Van Der Steen N (1981) Double-blind comparative study of clomipramine and amitryptiline in obsessive compulsive neurosis. Prog. Neuropsychopharmacol. 5:257-264.

Anderson GM, Pollak ES, Chatterjee D, Leckman JF, Riddle MA and Cohen DJ (1992) Postmortem analysis of subcortical monoamines and amino acids in Tourette syndrome. In Advances in Neurology 58: 123-133 (Ed. T. N. Chase, A. J. Friedhoff and D. J. Cohen), Raven Press Ltd., New York.

Andrade R, Malenka RC and Nicoll RA (1986) A G-protein couples serotonin and GABAB receptors to the same channels in hippocampus. Science 234:1261-1265.

Annunziato L, Cerrito F and Raiteri M (1981) Characteristics of dopamine release from isolated nerve endings of the tuberoinfundibular neurones. Neuropharmacol. 20:727-734.

Annunziato L, Di Renzo G, Amoroso S and Quattrone A (1984) Release of endogenous dopamine from tuberoinfundibular neurons. Life Sci. 35:399-407.

Armington JC (1981) Visually evoked cortical potentials accompanying blinks. Invest. Opthamol. Vis. Sci. 20:691-700.

Arnt J and Hyttel J (1989) Facilitation of 8-OH DPAT induced forepaw treading of rats by the 5-HT 2 agonist DOI. Eur. J. Pharmacol. 161:45-51.

Arnt J, Hyttel J and Larsen J J (1984) The citalopram/5-HTP-induced head shake syndrome is correlated to 5-HT2 receptor affinity and also influenced by other transmitters. Acta Pharmacol. Toxicol. 55:363-372.

Arnt J, Hyttel J and Perregaard J (1987) Dopamine D-1 receptor agonists combined with the selective D-2 agonist quinpirole facilitate the expression of oral stereotyped behaviour in rats, Eur. J. Pharmacol. 133:137-145.

Artigas F, Perez V and Alvarez E (1994) Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. Arch. Gen. Psych. 51:248-251.

Asberg M, Schalling D, Traskman-Bendz L and Wagner A (1987) Psychobiology of suicide, impulsivity, and related phenomena. In Pschopharmacology: Third Generation of Progress, pp 655-668, (Ed. H. Y. Meltzer), Raven Press, New York.

Asberg M, Traskman L and Thoreen P (1976) 5-HIAA in the cerebrospinal fluid: a biochemical suicide predictor? Arch. Gen. Psych. 33:1193-1197.

Backus LI, Sharp T and Grahame-Smith DG (1990) Behavioural evidence for a functional interaction between central 5-HT 1A and 5-HT 2 receptors. Br. J. Pharmacol. 100:793-799.

Baker GB, Bornstein RA, Douglass AB, Carroll A and King G (1991) Urinary excretion of metabolites of norepinephrine in Tourette's syndrome. Mol. Chem. Neuropath. 13:225-232.

Banki CM, Molnar G and Vojnik M (1981) Cerebrospinal fluid amine metabolites, tryptophan and clinical parameters in depression: psychopathological symptoms. J. Affective Dis. 3:1991.

Bannet J and Belmaker RH (Eds.) (1983) New Directions in Tardive Dyskinesia Research. Karger, Basel.

Baraban JM and Aghajanian GK (1980) Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor anatagonists. Neuropharmacol. 19:355.

Baraister M (1982) The Genetics of Neurological Disorders. Oxford University Press.

Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA and Weinshank RL (1993) Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylyl cyclase. J. Biol. Chem. 268:23422-23426.

Barnes JM, Barnes NM, Costall B, Naylor IL, Naylor RJ and Rudd JA (1990) Topographical distribution of 5-HT 3 receptor recognition sites in the ferret brain stem. N. S. Arch. Pharmacol. 342:17-21.

Beckett SRG, Lawrence AJ, Marsden CA and Marshall PW (1992) Attenuation of chemically induced defence response by 5-HT1 receptor agonists administered into the periaqueductal gray. Psychopharmacol. 108: 110-114.

Bedard P and Pycock CJ (1977) "Wet-dog"shake behaviour in the rat a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacol. 16:663-670.

Bednarczyk B and Vetulani J (1978) Antagonism of clonidine to shaking behaviour in morphine abstinence syndrome and to head twitches produced by serotonergic agents in the rat . Pol. J. Pharmacol. 30:307-322.

Bench CJ, Lammertsma AA, Pike VJ, Hume SP, Cliffe IA, Fletcher A and Grasby PM (1995) Imaging the 5-HT1A receptor in man using positron emission tomography and [^{14}C]-WAY100635. J. Psychopharmacol. 9(S):16

Bennett GW (1990) Functional interactions between neuropeptides and noradrenaline in the brain and spinal cord. In The Pharmmacology of Noradrenaline in the Central Nervous System, (Ed. D. J. Heal and C. A. Marsden), pp 454-494, pub. Oxford Medical Publications.

Berendsen HHG and Broekkamp CLE (1990) Behavioural evidence for functional interactions between 5-HT-receptor subtypes in rats and mice. Br. J. Pharmacol. 101:667-673.

Berg R (1985) A case of T.S. treated with nifedipine. Acta Psychiat. Scand. 72:400-401.

Bill DJ, Knight M, Forster EA and Fletcher A (1991) Direct evidence for an important species difference in the mechanism of 8-OH-DPAT-induced hypothermia. Brit. J. Pharmacol. 103:1857-1864.

Bjork L, Cornfield LJ, Nelson DL, Hillver SE, Anden NE, Lewander T and Hacksell U (1991) Pharmacology of the novel 5-hydroxytryptamine1A receptor antagonist (S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin: inhibition of (R)-8-hydroxy-2-(dipropylamino)tetralin-induced effects. J. Pharmacol. Exp. Ther. 258:58.

Blier P, Lista A and De Montigny C (1993) Differential properties of pre- and postsynaptic 5-hydroxytryptamine1A receptors in the dorsal raphe and hippocampus: 1. effects of spiperone. J. Pharmacol. Exp. Ther. 265:7-15.

Blier P, Steinberg S, Chaput Y and De Montigny C (1989) Electrophysiological assessment of putative antagonists of 5-hydroxytryptamine receptors: a single-cell study in the rat dorsal raphe nucleus. Can. J. Physiol. Pharmacol. 67:98-105.

Bliss J, Cohen DJ and Freedman OX (1980) Sensory experiences of Gilles de la Tourette's syndrome. Arch.Gen. Psych. 37:1343-1347.

Boddeke HWGM, Fargin A, Raymond JR, Schoeffter P and Hoyer D (1992) Agonist/antagonist interactions with cloned human 5-HT1A receptors: variations in intrinsic activity studied in transfected HeLa cells. Arch. Pharmacol. 345:257-263.

Bonnet KA (1982) Neurobiological dissection of Tourette syndrome: a neurochemical focus on a human neuroanatomical model. In Gilles de la Tourette Syndrome, Advances in Neurology, vol. 35, (Ed. A. J. Friedhoff & T. N. Chase), pub. Raven Press: New York.

Borison RL, Ang L, Chang S, Dysken M, Comaty JE and Davis JM (1982) New pharmacological approaches in the treatment of Tourette syndrome. Adv. Neurol. 35:377-382.

Bouhelal R, Smounya L and Bockhaert J (1988) 5-HT1B receptors are negatively coupled with adenylate cyclase in rat substantia nigra. Eur. J. Pharmacol. 151:189-196.

Boulenguez P, Chauveau J, Segu L, Morel A, Lanoir J and Delaage M (1992) Biochemical and pharmacological characterisation of serotonin-O-carboxymethylglcyl[125]iodotyrosinamide, a new radioiodinated probe for 5-HT1B and 5-HT1D binding sites. J. Neurochem. 58:951-959.

Boulton CS and Handley SL (1973) Factors modifying the head-twitch response to 5-hydroxytryptophan. Psychopharmologia (Berl) 31:205-214.

Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PPA, Middlemiss D. N, Miliecharane EJ, Richardson BP and Saxona PR (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacol. 25:563-576.

Brady KD, Han B and Tashjian AH (Jr) (1994) Kinetics and reversibility of thyrotropin-releasing hormone-stimulated guanine nucleotide exchange inmembranes from GH4C1 cells. Mol. Pharmacol. 46:644-652.

Branchey I, Branchey M, Shaw S and Lieber CS (1984) Depression, suicide and agression in alcoholics and their relationship to plasma amino acids. Psych. Res. 12:219-226.

Bristow LJ, Baucutt L, Thorn L, Hutson PH, Noble A, Beer A, Middlemass DN and Tricklebank MD (1991) Behavioural and biochemical evidence of the interaction of the putative antipsychotic agent, BMY 14802 with the 5-HT1A receptor. Eur. J. Pharmacol. 204:21-28.

Brooks DJ, Turjanski N, Swale GV, Playford ED and Lees AJ (1992) PET studies on the integrity of the pre and postsynaptic dopaminergic systems in Tourette syndrome. In Advances in Neurology, (Ed. T. N. Chase, A. J. Friedhoff and D. J. Cohen), pub. Raven Press Ltd., New York.

Brown DD and Chambers RA (1976) Physiological aspects of visual perception. I and II. Arch. Neurol. 33:219-242.

Brown GL, Ebert MH, Goyer PF, Jimerson DC, Klein WJ, Bunney WE and Goodwin FK (1982) Aggression, suicide, and serotonin: relationships to CSF amine metabolites. Am. J. Psych. 139:741-746.

Brown GL, Goodwin FK and Ballenger JC (1979) Aggression in humans correlates with cerebrospinal fluid amine metabolites. Psych. Res. A:131-139.

Bruun RD (1984) Gilles de la Tourette's syndrome: an overview of clinical experience. J. Am. Acad. Child Psych. 23:126-133.

Buisseret P and Maffei L (1982) Suppression of visual cortical activity during eyeblinks in the cat. Physiol. Soc. 19P.

Burke JG, Revely MA and Dursun SM (1995) A case study of Tourette's syndrome (TS) treated with buspirone. J. Psychopharmacol. 9(S):99.

Butler IJ, Koslow SH, Seifert WE, Caprioli RM and Singer HS (1979) Biogenic amine metabolism in Tourette syndrome. Ann. Rev. Neurol. 6:37-39.

Caine ED (1985) Gilles de la Tourette's syndrome: a review of clinical and research studies and consideration of future directions for investigations. Arch. Neurol. 42:393-397.

Caine ED, Polinsky RJ, Ebert MH, Rapoport JL and Mikkelson EJ (1979) Trial of chlorimipramine and desipramine for Gilles de la Tourette's syndrome. Ann. Neurol. 5:305-306.

Casey DE, Gerlach J and Christenson E (1980) Behavioural aspects of GABA interrelationships in the monkey. Brain Res. Bull. 5(s2):269-273.

Ceulemans DLS, Gelders YG, Hoppenbrouwers MLJA, Reyntjens AJM and Janssen PAJ (1985a) Effect of serotonin antagonism in schizophrenia: a pilot study with setoperone. Psychopharmacol. 85:329-332.

Charney DS and Heninger GR (1986) Serotonin function in panic disorders. The effect of intravenous tryptophan in healthy subjects and patients with panic disorder before and during alprazolam treatment. Arch. Gen. Psych. 43:1059-1065.

Charney DS, Woods SW, Goodman WK and Heninger GR (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist m-CPP in panic disorder patients and healthy subjects. Psychopharmacol. 92:14-24.

Ciprian J (1980) Three cases of Gilles de la Tourette's syndrome. Treatment with chlorimipramine: a preliminary report. J. Orthomol. Psych. 9:116-120.

Claustre Y, Rouquier L, Serrano A, Benavides J and Scatton B (1991) Effect of the putative 5-HT1A receptor antagonist NAN-190 on rat brain serotonergic transmission. Eur. J. Pharmacol. 204:71-77.

Cliffe IA, Brightwell CI, Fletcher A, Forster EA, Mansell HL, Reilly Y, Routledge C and White AC (1993) (S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)-piperazin-1-yl)-

2-phenylpropanamide [(S)-WAY-100135]: A selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. J. Med. Chem. 36:1506-1510.

Cohen B and Feldman M (1968) Relationship of electrical activity in the pontine reticular formation and lateral geniculate body to rapid eye movements. J. Physiol. 31:809-817.

Cohen DJ, Detlor J, Young JG and Shaywitz BA (1980) Clonidine ameliorates Gilles de la Tourette syndrome. Arch. Gen. Psych. 37:1350-1357.

Cohen DJ, Leckman JF and Shaywitz BA (1984) Tourette's syndrome: assessment and treatment. In diagnosis and treatment (Ed. D. Shaffer, A. A. Ehrhardt & L. Greenbill) Macmillan Free Press, New York.

Cohen DJ, Young JG, Nathanson JA, Lieberman D, Bowers MB and Maas JW (1979) Central biogenic amine metabolism in children with the syndrome of chronic multiple tic of Gilles de la Tourette: norepinephrine, serotonin and dopamine. J. Am. Child Psych. 18:320-341.

Colpaert FC and Janssen PAJ (1983a) The head-twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: Antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, a LSD antagonist. Neuropharmacol.22:993-1000.

Comings DE (1990a) Blood serotonin and tryptophan in Tourette's syndrome. Am. J. Med. Gen. 36:418-430.

Comings DE (1990b) Tourette syndrome and human behaviour. Hope Press, Duarte, California.

Comings DE and Comings BG (1986) Evidence for a X-linked modifer gene affecting the expression of Tourette syndrome and its relevance to the increased frequency of speech, cognitive, behavioural disorders in males. Proc. Nat. Acad. Sci. 83:2551-2555.

Comings DE and Comings BG (1987a) A controlled study of Tourette's syndrome. I-VII. Am. J. Hum. Gen. 41:701-866.

Comings DE and Comings BG (1987b) Hereditary agorophobia and obsessive compulsive behaviour in relatives of patients with Gilles de la Tourette syndrome. Br. J. Psych. 151:195-199.

Comings DE and Comings BG (1985) Tourette syndrome: clinical and psychological aspects of 250 cases. Am. J. Hum. Gen. 37:435-450.

Comings DE and Comings BG (1992) Alternative hypotheses on the inheritance of Tourette syndrome. In Advances in Neurology, (Ed. T. N. Chase, A. J. Friedhoff and D. J. Cohen), pub. Raven Press Ltd., New York.

Comings DE, Muhleman D, Dietz G, Dino M, LeGro R and Gade R (1993) Association between Tourette's syndrome and homozygosity at the dopamine D3 receptor gene. Lancet 341:906.

Conn PJ and Sanders-Bush E (1984) Selective 5-HT2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacol. 23:993-996.

Conn PJ and Sanders-Bush E (1985) Serotonin-stimulated phosphoinositide turnover: mediation by the S2 binding site in rat cerebral cortex but not in subcortical regions. J. Pharmacol. Exp. Ther. 234:195-203.

Conn PJ and Sanders-Bush E (1986) Agonist-induced phosphoinositide hydrolysis in choroid plexus. J. Neurochem. 47:1754-1760.

Conn PJ, Sanders-Bush E, Hoffman BJ and Hartig PR (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. Proc. Natl. Acad. Sci. USA 83:4086-4088.

Corne SJ and Pickering RW (1967) Apossible correlation between drug-induced hallucinogens in man and a behavioural response in mice. Psychopharmacologia 11:65-78.

Corne SJ, Pickering RW and Warner BT (1963) A method for assessing drugs on the central action of 5-hydroxytryptamine. Br. J. Pharmacol. 20:106-120.

Costall B, Hui S-CG, Metcalf G and Naylor RJ (1979) A study of the changes in motor behaviour caused by TRH on intracerebral injection. Eur. J. Pharmacol. 53:143-150.

Crespi F, Keane PE, and Morre M (1986) In vivo evaluation by differential pulse voltammetry of the effect of thyrotrophin-releasing-hormone (TRH) on dopanergic and serotonergic synaptic activity in the striatum and nucleus accumbens of the rat. Exp. Brain Res. 62: 329-334.

Crosley CJ (1979) Decreased serotoninergic activity in Tourette syndrome. Ann. Neurol. 5(6):596.

Dahlstrom A and Fuxe K (1965) Evidence for the existence of monoamine containing neurone in the central nervous system. Demonstration of monoamine in the cell bodies of brain stem neurons. Acta. Physiol. Scand. (S) 232:1-55.

Darby CE, De Korte RA, Binnie CD and Wilkins AJ (1980) The self-induction of epileptic seizures by eye closure. Epilepsia, 40:103-105.

Darmani NA, Martin BR, Pandey U and Glennon RA (1990) Do functional relationships exist between 5-HT 1A and 5-HT 2 receptors? Pharmacol. Biochem. Behav. 36:901-906.

De La Pena P, Delgado LM, Del Camino and Barros F (1992) Cloning and expression of the thyrotropin-releasing hormone receptor from GH3 rat anterior pituitary cells. Biochem. J. 284:891-899.

De la Tourette G (1885) Etude sur une affection nerveuse caracterisee par de l'incoordination matrice accompagnee de echolalie et de copralalie. Arch. Neurol. 9:19-42, 158-200.

Derkach V, Surprenant AM and North RA (1989) 5-HT3 receptors are membrane ion channels. Nature (Lond.) 339:706-709.

De Vivo M and Maayani S (1985) Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists. Eur. J. Pharmacol. 119:231-234.

De Vivo M and Mayyani S (1986) Characterisation of 5-hydroxytryptamine1A receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea-pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther. 238:248-252.

Devor EJ (1984) Brief communication-complex seggregation analysis of Gilles de la Tourette syndrome: further evidence for a major locus mode of transmission. Am. J. Hum. Gen. 36:704-709.

Diagnostic and Statistical Manual of Mental Disorders (Third Edition- revised) (1987) Pub. The Am. Psychiatric Assoc., Washington, DC, 79-80.

Diamond BI, Reyes MG and Borison R (1982) A new animal model for Tourette syndrome. In Gilles de la Tourette Syndrome, Advances in Neurology, vol. 35, (Eds. A. J. Friedhoff & T. N. Chase) Raven Press: New York.

Dourish CT (1992) 5-HT receptor subtypes and feeding behaviour. In Serotonin, CNS Receptors and Brain Function, (Ed. P. B. Bradley, S. L. Handley, S. J. Cooper, B. J. Key, N. M. Barnes and J. H. Coote), Adv. Biosci. 85:pp179-202.

Dumuis A, Sebben M and Bockaert J (1988) A non-classical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. Mol. Pharmacol., 34: 880-887.

Dumuis A, Sebben M and Brockaert J (1989a) BRL 24924: a potent agonist at a non classical 5-HT receptor positively coupled with adenylate cyclase in colliculi neurons. Eur. J. Pharmacol. 162:381-384.

Dumuis A, Sebben M and Bockaert J (1989b) The gastrointestinal prokinetic benzamine derivatives are agonists at the non-classical 5-HT receptor (5-HT4) positively coupled to adenylate cyclase in neurons. N. S. Arch. Pharmacol. 340:403-410.

Dursun SM (1992) An investigation into the pharmacology of tics and tic-like movements. Ph.D. Thesis the University of Aston in Birmingham, England.

Dursun SM, Burke JG and Reveley MA (1995) Buspirone treatment of Tourette's syndrome. Lancet 345:1366.

Dursun SM, Farrar G, Handley SL, Rickards H, Betts T and Corbett JA (1994) Elevated plasma kynurenine in Tourette Syndrome. Molec. Chem. Neuropath. 21:55-59.

Dursun SM and Handley SL (1991) Tic-like behaviours induced by TRH-amide: differential effects of ritanserin and haloperidol. Br. J. Pharmacol. 102:229P.

Dursun SM and Handley SL (1992) Comparison of ritanserin, haloperidol,pimozide and clonidine effects on spontaneous and DOI head-shake. Br. J. Pharmacol. 105S:225P.

Dursun SM and Handley SL (1993) The effects of alpha2-adrenoceptor antagonists on the inhibition of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes by 5-HT1A receptor agonists in the mouse. Br. J. Pharmacol. 109:1046-1052.

Dursun SM and Handley SL (1994) Complex of rapid movements and vocalization induced by RX336-M in mice: possible relevance to Tourette's syndrome. J. Psychopharmacol. 8:27-31.

Duxon MS, Reavley AC, Flanigan TP, Baxter G, Blackburn TP and Fone KCF (1995) Expression of the 5-HT28 receptor protein in the rat brain. Br. J. Pharmacol. 115(S.):105P.

Eilam D, Talangbayan H, Canaran G and Szechtman H (1992) Dopaminergic control of locomotion, mouthing, snout contact, and grooming: opposing roles of D1 and D2 receptors. Psychopharm. 106:447-454.

Eisenberg L, Ascher E and Kanner L (1959) A clinical study of Gilles de la Tourette's disease (maladie des tics) in children. Am. J. Psych. 115:715-723.

Eison AS and Wright RN (1992) 5-HT1A and 5-HT2 receptors mediate discrete behaviors in the mongolian gerbil. Pharmacol. Biochem. Behav. 43:131-137

Elsworth JD, Lawrence MS, Roth RH, Taylor JR, Mailman RB, Nichols DE, Lewis MH and Redmond DE (Jr) (1991) D1 and D2 dopamine receptors independently regulate spontaneous blink rate in the vervet monkey. J. Pharmacol. Exp. Ther. 259:595-600.

Erenburg G, Cruse RP and Rothner AD (1986) Tourette syndrome ; an analysis of 200 pediatric and adolescent cases. Clev. Clin. Quart. 53:127-131.

Erenberg G, Cruse RP and Rothner AD (1987) The natural history of Tourette syndrome: a follow up syndrome. Ann. Neurol. 22:383-385.

Erickson Hm (Jr), Goggin JE and Messiha FS (1977) Comparison of lithium and haloperidol therapy in Gilles de la Tourette syndrome. Adv. Exp. Med. Biol. 90:197-205.

Erlander MG, Lovenberg TW, Baron BM, Delecea L, Danielson PE, Racke M, Slone AL, Siegel BW, Foye PE, Cannon K, Bruns JE and Sutcliffe JG (1993) Two members of a distinct subfamily of 5-hydroxytryptsmine receptors differentially expressed in rat brain. Proc. Natl. Acad. Sci. U. S. A. 90:3452-3456.

Escandon NA, Zimmermann DC and McCall RB (1994) Characterization of the serotonin1A receptor antagonist activity of WAY-100135 and spiperone. J. Pharmacol. Exp. Ther. 268:441-447.

Essman WB and Essman EJ (1982) Comparitive features of an animal model for behavioural and neurochemicalchange. In: G.T.S. (Eds. A. J. Friedhoff and T. N. Chase) pp. 227-282, Raven Press, New York.

Faden AI, Sacksen I and Noble LJ (1988) Structure activity relationships of TRH analogs in experimental spinal injury. Brain Res. 448:287-293.

Faux EJ (1966) Gilles de la Tourette syndrome, social psychiatric management. Arch. Gen. Psych. 14:139-142.

Fischer-Cornelssen KA and Ferner UJ (1976) An example of European multicenter trials: multispectral analysis of clozapine. Psychopharm. Bull. 12:34-39.

Fleming AA and Todd AJ (1994) Thyrptropin-releasing hormone- and GABA-like immunoreactivity coexist in neurons in the dorsal horn of the rat spinal cord. Brain Res. 638:347-351.

Fletcher A, Bill DJ, Bill SJ, Cliffe IA, Dover GM, Forster EA, Haskins JT, Jones D, Mansell HL and Reilly Y (1993b) WAY100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. Eur. J. Pharmacol. 237:283-291.

Fletcher A, Bill DJ, Cliffe IA, Firster EA, Jones D and Reilly Y (1994) A pharmacological profile of WAY-100135, a potent and selective 5-HT1A receptor antagonist. Br. J. Pharmacol. 112S:91P.

Fletcher A, Cliffe IA and Dourish CT (1993a) Silent 5-HT1A receptor antagonists: utility as research tools and therapeutic agents. Trends in Pharamcol. Sci. 14:441-447.

Fliman M, Dickman M and Perl E (1991) Gilles de la Tourette syndrome: onset in old age. J. Am. Ger. Soc. 39:277-279.

Fone KCF, Bennett GW, Bennett GW and Marsden CA (1987) Involvement of catecholamunergic neurones and alpha-adrenoceptors in the forepaw licking behaviour produced by the intrathecal injection of an analogue of thyrotrophin releasing hormone (CG3509). Neuropharmacol. 26:1147-1155.

Fone KCF, Dix P, Tomlinson DR, Bennett GW and Marsden CA (1988) Spinal effects of chronic intrathecal administration of the thyrotrophin-releasing hormone analogue (CG3509) in rats. Brain Res. 455:157-161.

Fone KCF, Johnson JV, Bennett GW and Marsden CA (1989a) Involvement of 5-HT2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analog (CG3509) to rats. Br. J. Pharmacol. 96:599-608.

Fone KCF, Johnson JV, Marsden CA and Bennett GW (1989b) Comparative behavioural and biochemical effects of repeated intrathecal administration of thyrotropin-releaseing hormone (TRH) or two analogues of TRH in adult rats. Neuropharmacol. 28:867-875.

Fone KCF and Sharma A (1993) Effect of chronic intracerebroventricular 5-HT1C receptor antiserum infusion on the 5-HT behavioural syndrome and 5-HT turnover. Br. J. Pharmacol. 108S:97P.

Foquet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, Stuhmer W and Lubbert H (1992) Cloning and functional characterisation of the rat stomach fundus serotonin receptor. EMBO J. 11:3481-3487.

Forbes IT, Kennett GA, Garde A, Ham P, Hayward CJ, Martin RT, Thompson M, Wood MD, Baxter GS, Glen A, Murphy OE, Stewart BA and Blackburn TP (1993) N-(1-methyl-5-indolyl)-N-(3-pyridyl)urea hydrochloride: the first selective 5-HT1C receptor atagonist. J. Med. Chem. 36:1104-1107.

Fornal CA, Litto WJ, Metzler CW, Marrosu F, Tada K and Jacobs BL (1994a) Single-unit responses of serontonergic dorsal raphe neurons to 5-HT1A agonist and antagonist drug administration in behaving cats. J. Pharmacol. Exp. Ther. 270:1345-1358.

Fornal CA, Marrosu F, Metzler CW, Tada K and Jacobs BL (1994b) Effects of the putative 5-hydroxytryptamine1A antagonists BMY 7378, NAN 190 and (-)-propranolol on serotonergic dorsal raphe unit activity in behaving cats. J. Pharmacol. Exp. Ther. 270:1359-1366.

Fornal CA, Metzler CW, Gallegos RA, Veasey SC, McCreary AC and Jacobs BL (1994) WAY-100635, a potent and selective 5-HT1A receptor antagonist, increases serotonergic neuronal activity and blocks the action of 8-OH DPAT in behaving cats. Soc. Neurosci. Abs. 20:634.1.

Fornal CA, Metzler CW, Veasey SC, McCreary AC, Dourish CT and Jacobs BL (1994c) Single-unit recordings from freely-moving animals provide evidence that WAY-100635, but not (S)-WAY-100135, blocks the action of endogenous serotonin at the 5-HT autoreceptor. Br. J. Pharmacol. 112:92P.

Forno LS (1981) Pathology of Parkinson's disease. In Movement disorders (Eds. C. D. Marsden and S. Fahn) Butterworth Scientific, London. 25-40.

Fox PT, Fox JM, Raichle ME and Burde RM (1985) The role of cerebral cortex in the generation of voluntary saccades: a positron emmission tomographic study. J. Neurophysiol. 54:2.

Fozard JR, Mobarok Ali ATM and Newgrosh G (1979) Blockade of serotonin receptors on autonomic neurones by (-)-cocaine and some related compounds. Eur. J. Pharmacol. 59:195-210.

Freed WJ, Karson CN, Kleiman JE and Wyatt RJ (1981) Increased spontaneous eye blinks in cerebellctomized rats. Biol. Psych. 16:789-792.

Friedhoff AJ (1982) Receptor maturation in pathogenesis and treatment of Tourette syndrome. In Gilles de la Tourette Syndrome, Advances in Neurology, vol. 35, (Eds. A. J. Friedhoff & T. N. Chase), Raven Press: New York.

Friedman and Dallob A (1979) Enhanced serotonin receptor activity after chronic treatment with imipramine and amitryptyline.Cmmun. Psychopharmacol. 3:89.

Fuller RW and Perry KW (1989) Effects of buspirone and its metabolite, 1-(2-pyrimidinyl)piperazine, on brain monoamines and their metabolites in rats. J. Pharmacol. Exp. Ther. 248:50-56.

Funatsu K, Teshima S and Inanaga K (1985) Variuos types of thyrotropin-releasing hormone receptors in discrete brain regions and pituitary of the rat. J. Neurochem. 45:390-397.

Gaddum JH and Picarelli ZP (1957) Two kinds of tryptamine receptor. Br. J. Pharmacol. 12:323-328.

Gale JD, Grossman CJ, Whitehead JWF, Oxford AW, Bunce KT and Humphrey PPA (1994)GR113808: a novel, selective antagonist with high affinity at the 5-HT4 receptor. Br. J. Pharmacol. 111:332-338.

Garattini S and Samanin R (1976) Anorectic drugs and neurotransmitters. In food intake and appetite. (Ed. T. Silverstone), Berlin.

Garcia-Rill E and Skinner RD (1990) Modulation of rhythmic functions by the brainstem. In Neurobiology of human locomotion (Ed. M. Shimamura) J. Wiley & Sons, New York.

Gartside SE, Umbers V, Hajos M and Sharp T (1995a) Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. Br. J. Pharmacol. 115:1064-1070.

Gartside SE, Umbers V and Sharp T (1995b) Evidence for 5-HT-enhancing effects of an MAOI and 5-HT1A antagonist in combination. J. Psychopharmacol. S9:3,93

Gelders Y, Bussche GV, Reyntjens A and Janssen PAJ (1986) Serotonin S2 receptor blockers in the treatment of chronic schizophrenic. Clin. Psychopharm. 9:325-327.

George MS, Trimble MR and Robertson MM (1993) Fluvoxamine and sulpiride in comorbid obsessive-compulsive disorder and Gilles de la Tourette syndrome. Hum. Psychopharmacol. 8:327-334.

Gershengorn MC (1986) Mechanism of thyrotropin releasing hormone stimulation of pituitary hormone secretion. Ann. Rev. Physiol. 48:515-26.

Gibbons RD and Davis JM (1986) Consistent evidence for a biological subtype of depression characterised by low CSF 5-HIAA monoamine levels. Acta Psych. Scand. 74:8-12.

Gilbert RFT, Emson PC, Hunt SP, Bennett GW, Marsden CA, Sandberg BEB, Steinbusch HWM and Verhofstad AAJ (1982) The effects of monoamine neurtoxins on peptides in the rat spinal cord. Neurosci. 7:69-87.

Giovannini MG, Casamenti F, Nistri A, Paoli F and Pepeu G (1991) Effect of thyrotropin releasing hormone (TRH) on acetylcholine release from different brain areas investigated by microdialysis. Br. J. Pharmacol. 102:363-368.

Glaze DG, Jankovic J and Frost JD (1982) Sleep in Gilles de la Tourette syndrome: disorder of arousal. Neurol. 32:586-592.

Glennon R (1987) Central serotonin receptors as targets for drug research. J. Med. Chem. 30:1-12.

Glennon RA and Lucki I (1989) Behavioural models of serotonin receptor activation. In The Serotonin Receptors (Ed. E. Saunders-Bush), pp. 253-293, Humana, Clifton, New Jersey.

Glennon RA, McKenney JD, Lyon RA and Titeler M (1986) 5-HT1 and 5-HT2 binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane analogues. J. Med. Chem. 29: 194-199

Gobert A, Lejeune F, Rivet J-M, Audinot V, Newman-Tancredi A and Millan MJ (1995) Modulation of the activity of central serotoninergic neurons by novel serotonin1A receptor agonists and antagonists: a comparison to adrenergic and dopaminergic neurons in rats. J. Pharmacol. Exp. Ther. 273:1032-1046.

Goetz CG, Tanner CM, Wilson RS, Carroll VS, Como PG and Shannon KM (1987) Clonidine and Gilles de la Tourette's syndrome: Double blind study using objective rating methods. Ann. Neurol. 21:307-310.

Golden GS (1978) Tics and Tourette's : a continuum of symptoms. Ann. Neurol. 4:145-148.

Golden GS (1987) Tic disorders in childhood. Ped. Rev. 8:229-234.

Goldstein JA (1984) Nifedipine treatment of Tourette's syndrome. J. Clin. Psychiatr. 45:360.

Gonce M and Barbeau A (1977) Seven cases of Gilles de la Tourette's syndrome partial relief with clonazepam: a pilot study. Can. J. Neuro. Sci. 4:279-283.

Goodman WK, Price LH and Rasmussen SA (1989) Efficacy of fluvoxamine in obsessive compulsive disorders. Arch. Gen Psych. 146:36-44.

Goodwin GM, De Souza RJ and Green AR (1986) The effects of 5-HtT1 receptor ligand ipsapirone (TVXQ 7821) on 5-HT synthesis and behavioural effects of 5-HT agonists in mice and rats. Psychopharmacology (Berlin), 89:382-387.

Goodwin GM and Green AR (1985) A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT 1 and 5-HT 2 receptors. Br. J. Phamacol. 84:743-753.

Gozlan H, El Mestikawy S, Pichat L, Glowinski J and Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: 3H-PAT. Nature 305:140-142.

Green AR, De Souza RJ and Cross AJ (1990) The effects of Ca++ antagonists on central 5-hydroxytrypamine biochemistry and function in rats and mice. Br. J. Pharmac. 99:41-46.

Green AR and Grahame-Smith DG (1976) Effects of drugs on the processes regulating the functional activity of brain 5-HT. Nature 260:487-491.

Green AR and Heal DJ (1978) Release of dopamine in the nucleus accumbens of rats by thyrotrophin releasing hormone. Brit. J. Pharmacol. 63: 385P.

Green AR and Heal DJ (1985) The effects of drugs on serotonin-mediated behavioural models. In: Neuropharmacology of serotonin (Ed: Green AR), pp. 326-365. Oxford University Press.

Green AR, O'Shaugnessy K, Hammond M, Schachter M and Grahame-Smith DG (1983) Inhibition of 5-hydroxytryptamine-mediated behaviour by the putative 5-HT2 antagonist pirenperone. Neuropharmacol. 22:573-578.

Greuel JM and Glasser T (1992) The putative 5-HT1A receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphe nuceus in vitro. Eur. J. Pharmacol. 211:211-219.

Grossman CJ, Kilpatrick GJ and Bunce KT (1993) Development of a radioligand binding assay for 5-HT4 receptors in gunea-pig and rat brain. Br. J. Pharmacol. 109:618-624.

Gurling J, Ashworth-Preece MA, Dourish CT and Routledge C (1994a) Effects of acute and chronic treatment with the selective 5-HT1A receptor antagonist WAY-100635 on hippocampal 5-HT release in vivo. Br. J. Pharmacol. 112S:299P.

Gurling J, Ashworth-Preece MA, Hartley JE, Fletcher A, Dourish CT and Routledge C (1993) Effects of the putative 5-HT1A receptor antagonist SDZ 216,525 in two models of somatodendritic 5-HT1A autoreceptor function. Br. J. Pharmacol. 108S:255P.

Gurling J, Shilliam C, Routledge CR and Dourish CT (1994b) Increased 5-HT release induced by a 5-HT1A antagonist is determined by arousal state. J. Psychopharmacol. Abstr. Cambridge meeting of British Association of Psychopharmacology 1994 A13:50.

Haber SN, Kowall NW, Vonsattell JP, Bird ED and Richardson EP (1986) Gilles de la Tourette's syndrome: a postmortem neuropathological and immunohistochemical study. J. Neurol. Sci. 75:225-241.

Hamon M, Gallissot MC, Menard F, Gozlan H, Bourgoin S and Verge D (1989) 5-HT3 receptor binding sites are on capsacin-sensitive fibres in the rat spinal cord. Eur. J. Pharmacol. 164:315-322.

Handley SL (1995) 5-hydroxytryptamine pathways in anxiety and its treatment. Pharmacol. Ther. 66:103-148

Handley SL and Brown J (1982) Effects of 5-hydroxytryptamine- induced head twitch actions on alpha-1 and alpha-2 adrenoceptors. Neuropharmacol. 21:507-510.
Handley SL and Dursun SM (1992) Serotonin and Tourette's syndrome: movements such as head shakes and wet dog shakes may model human tics. Adv. Biosci. 85:235-253.
Handley SL and McBlane JW (1991) 5-HT the disengaging transmitter? J. Psychopharmacol. 5:322-326.
Handley SL and Miskin RC (1977) The interaction ofsome kynurenine pathway metabolites with 5-hydroxytryptophan and 5-hydroxytryptamine. Psychopharmacol. 51:305-309.
Handley SL, Singh A and Singh L (1986) Ritanserin reduces morphine and clonidine withdrawal tics. Br. J. Pharmacol. 89:647P.
Handley SL and Singh L (1986a) Neurotransmitters and shaking behaviour- more than a "gut-bath" for the brain? Trends in Pharmacol. Sci. 7:324-328.
Handley SL and Singh L (1986b) The modulation of head-twitch behaviour by drugs acting on beta-adrenoceptors: evidence for the involvement of both beta 1- and beta 2-adreneceptors. Psychopharmacol. 88:320-324.
Harkness DH and Brownfield MS (1986) A thyrotrophin-releasing hormone-containing system in the rat dorsal horn seperate from serotonin. Brain Res. 384:323-333.
Hartley JE and Fletcher A (1994) The effects of WAY-100135 and 8-hydroxy-2-(di-n-propylamino)tetralin on feeding in the rat. Eur. J. Pharmacol. 252:329-332.
Hartley JE, Forster EA and Fletcher A (1994) The effects of WAY-100635, a potent and selective 5-HT1A receptor antagonist, on feeding and 8-OH-DPAT-induced feeding in the rat. Soc. Neurosci. Abstr. 20:246.12.
Hashimoto T, Kitamur N, Kajimoto Y, Shirai Y, Shirakawa O, Mita T, Nishino N and Tanaka C (1993) Differential changes in serotonin 5-HT1A and 5-HT2 receptor binding in patients with chronic schizophrenia. Psychopharmacol. 112:S35-S39.
Heal DJ, Philpot J, O'Shaghnessy KM and Davis JL (1986) The influence of central noradrenergic function on 5-HT2 mediated head-twitch responses in mice: possible implications for the actions of antidepressant drugs. Psychopharmacol. 89:414-420.
Heal DJ, Sabbagh A, Youdim MBH and Green AR (1981) Behavioural effects of central and peripheral injection of various analogues and metabolites of thyrotropin releasing hormone (TRH). Neuropharmacol. 20:947-957.
Heaton JCP (1992) Modulation of certain 5-HT related behaviours by serotonergic and nor-adrenegic systems> PhD Thesis, Aston University in Birmingham, UK.

Heaton JCP and Handley SL (1989)Comparison of DOI-induced scratching and head twitch behaviours; differential action of 8-OH-DPAT. Br. J. Pharmacol. 98S:687P.

Helms PM and Godwin CD (1985) Abnormalities of blink rate in psychoses: A preliminary report. Biol. Psych. 20:103-106.

Heuring RE and Peroutka SJ (1987) Characterization of a novel 3H-5-hydroxytryptamine binding site subtypes in bovine brain membranes. J. Neurosci. 7:894-903.

Hillver SE, Bjork L, Li YL, Svensson B, Ross S, Anden NE and Hacksell U (1990) (S)-5-Fluoro-8-hydroxy-2-(dipropylamino)tetralin: a putative 5-HT1A-receptor antagonist. J. Med. Chem. 33:1541.

Hjorth S (1992) (-)-Penbutolol as a blocker of central 5-HT1A receptor-mediated responses. Eur. J. Pharmacol. 222:121-127.

Hjorth S (1993) Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. J. Neurochem. 60:776-779.

Hjorth S and Carlsson A (1986) Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptor? 129: 131-138.

Hjorth S and Sharp T (1990) Mixed agonist/antagonist properties of NAN-190 at 5-HT1A receptors: behavioural and in vivo brain microdialysis studies. Life Sci. 46:955-963.

Hjorth S and Sharp T (1993) In vivo microdialysis evidence for central serotonin1A and serotonin1B autoreceptor blocking properties of the beta adrenoceptor antagonist (-)-penbutolol. J. Pharmacol. Exp. Ther. 265:707-712.

Hoyer D (1988a) Molecular pharmacology and biology of 5-HT1C receptors. Trends in Pharmacol. Sci. 9:89-94.

Hoyer D (1988b) Functional correlates of serotonin 5-HT1 recognition sites. J. Rec. Res. 8:59-81.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR and Humphrey PPA (1994) International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46:157-203.

Hoyer D, Pazos A, Probst A and Palacois JM (1986a) Serotonin receptors in the human brain. 1. Characterization and autoradiographic localization of 5-HT1A recognition sites. Apparent absence of 5-HT 1B recognition sites. Brain Res. 376:85-96.

Hoyer D, Pazos A, Probst A and Palacois JM (1986b) Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT1C and 5-HT2 recognition sites. Brain Res. 376:97-107.

Hoyer D and Schoeffter P (1988) 5-HT1D receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in calf substantia nigra. Eur. J. Pharmacol. 147:145-147.

Humphrey PPA, Hartig P and Hoyer D (1993) A proposed new nomenclature for 5-HT receptors. Trends in Pharmacol. Sci. 14:233-236.

Hutson PH, Semark JE and Middlemiss DN (1990) The TRH analogue MK-771, increases acetylcholine release in hippocampus but not striatum of the conscious rat. Neurosci. Lett. 116:149-155.

Incagnoli T and Kane R (1983) Developmental perspectives of the Gilles de la Tourette's syndrome. Perceptual and Motor Skills 57:1271-1281.

Insel TR, Murphy DL, Cohen RM, Alterman I, Kilts C and Linnoila M (1983) Obsessive compulsive disorder: a double blind trial of clomipramine and clorgyline. Arch. Gen. Psych. 40:605-612.

Itard JMG (1825) Memoire sur quelques fonctions involontaires des appareils de la locomotion de la prehension et de la voix. Arch. Gen. Med. 8:385-407.

Jackson HC (1990) Behavioural profile of the TRH analogue RX77368 in developing rats. Peptides 11:897-901.

Jackson HC and Nutt DJ (1992) Are 5-Ht receptors or beta-adrenoceptors involved in idazoxan-induced food and water intake? Neuropharmacol. 31:1081-1087.

Jacobs BL (1976) An animal behaviour model for studying central serotonergic synapses. Life Sci. 19:777-786.

Jacobs BL and Fornal CA (1993) 5-HT and motor control: a hypothesis. Trends in Neuro. Sci. 16: 346-352.

Jacobs BL, Trullson M, Heim J and Steinfeld G (1982) On the role of CNS serotonin in the motor abnormalities of Tourette syndrome: behavioural and single unit studies. In: Gilles de la Tourettes Syndrome (Eds. A. J. Friedhoff and T. N. Chase) pp.69-76, Raven Press. New York.

Jankovic J, Glaze DG and Frost JD (1984) Effects of tetrabenazine on tics and sleep of Gilles de la Tourette syndrome. Neurol. 34:688-692.

Johansson O, Hokfelt T, Jeffcoate SL, White N and Spindel E (1983) Light and electron microscopic immunohistochemical studies on TRH in the central nervous system of the rat. In Thyrotropin-Releasing Hormone, (Ed. E. C. Griffiths and G. W. Bennett), pp19-32, pub. Raven Press, New York.

Johansson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbusch HWM, Verhofstad AAJ, Emson PC and Spindel E (1981) Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neurosci. 6:1857-1881.

Johnson JV, Fone KCF, Havler ME, Tulloch IF, Bennett GW and Marsden CA (1989) A comparison of the motor behaviours produced by the intrathecal administration of thyrotrophin releasing hormone analogues in the conscious rat. Neurosci. 29:463-470.

Jones DNC, Barnes NM, Costall B, Domeney AM, Kilpatrick GJ, Naylor RJ and Tyers MB (1992) The distribution of 5-HT3 recognition sites in the marmoset brain. Eur. J. Pharmacol. 215:63-68.

receptor and the triggering of malignant transformation. Science 244:1057-1062.

Kahn RS, Davidson M, Siever L, Gabriel S, Apter S and Davis KL (1993) Serotonin function and treatment response to clozapine in schizophrenic patients. Am. J. Psych. 150:1337-1342.

Kahn RS and Van Praag HM (1988) A serotonin hypothesis of panic disorder. Hum. Psychopharmacol. 3:285-288.

Kahn RS and Van Praag HM (1990) Panic disorder :a presynaptic serotonin defect? Psch. Res. 31:209-210.

Kahn RS, Van Praag HM, Wetzler S, Asnis GM and Barr G (1988) Serotonin and anxiety revisited. Biol. Psych. 23:189-208.

Kahn RS, Wetzler S, Asnis GM and Van Praag HM (1990) The effects of m-chlorophenylpiperazine in normal subjects: a dose response study. Psychopharmacol. 100:339-344.

Karler R, Calder LD and Turkanis SA (1990) Reverse tolerance to amphetamine evokes reverse tolerance to 5-HTP. Life Sci. 46:1173-1780.

Karson CN (1983) Spontaneous eye blink rates and dopaminergic systems. Brain 106:643-653.

Karson CN (1988) Physiology of normal and abnormal blinking. In Advances in Neurology, Vol 49: Facial dyskinesias (Eds. J. Jankovic and E. Tolosa) , Raven Press, New York.

Karson CN (1989) Blinking. Bull. Soc. Belge Ophtamol. 237:443-457.

Karson CN, Bigelow LB, Kleinman JE, Weinberger DR and Wyatt RJ (1982) Haloperidol-induced changes in blink rates correlate with changes in BPRS-score. Br. J. Psych. 140:503-507.

Karson CN, Fred WJ, Kleinman JE, Bigelow LB and Wyatt RJ (1981) Neuroleptics decrease blinking in schizophrenic subjects. Biol. Psych. 16:679-682.

Karson CN, Kaufmann CA, Shapiro AK, *et al.* (1985) Eye blink rate in Tourette's syndrome. J. Nerv. Mental Disease 173:566-568.

Karson CN, Kleinman JE, Berman KF, Phelps BH, Wise CD, DeLise LE and Jeste DV (1983) An inverse correlation between spontaneous eye blink rate and platelet monoamine oxidase activity. Br. J. Psych. 142:43-46.

Kennett GA (1992) 5-HT1C receptor antagonists have anxiolytic-like actions in the rat social interaction model. Psychopharmacol. 107:379-384.
Kennett GA and Curzon G (1988) Evidence that mCPP may have behavioural effects mediated by central 5-HT1C receptors. Br. J. Pharmacol. 94:137-147.
Kennett GA and Curzon G (1991) Potencies of antagonists indicate that 5-HT1c mediate the 1-3(chlorophenyl) piperazine-induced hypophagia. Br. J. Pharmacol. 103:2016-2020.
Kennett GA, Pittaway K and Blackburn TP (1994b) Evidence that 5-HT2C receptor antagonists are anxiolytic in the rat Geller-Seifter model of anxiety. Psychopharmacol. 114:90-96.
Kennett GA, Whitton P, Shah K and Curzon G (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. Eur. J. Pharmacol. 164:445-454.
Kennett GA, Wood MD, Glen A, Grewal S, Forbes I, Gadre A and Blackburn TP (1994a) *In vivo* properties of SB200646A, a 5-HT2C/2B receptor antagonist. Br. J. Pharmacol. 111:797-802.
Kerwin RW and Pycock CJ (1979) Thyrotrophin releasing hormone stimulates release of [3H]-dopamine from slices of rat nucleus accumbens in vitro. Br. J. Pharmacol. 67:323-325.
Kilpatrick GJ, Jones BJ and Tyers MB (1987) Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. Nature 330:746-748.
Kimura J (1988) Blink reflex in facial dyskinesia. In Advances in neurology, Vol 49, Facial dyskinesias (Eds. J. Jankovic and E. Tolosa)Raven Press, New York.
Kimura J and Lyons LW (1972) Orbicularis oculi reflex on the Wallenberg syndrome: alteration of the late reflex by lesions of the spinal tract and nucleus of the trigminal nerve. J. Neurol. Neurosurg. Psych. 35:228-233.
Kitamura Y, Nagatani T and Watanabe T (1994) Buspirone enhances head twitch behavior in mice. Eur. J. Pharmacol. 253:297-301.
Klawans HL, Goetz CG and Perlik S (1980) Tardive dyskinesia: Review and update. Am. J. Psych. 137:900-911.
Kleinman JE, Karson CN, Weinberger DR, Freed WJ, Berman KF and Wyatt RJ (1984) Eye blinking and cerebral ventricular size in chronic schizophrenic patients. Am. J. Psych. 141:1430-1432.
Klint T, Dahglen IL and Larson K (1992) The selective 5-HT2 receptorantagonist amperozide attenuates 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-induced inhibition of male rat sexual behavioir. Eur. J. Pharmacol. 212:241-246.
Knott PJ and Hutson PH (1982) Stress-induced stereotypy in the rat: neuropharmacological similarities to Tourette syndrome. In: Gilles de la Tourette

Syndrome, Advances in Neurology, vol. 35 (Eds. A. J. Friedhoff & T. N. Chase), Raven Press, New York.

Kosofsky BE and Molliver ME (1987) The serotonergic innervation of cerebral cortex: Different classes of axon terminals arise from dorsal and median raphe nuclei. Synapse 1:153-168.

Kugelberg E (1952) Facial reflex. Brain 75:385-396.

Kurlan R (1989) Tourette's syndrome current concepts. Neurol. 39:1625-1630.

Kurlan R, Behr J, Medved L, Shoulson J, Pauls D and Kidd KK (1987) Severity of Tourette's syndrome in one large kindred. Implications for determination of disease prevelance rate. Arch. Neurol. 44:268-269.

Kursar JD, Nelson DL, Wainscott DB, Cohen ML and Baez M (1992) Molecular cloning, functional expression and pharmacological characterisation of a novel serotonin receptor (5-hydroxytryptamine2F) from rat stomach fundus. Mol. Pharmacol. 42:549-557.

Kwang LL, Smith ER, Davidson JM and Peroutka SJ (1986) Differential interactions of prosexual drugs with 5-hydroxytryptamine 1A and Alpha-2-Adrenergic receptors. Behav. Neurosci. 100:664-668.

Lanfumey L, Haj-Dahmane S and Hamon M (1993) Further assessment of the antagonist properties of the novel and selective 5-HT1A receptor ligands (+)-WAY100135 and SDZ216-525. Eur. J. Pharmacol. 249:25-35.

Lawrence MS and Redmond DE (Jr) (1991) MPTP lesions and dopaminergic drugs alter eye blink rate in African green monkeys> Pharmacol. Biochem. Behav. 38:869-874.

Leckman JF, Riddle MA, Berettin WH, Anderson GM, Hardin M, Chappell P, Bissette G, Nemeroff CB and Goodman WK (1988) Elevated CSF dynorphan A(1-8) in Tourette's syndrome. Life Sci. 43:2015-2023.

Lees AJ, Robertson M, Trimble MR, *et al.* (1984) A clinical study of Gilles de la Tourette syndrome in the U. K. J. Neurosur. Psych. 47:1-8.

Leon CA (1979) Therapeutic effects of clozapine: a four year follow-up of a controlled clinical trial. Acta Psychiatr. Scand. 59:471-480.

Leonard H, Swedo S, Rapoport JL, *et al.* (1988) Treatment of childhood obsessive disorder with clomipramine and desmetylimipramine: a double blind crossover comparison. Psychopharmacol. Bull. 24:93-95.

Leonhardt S, Herrick-Danis K and Teitler M (1989) Detection of a novel serotonin receptor subtype (5-HT1E) in human brain: interaction with a GTP-binding protein. J. Neurochem. 53:465-471.

Levy AD, Li Q, Alvarez Sanz MC, Rittenhouse PA, Brownfield MS and Van de Kar LD (1992) Repeated cocaine modifies the neuroendocrine responses to the 5-HT1C/5-HT2 receptor agonist DOI. Eur. J. Pharmacol. 221:121-127.

Leysen J (1983) Serotonin receptor-binding sites. Is there pharmacological and clinical significance. Med. Biol. 61:139-143.

Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J and Janssen PAJ (1981) Receptor binding profile of R 41 468, a novel antagonist at 5-HT2 receptors. Life Sci. 28:1015-1022.

Leysen JE, Niemegeers CJE, Van Nueten JM and Laduron PM (1982) [3H]Ketanserin (R41 468), a selective 3H-ligand for serotonin2 receptor binding sites. Mol. Pharmacol. 21:301-314.

Lichter D, Majumdar L and Kurlan R (1988) Opiate withdrawal unmasks Tourette's Syndrome. Clin. Neuropharmacol. 11: 559-564.

Lighton C, Marsden CA and Bennett GW (1984) The effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine on thyrotrophin-releasing hormone in regions of the brain and spinal cord of the rat. Neuropharmacol. 23:55-60.

Linthorst ACE, Broekhoven MH, De Jong W, Van Wimersma Greidanus TB and Versteeg DHG. (1992) Effects of SCH23390 and quinpirole on novelty-induced grooming behaviour in spontaneously hypertensive rats and Wistar-Kyoto rats. Euro. J. Pharmacol. 219:23-28.

Linton M and Gallo PS (1975) The Practical Statistician: Simplified Handbook of Statistics. Pub. Brooks/Cole Publishing Co., Montercy, California.

Lovenberg TW, Baron BM, De Lecea L, Miller JD, Prosser RA, Rea MA, Foye PE, Racke M, Slone AL, Siegel BW, Danielson PE, Sutcliffe JG and Erlander MG (1993b) A novel adenylyl cyclase-activating serotonin receptor (5-HT7) implicated in the regulation of mammalian circadian rhythms. Neuron. 11:449-458.

Lovenberg TW, Erlander MG, Baron MG, Racke M, Slone AL, Siegel BW, Craft CM, Burns JE, Danielson PE and Sutcliffe JG (1993a) Molecular cloning and functional expression of 5-HT1E-like rat and human 5-hydroxytrptamine receptor genes. Proc. Natl. Acad. Sci. U. S. A. 90:2184-2188.

Lucki I, Nobler MS and Frazer A (1984) Differential action of serotonin antagonists on two behavioural models of serotonin receptor activation in the rat. J. Pharmacol. Exp. Ther. 228:133-139.

Ludlow CL, Polinsky RJ, Caine ED, *et al.* (1982) Language and speech abnormalities in Tourette syndrome . In: G.T.S. (Eds. A. J. Friedhoff and T. N. Chase),pp.351-361,Raven Press, New York.

Lum JT and Piercey MF (1988) Electrophysiological evidence that spiperone is an antagonist of 5-HT1A receptors in the dorsal raphe nucleus. Eur. J. Pharmacol. 149:9-15.

Mackay AVP Iversen LL, Rossor M, Spokes E, Bird E, Arregui A, Creese I and Snyder SH (1982) Increased brain dopamine and dopamine receptors in schizophrenia. Arch. Gen. Psych. 39:991-997.

Mackert A, Fletchtner K, Woyth C and Frick K (1991) Increased blink rates in schizophrenics: Influences of neuroleptics and psychopathology. Schizophren. Res. 4:41-47.

Malick JB, Dosen E and Barnett A (1977) Quipazine induced head-twitch in mice. Pharmacol. Biochem. Behav. 6:325-329.

Marcinkiewicz M, Verge D, Gozlan H, Pichat L and Hamon M (1984) Autoradiographic evidence for the heterogeneity of 5-HT1 sites in the rat brain. Brain Res. 291:159-163.

Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, Muscatelli G, Mattei MG and Hen R (1993) Mouse 5-hydroxytryptamine5A and 5-hydroxytryptamine5B receptors define a new family of serotonin receptors: cloning, functional expression and chromosomal localisation. Mol. Pharmacol. 43:313-319.

Matthews WD and Smith CD (1980) Pharmacological profile of a model for central serotonin receptor activation. Life Sci. 26:1397-1403.

McAllister G, Charlesworth A, Snodin C, Beer MS, Noble AJ, Middlemiss DN, Inversen LL and Whiting P (1992) Molecular cloning of a serotonin receptor from human brain (5-HT1E): a fifth 5-HT1-like subtype. Proc. Natl. Acad. Sci. USA 89:5517-5521.

McCreary AC and Handley SL (1995) Kynurenine potentiates the DOI head shake in mice. J. Psychopharmacol. 9:69-70.

McKinney WT and Bunney WE (1969) Animal models of depression. Arch. Gen. Psychiat. 21:240-248.

Meibach RC, Maayani S and Green JP (1980) Characterisation and radioautography of [3H]LSD binding by rat brain slices in vitro: the effect of 5-hydroxytryptamine. Eur. J. Pharmacol. 67:371-382.

Meller E, Goldstein M and Bohmaker K (1990) Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: Possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. Mol. Pharmacol. 37:231-237.

Meltzer HY and Lowy MT (1987) The serotonin hypothesis of depression. In Psychopharmacology: The Third Generation of Progress, pp 513-526, (Ed. H. Y. Meltzer), Raven Press, New York.

Mendez M, Moran J, Wilk S, Joseph-Bravo P and Charli J-L (1993) Assessment of the role of TRH in the release of [3H]-dopamine from rat nucleus accumbens-lateral septum slices. Brain Res. Bull. 31:621-625.

Mengod G, Nguyen S, Le H, Waeber C, Lubbert H and Palacios JM (1990a) The distribution and cellular localisation of the serotonin1C receptor mRNA in the rodent brain examined by in situ hybridisation histochemistry. Comparison with receptor binding distribution. Neurosci. 35:577-591.

Mengod G, Pompeiano M, Martinez-Mir MI and Palacios JM (1990b) Localisation of the mRNA for the 5-HT2 receptor by in situ hybridisation histochemistry. Correlation with the distribution of receptor sites. Brai Res. 524:139-143.

Messiha FS (1988)Biochemical pharmacology of Gilles de la Tourette syndrome. Neurosci. Biobehav. Rev. 12:295-305.

Messiha FS, Erickson HM and Goggin JE (1976) Lithium carbonate in Gilles de la Tourette's disease. Res. Commun. Chem. Pathol. Pharmacol. 15:609-612.

Meyerhof W, Obermuller F, Fehr S and Richter D (1993) A novel rat serotonin receptor: primary structure, pharmacology and expression pattern in distinct brain regions. DNA Cell Biol. 12:401-409.

Michelli F, Gatto M, Lukhuniec E, Mangone C, Pardal MF, Pikielny R and Parera IC (1990) Treatment of Tourettes syndrome with calcium antagonists. Clin. Neuropharmacol. 13:77-83.

Millan MJ, Canton H, Gobert A, Lejeune F, Rivet J-M, Bervoets K, Brocco M, Widdowson P, Mennini T, Audinot V, Honore P, Renouard A, Le Marouille-Girardon S, Verriele L, Gressier H and Peglion J-L (1995) Novel benzodioxopiperazines acting as antagonists at postsynaptic 5-HT1A receptors and as agonists at 5-HT1A autoreceptors: a comparative pharmacological characterization with proposed 5-HT1A antagonists. J. Pharmacol. Exp. Ther. 268:337-352.

Millan MJ, Rivet J-M, Canton H, Lejeune F, Bervoets K, Brocco M, Gobert A, Lefebvre De Ladonchamps B, Le Marouille-Girardon S, Verriele L, Laubie M and Lavielle G (1992) S 14671: A naphtylpiperazine 5-hydroxytryptamine1A agonist of exceptional potency and high efficacy possessing antagonist activity at 5-hydroxytryptamine1C/2 receptors. J. Pharmacol. Exp. Ther. 262:451-463.

Millan MJ, Rivet J-M, Canton H, Le Marouille-Girardon Sm and Gobert A (1993) Induction of hypothermia as a model of 5-hydroxytryptsmine1A receptor-mediated activity in the rat: apharmacological characterization of the actions of novel agonists and antagonists. J. Pharmacol. Exp. Ther. 264:1364-1376.

Minitab Reference Manual, Release 8 (PC Version) (1991) Published by Media Cybernetics.

Miyamoto M and Nagawa Y (1977) Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. Eur. J. Pharmacol. 44:143-152.

Miyamoto M, Narumi S, Nagai Y, Shima T and Nagawa Y (1979) Thyrotropin releasing hormone: Hyperactivity and mesolimbic dopamine system in rats. Jpn. J. Pharmacol. 29:335-347.

Molloy AG and Waddington JL (1984) Dopaminergic behaviour stereospecifically promoted by the D1 agonist R-SK & F38393 and selectively blocked by the D1 antagonist SCH23390. Psychopharmacol. 82:409-410.

Molloy AG and Waddington JL (1987a) Assessment of grooming and other behavioural responses to the D1 dopamine receptor agonist SK & F38393 and its R- and S-enantiomers in the intact adult rat. Psychopharamcol. 92:164-168.

Molloy AG and Waddington JL (1987b) Pharmacological characteristion in the rat of grooming and other behavioural responses to the D-1 dopamine receptor agonist R-SK&F 38393. J. Psychopharmacol. 1:177.

Monferini E, Gaetani P, Rodriguez Y, Baena R, Giraldo E, Parenti M, Zocchetti A and Rizzi CA (1993) Pharmacological characterisation of the 5-hydroxytryptamine receptor coupled to adenylyl cyclase stimulation in human brain. Life Sci. 52:61-65.

Monsma FJ (Jr), Shen Y, Ward RP, Hamblin MW and Sibley DR (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol. Pharmacol. 43:320-327.

Moser PC (1991) The effect of putative 5-HT1A receptor antagonists on 8-OH-DPAT-induced hypothermia in rats and mice. Eur. J. Pharmacol. 193:165-172.

Mueller EA, Sunderland T and Murphy DL (1985) Neuroendocrine effects of m-CPP, a serotonin agonist, in humans. J. Clin. Endocrinol. Metab. 61:1179-1184.

Muesser KT, Dysken MW, Sussman S, Lyons JS and Davis JM (1984) Development of tolerance to neuroleptic-induced decreases in blink rate. Biol. Psych. 19:617-621.

Mundey MK, Fletcher A and Marsden CA (1994a) Effect of the putative 5-HT1A antagonists WAY100135 and SDZ 216-525 on 5-HT neuronal firing in the guinea-pig dorsal raphe nucleus. Neuropharmacol. 33:61-66.

Mundey MK, Fletcher A and Marsden CA (1994b) Effect of the putative 5-HT1A antagonist WAY100635 on 5-HT neuronal firing in the guinea-pig dorsal raphe nucleus. Br. J. Pharmacol. 112S:93P.

Mundey MK, Fletcher A, Marsden CA and Fone KCF (1995) Effect of iontophoretic application of the 5-HT1A antagonist WAY100635 on neuronal firing in the guinea-pig dorsal raphe nucleus. Br. J. Pharmacol. 115S:84P.

Murray AM and Waddington JL (1989) The induction of grooming and vacuous chewing by a series of selective D1 dopamine receptor agonists: two directions of D1: D2 interaction. Eur. J. Pharmacol. 160:377-384.

Murray AM and Waddington JL (1990) New putative selective agonists at the D1 dopamine receptor: behavioural and neurochemical comparison of CY208-243 with SK & F101384 and SK & F103243. Pharmacol. Biochem. Behav. 35:105-110.

Nakumura M and Fukushima H (1978) Effects of 5,6-dihydroxytryptamine on the head twitches induced by 5-HTP, 5-HT, mescaline and fludiazepam in mice. J. Pharm. Pharmacol. 30:56-58.

Niemegers CJE, Colpaert FC, Leysen JE, Awouters F and Janssen PAJ (1983) Mescaline induced head twitches in the rat : an in-vivo method to evaluate serotonin S2 antagonists. Drug Develop. Res. 3:123-135.

Nishikawa Y, Ikegami H, Jikihara H, Koike K, Masumoto N, Kasahara K, Tasaka K, Hirota K, Miyake A and Tanizawa O (1993) Effects of thyrotropin-releasing hormone and phorbol ester on dopamine release from dispersed rat tuberoinfundibular dopaminergic neurons. Peptides 14:839-844.

Nomikos GG, Arborelius L and Svensson TH (1992) The novel 5-HT1A receptor antagonist (S)-UH-301 prevents (R)-8-OH-DPAT-induced decrease in interstitial concentrations of serotonin in the rat hippocampus. Eur. J. Pharmacol. 216:373-378.

Ogren SO and Fuxe K (1989) Intraventricular injections of galanin counteract developement of head twitches induced by the 5-HT2 agonist 1-(2,5-dimethoxy-phenly-4-bromo)-2-aminopropane. Acta. Physiol. Scand. 136:297-298.

Ono H, Ono S and Fukuda H (1991) 5-HT receptor-mediated regulation of thyrotropin-releasing hormone release in rat spinal cord. Eur. J. Pharmacol. 203:283-285.

Palacios JM, Waeber C, Bruinvels AT and Hoyer D (1992) Direct visualisation of serotonin1D receptors in human brain using a new iodinated ligand. Mol. Brain Res. 346:175-179.

Paul SM (1990) Introduction: serotonin and its effects on human behaviour. J. Clin. Psych. 51:4(S.).

Pauls DL and Leckman JF (1986) The inheritance of Gilles de la Tourette syndrome and associated behaviours. New Eng. J. Med. 315:993-997.

Pauls DL, Pakstis AJ, Kurlan R, Kidd KK, Leckman JF, Cohen DJ, Kidd JR, Como P and Sparkes R (1990) Segregation and linkage analysis of Tourette's syndrome and related disorders. J. Am. Child Adolesc. Psych. 43:1180-1182.

Pazos A, Hoyer D and Palacios JM (1984) The binding of serotonergic ligands to the porcine choroid plexus: characterisation of a new type of serotonin recognition site. Eur. J. Pharmacol. 106:539-546.

Pazos A and Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res. 346:205-230.

Pazos A, Probst A and Palacios JM (1987a) Serotonin receptors in the human brain. III. Autoradiographic mapping of serotonin-1 receptors. Neurosci. 21:97-122.

Pazos A, Probst A and Palacios JM (1987b) Serotonin receptors in the human brain. IV. Autoradiograpic mapping of serotonin-2 receptors. Neurosci. 21:123-139.

Pedigo NW, Yamamura HI and Nelson DL (1981) Discrimination of multiple [3H]-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem. 36:220-226.

Peroutka SJ, Lebovitz RM and Snyder SH (1981) Distinct central serotonin receptors with different physiological functions. Science 212:827-829.

Peroutka SJ and Snyder SH (1979) Multiple serotonin receptors: Differential binding of [3H]-5-hydroxytryptamine, [3H]-lysergic acid diethylamide and [3H]-spiroperidol. Mol. Pharmacol. 16:687-699.

Perse TL, Greist JH, Jefferson JW, *et al.* (1987) Fluvoxamine treatment of obscessive compulsive disorder. Am. J. Psych. 144:1543-1548.

Phelps ME and Kuhl DE (1981) Metabolic mapping of the brain's response to visual stimulation: studies in humans. Science 211:1445-1448.

Pifl C, Reither H and Hornykiewicz O (1991) Lower efficacy of the dopamine D1 agonist, SKF 38393, to stimulate adenyly cyclase activity in primate than in rodent striatum. Eur. J. Pharmacol. 202:273.

Plassat JL, Amlaiky N and Hen R (1993) Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. Mol. Pharmacol. 44:229-236.

Plassat J, Boschert U, Amlaiky N and Hen R (1992) The mouse 5HT2 receptor reveals a remarkable heterogeneity within the 5HT1D receptor family. EMBO J. 11:4779-4786.

Plotnikoff NP, Bresse GR and Prange AJ (Jr) (1975) Thyrotropin-releasing hormone (TRH): DOPA potentiation and biogenic amine studies. Pharmacol. Biochem. Behav. 3:665.

Plotnikoff NP, Prange AJ (Jr), Breese GR, Anderson MS and Wilson IC (1972) Thyrotropin releasing hormone: enhancement of DOPA activity by a hypothalmic hormone. Science 178:417.

Powers JM (1985) Blepharospasm due to unilateral diencephalon infarction. Neurol. 35:283-284.

Pranzatelli MR (1988) The comparative pharmacology of the behavioural syndromes induced by TRH and by 5-HT in the rat. Gen. Pharmac. 19:205-211.

Pratt GD, Bowery NG, Kilpatrick GJ, Leslie RA, Barnes NM, Naylor RJ, Jones BJ, Nelson DR, Palacios JM, Slater P and Reynolds DJM (1990) Consensus meeting agrees distribution of 5-HT3 receptors in mammalian hindbrain. Trends in Pharmacol. Sci. 11:135-137.

Price RA, Pauls DL and Caine ED (1984). Pedigree and segregation analysis of clinically defined subgroups of Tourette syndrome. Am. J. Hum. Gen. 36:178s.

Prisco S, Cagnotto A, Talone D, De Blasi A, Mennini T and Esposito E (1993) Tertatolol, a new beta-blocker, is a serotonin (5-hydroxytryptamine1A) receptor antagonist in rat brain. J. Pharmacol. Exp. Ther. 265:739-744.

Przegalinski E and Jaworska L (1990) The effect of repeated administration of anti-depressant drugs on the thyrotropin-releasing hormone (TRH) content of rat brain structures. Psychoneuroendoc. 15:147-153.

Przegalinski E, Jaworska L and Golembiowska K (1990) The effect of fenfluramine on the thyrotropin-releasing hormone (TRH) content in therat brain structures and lumbar spinal cord. Neuropep. 15:195-200.

Rapport MM and Green AA (1947) Purification of the substance which is responsible for vasocostricter activty of serum. Fed. Proc. 6:184.

Rastogi RB, Singhal RL and Lapierre YD (1981) Effects of MK-771, a novel TRH analog, on brain dopaminergic and serotonergic systems. Eur. J. Pharmacol. 73:307-312.

Regeur L, Pakkenberg B, Fog R *et al.* (1986) Clinical features and long term treatment with pimozide in 65 patients with Gilles de la Tourette syndrome. J. Neurol., Neurosurg. Psych. 49:791-795.

Renyi L and Jimenez P (1994) After acute and repeated treatment with the 5-HT1A receptor antagonists (-)UH-301 and WAY100135 tolerance develops to a 5-HT1A receptor-mediated functional response. Soc. Neurosci. Abstr. 20:634.3.

Riddle MA, Leckman JF, Hardin MT, Anderson GM and Cohen DJ (1988) Fluoxetine treatment of obsessions and compulsions in patients with Tourette's syndrome. Am. J. Psychiatry 145:1173-1174.

Robertson MM (1989) The Gilles de la Tourette syndrome: the current status. Br. J. Psych. 154:147-169.

Robertson MM and Gourdie A (1990) Familial Tourettes syndrome in a large Britsh pedigree: associated psychopathology, severity and potential for linkage analysis. Brit. J. Psych. 156:515-521.

Robertson MM, Schnieden V and Lees AJ (1990) Management of Gilles de la Tourette syndrome using sulpiride. Clin. Neuropharmacol. 13(3):229-235.

Robertson M, Trimble MR and Lees AJ (1988) The psychopathology of the Gilles de la Tourette syndrome: a Phenomennological analysis. Br. J. Psych. 152:383-390.

Routledge C, Gurling J, Wright IK and Dourish CT (1993) Neurochemical profile of the selective and silent 5-HT1A receptor antagonist WAY100135: an in vivo microdialysis study. Eur. J. Pharmacol. 239:195-202.

Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R and Schwartz JC (1993a) A novel rat serotonin (5-HT6) receptor-molecular cloning,

localisation and stimulation of cAMP accumulation. Biochem. Biophys. Res. Commun. 193:268-276.

Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, Arrang JM and Schwartz JC (1993b) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. Proc. Natl. Acad. Sci. U.S.A. 90:8547-8551.

Saller CF and Salama AI (1986) D-1 and D-2 dopamine receptor blockade: interactive effects in vivo and in vitro. J. Pharmacol Exp. Ther. 236:714-720.

Sandyk R (1989) Alpha melanocyte stimulating hormone (MSH) in Tourette's syndrome. Int. J. Neurosci. 44: 161-164.

Sandyk R and Fisher H (1988) Serotonin in involuntary movement disorders. Int. J. Neurosci. 42:185-205.

Sasek CA, Wessendorf MW and Helke CJ (1990) Evidence for co-existence of thyrotropin-releasing hormone, substance P and serotonin in ventral medullary neurons that project to the intermediolateral cell column in the rat. Neurosci. 35:105-119.

Schelkunov EL (1979) Influence of catecholaminergic drugs on the serotonergic head twitching in mice. Formakol. Toxikol. 5: 470-474, (in Russian).

Schelkunov EL, Kenunen OG, Pushkov VV and Charitonov RA (1986) Heart rate, blood pressure regulation and neurotransmitter balance in Tourette's syndrome. J. Am. Acad. Child Psych. 25:645-652.

Schoeffter P and Hoyer D (1988) Centrally acting hypotensive agents with affinity for 5-HT1A binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. Br. J. Pharmacol. 95:975-985.

Schoeffter P, Fozard JR, Stoll A, Siegl H, Seiler MP and Hoyer D (1993) SDZ 216-525, a selective and potent 5-HT1A receptor antagonist. Eur. J. Pharmacol. 244:251-257.

Schoeffter P, Waeber C, Palacios JM and Hoyer D (1988) The serotonin 5-HT1D receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. N. S. Arch. Pharmacol. 337:602-608.

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S and Millan MJ (1995) (1-(2,5-Dimethoxy-4 Iodophenyl)-2-Aminopropane)-induced head twitches in the rat are mediated by 5-Hydroxytryptamine (5-HT)2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. J. Pharmacol. Exp. Ther. 273:101-112.

Schweitzer JW and Friedhoff AJ (1988) Gilles de la Tourette syndrome. In Receptors and Ligands in Neurological Disorders, (Eds. A. K. Sen & T. Lee), Cambridge University Press, Cambridge.

Segu L, Chauveau J, Boulenguez P, Morel A, Lanoir J and Delaage M (1991) Synthesis and pharmacological study of radioiodinated serotonin derivative specific for 5-HT1B and 5-HT1D binding sites in the central nervous system. CR Acad. Sci. (Paris) 312:655-661.

Shahani BT and Young RR (1972) Human orbicularis oculi reflexes. Neurology (Minneap.) 22:149-154.

Shapiro AK and Shapiro E (1981) The treatment and etiology of tics and Tourette syndrome. Comp. Psych. 22(2):193-205.

Shaprio AK, Shapiro E, Brown RD and Sweet RD (1978) Gilles de la Tourette syndrome. Raven Press, New York.

Shapiro AK, Shaprio E and Sweet (1981) Treatment of tics and Tourette syndrome. In Disorders of Movement, (Ed. A. Barbeau) MTP Press Ltd, Lancaster, England.

Shapiro AK, Shapiro E and Wayne HC (1973) The symptomology and diagnosis of Gilles de la Tourette syndrome. J. Am. Acad. Child Psych. 12:702-723.

Shapiro AK, Shapiro E, Young JG and Feinberg TE (1988) Gilles de la Tourette syndrome, 2nd ed. Raven Press, New York.

Sharp T, Bennett GW and Marsden CA (1982) Thyrotrophin-releasing hormone analogues increase dopamine release from slices of rat brain. J. Neurochem. 39:1763-1766.

Sharp T, Branwell SR, Hjorth S and Grahame-Smith DG (1989) Pharmacological charcterization of 8-OH-DPAT-induced inhibition of rat hippocampal 5-HT release in vivo as measured by microdialysis. Br. J. Pharmacol. 98:989-997.

Sharp T, Backus LI, Hjorth S, Branwell SR and Grahame-Smith DG (1990) Further investigation of the in vivo pharmacological properties of the putative 5-HT1A antagonist, BMY 7378. Eur. J. Pharmacol. 176:331-340.

Sharp T, McQuade R, Fozard JR and Hoyer D (1993) The novel 5-HT1A receptor antagonist, SDZ216-525, decreases 5-HT release in rat hippocampus in vivo. Br. J. Pharmacol. 109:699-702.

Shaywitz BA, Wolf A, Shaywitz SE, Loomis R and Cohen DJ (1982) Animal models of neuropsychiatric disorders and their relevance for Tourette syndrome. In Gilles de la Tourette syndrome, Advances in Neurology, vol. 35, (Eds. A. J. Friedhoff & T. N. Chase), Raven Press, New York.

Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW and Sibley DR (1993) Molecular cloning and expression of 5-hydroxytryptamine7 serotonin receptor subtype. J. Biol. Chem. 268:18200-18204.

Shopsin B, Klein H, Aaronson M and Collora M (1979) Clozapine, chlorpromazine and placebo in newly hospitalised acutely schizophrenic patients. Arch. Gen. Psych. 36:657-664.

Siever LJ, Kahn RS, Lawlor BA, Trestman RL, Lawrence TL and Coccaro EF (1991) Critical issues in defining the role of serotonin in psychiatric disorders. Pharmacol. Rev. 43:509-525.

Silver H, Blacker M, Weller MP and Lerer B (1989) Treatment of chronic schizophrenics with cyproheptadine. Biol. Psych. 25:502-504.

Simasko SM and Hortia A (1985) Treatment of rats with the TRH analog MK771: Down-regulation of TRH receptors and behavioural tolerance. Neuropharmacol. 24:157-165.

Singer HS, Bulter IJ, Tune LE, Seifert WE and Coyle JT (1982) Dopaminergic dysfunction in Tourette syndrome. Ann. Neurol. 12:361-366.

Singer HS, Hahn IH and Moran TH (1990) Abnormalities of dopaminergic markers in postmortem striatal specimens from patients with Tourette syndrome.(abstract) Ann. Neurol. 28:441.

Singer HS, Oshida L and Coyle JT (1984) CSF cholinesterase activity in Gilles de la Tourette's syndrome. Ann. Neurol. 41:756-757.

Singer HS and Walkup JT (1991) Tourette syndrome and other tic disorders. Diagnosis, pathophysiology and treatment. Medicine 70:15-32.

Singer HS, Wong DF, Brown JE, Brandt J, Krafft L, Shaya E, Dannals RF and Wagner HN (Jr) (1992) Positron emission tomography evaluation of dopamine D-2 receptors in adults with Tourette syndrome. In Advances in Neurology, (ED. T. N. Chase, A. J. Friedhoff and D. J. Cohen), pub. Raven Press Ltd., New York.

Skingle M, Cole N, Higgins G and Feniuk W (1991) Inhibition of DOI-induced wet-dog shakes in the guinea-pig by 5-HT2 receptor antagonists. J. Psychopharmacol. 5:196-201.

Smith CG (1969) Gilles de la Tourette syndrome treated with LSD . Irish J. Med. Sci. 2:269-271.

Smith SJM and Lees AJ (1989) Abnormalities of the blink reflex in Gilles de la Tourette syndrome. J. Neurol., Neurosurg. Psych. 52:895-898.

Sprouse JS and Aghajanian GK (1986) (-)-propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT1A selective agonists. Eur. J. Pharmacol. 128:295-298.

Stanley M and Stanley B (1990) Postmortem evidence for serotonin's role in suicide. J. Clin. Psych. 51:4 (S.).

Starr BS and Starr MS (1986a) Grooming in the mouse is stimulated by the dopamine D1 agonist SKF38393 and by low doses of the D1 antagonist SCH23390, but is inhibited by dopamine D2 agonists, D2 antagonists and high doses of SCH23390. Pharmacol. Biochem. Behav. 24:837-839.

Starr BS and Starr MS (1986b) Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Implications for the roles of D1 and D2 recpteors. Neuropharmacol. 25:455-463.

Stern RS, Marks IM and Mawson D (1980) Clomipramine and exposure for compulsive rituals: plasma levels, side effects and outcome. Br. J. Psych. 136 161-166.

Stevens H (1964) The syndrome of Gilles de la Tourette and its treatment: report of a case. Med. Ann. of the District of Columbia, 33:277-279.

Stevens JR (1978) Eye blink and schizophrenia: Psychosis or tardive dyskinesia? Am. J. Psych. 135:223-227.

Straub RE, Frech GC, Joho RH and Gershengorn MC (1990) Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc Natl. Acad. Sci. USA 87:9514-0518.

Swedo SE, Leonard HL and Kiessling LS (1994) Speculations on antineuronal antibody-mediated neuropsychiatric disorders of childhood. Pediatrics 93:323-326.

Sweet RD Bruun RD, Shapiro, *et al.* (1976). The pharmacology of Gilles de la Tourette syndrome (chronic multiple tic). Clin. Neuropharmacol. 1:1397-1408.

Tagrum SD and Marshall LE (1990) Fenfluramine provocation of anxiety in patients with panic disorder. Psych. Res. 28:95-306.

Tamai H, Komaki G, Kubota S, Kobayashi N, Matsubayashi S, Mori K, Nagakawa T, Takayama T, Kimura M and Kumaga LF (1990) The clinical efficacy of a 5-HT 1A agonist, SM-3997 in the treatment of Bulimia. Int. J. Obesity 14:289-292.

Taylor JR, Lawrence MS, Redmond DE (Jr), Elsworth JD, Roth RH, Nichols DE and Mailman RB (1991) Dihydrexidine, a full dopamine D1 agonist, reduces MPTP-induced parkinsonism in monkeys. Eur J. Pharmacol. 199:389-391.

Terzano MG, Parrino L, Manzoni GC and Mancia D (1983) Seizures triggered by blinking when beginning to speak. Arch. Neurol. 21:31-42.

Thoren P, Asberg M, Cronholm B, Jornestedt L and Traskman L (1980) Clomipramine treatment of obsessive compulsive disorder. 1. A controlled clinical trial. Arch. Gen. Psych. 37: 1281-1289.

To ZP, Bonhaus DW, Eglen RM and Jakeman LB (1995) Characterization and distribution of putative 5-ht7 receptors in gunea-pig brain. Br. J. Pharmacol. 115:107-116.

Traskman-Bendx L, Asberg M, Bertilsson L and Thoren P (1984) CSF monoamine metabolites of depressed patients during illness and after recovery. Acta Psych. Scand. 69:333-342.

Tricklebank MD, Forler C and Fozard J (1984) The involvement of subtypes of the 5-HT1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino) tetralin in th rat. Eur. J. Pharmacol. 106:271-282.

Tsou AP. Kosaka A, Bach C, Zuppan P, Yee C, Tom L, Alvarez R, Ramsey S, Bonhaus DW, Stefanich E, Jakeman L, Eglen RM and Chan HW (1994) Cloning and expression of a 5-hydroxytryptamine7 receptor positively coupled to adenylyl cyclase. J. Neurochem. 63:456-464.

Twarog BM and Page IH (1953) Serotonin content of some mammalian tissues and urine and a method for its determination. Am. J. Physiol. 175:157-161.

Van Der Maelen CP and Aghajanian GK (1983) Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellularly and intracellulary in rat brain slices. Brain Res. 289:109.

Van Woert MJ, Rosenbaum D and Enna SJ (1982) Overview of pharmacological approaches to therapy for Tourette syndrome. Adv. Neurol. 35:369-375.

Veber DF, Holly FW, Varga SL, Hirschnamm R, Nutt RF, Lotti VJ and Porter CC (1976) The dissociation of hormonal and CNS effects in analogs of TRH. In Proceedings 14th European Peptide Symposium (Ed. A. Loffet), University of Brussels Press, Belgium.

Vialli M and Erpsamer V (1933) Cellule enterocromaffine e cellule basigranulose acidofile nei vertebrati. 2. Zellforsch. Mikrosk. Anat. 19:743.

Volkmann FC, Riggs LA and Moore RK (1979) A comparison of saccades and blinks in suppression of vision. Invest. Ophthalmol. Vis. Sci. 18 (S.):140.

Waeber C, Dietl MM, Hoyer D, Probst A and Palacois JM (1988) Visualisation of a novel serotonin recognition site (5-HT 1D) in the human brain by autoradiography. Neurosci. Lett. 88:11-16.

Waddington JL, Molloy AG, O'Boyle KM and Mashurano M (1986) Enantiomeric analogues of SCH23390 as new probes for behavioural interactions between D1 and D2 dopaminergic function. Adv. Med. Biol. 204: 125-136.

Waeber C, Sebben M, Grossman C, Javoy-Agid F, Bockaert J and Dumuis A (1993) [3H]-GR113808 labels 5-HT4 receptors in the human and guinea-pig brain. Neuroreport 4:1239-1242.

Walsh TL, Lavenstein B, Licamele WL, Bronheim S and O'Leary J (1986) Calcium antagonist in the treatment of Tourette's disorder. Am. J. Psych. 143:1467-1468.

Watson CD, Hewitt MJ, Fone KCF, Dickinson SL and Bennett GW (1994) Behavioural effects of scopolamine and the TRH analogue RX77368 on radial arm maze performance in the rat. J. Psychopharmacol. 8:88-93.

Wei E (1973) Brain lesions attenuating "wet shake" behavior in morphine-abstinent rats. Life Sci. 12:385-392.

Wei E (1975) Thyrotropin-releasing hormone and shaking behaviour in rat. Nature 253:739-740.

Weiner WJ, Carvey P, Nausieda P, Goetz CG and Klawans HL (1982) Effect of chronic levodopa on haloperidol-induced behavioural supersensitivity in the guinea pig. In Gilles de la Tourette Syndrome (Ed. A. J. Friedhoff and T. N. Chase), pp213-219, Raven Press, New York.

Weiss S, Sebben M, Kemp DE and Bockaert J (1986) Serotonin 5-HT1 receptors mediate inhibition of cyclic AMP production in neurones. Eur. J. Pharmacol. 120:227-230.

Wilkinson LO and Dourish CT (1991) Serotonin and animal behaviour. In Serotonin Receptor Subtypes: Basic and Clinical Aspects, pp 147-210, (Ed. S. J. Peroutka), Wiley-Liss, Inc.

Williams AR and Dourish CT (1992) Effects of the putative 5-HT1A receptor antagonist NAN-190 on free feeding and on feeding induced by the 5-HT1A receptor agonist 8-OH-DPAT in the rat. Eur. J. Pharmacol. 219:105-112.

Winer BJ (1971)Statistical Principles in Experimental Design. Pub. McGraw-Hill Inc.

Wong DF, Pearlson GD, Young CT, Singer HS, Villemagne V, *et al.* (1989) D2 dopamine receptors are elevated in neuropsychiatric disorders other than schizophrenia. J. Cerebral Blood Flow Metabl. 9:(S1) s593.

Xu M, Takeshi K and Nakamura Y (1990) In vivo striatal dopamine release by TRH analog YM-14673 is blocked by M1 muscarinic receptor antagonist. Neurochem Int. 16:281-285.

Yamada K, Demarest KT and Moore KE (1984) Effects of behaviorally active doses of thyrotrpin-releasing hormone and its analog MK-771 on dopaminergic neuronal systems in the brain of the rat. Neuropharmacol. 23:735-739.

Yap CY and Taylor DA (1983) Involvement of 5-HT2 receptors in the wet dog shake behaviour induced by 5- hydroxytryptophan in the rat. Neuropharmacol. 22:801-804.

Yarbrough GG (1978) Studies on the neuropharmacology of thyrotropin releasing hormone (TRH) and a new TRH analog. Eur. J. Pharmacol. 48:19-27.

Yaryura-Tobias JA (1975) Chlorimipramine in Gilles de la Tourette's disease. Am. J. Psych. 132:11.

Yaryura-Tobias JA and Neziroglu FA (1977) Gilles de la Tourette syndrome: a new clinico-therapeutic approach.Prog. Neuro-Psychopharmac. 1:335-338.

Yocca FD, Wright RN, Margraf RR and Eison AS (1990) 8-OH DPAT and buspirone analogs inhibit the ketanserin-sensitive quipazine-induced head shake response in rats. Pharmacol. Biochem. Behav. 35:251-254.

Zausmer DM and Dewey ME (1987) Tics and heredity. Br. J. Psych. 150:628-634.

Zhao D, Yang J, Jones KE, Gerald C, Suzuki Y, Hogan PG, Chin WW and Tashjian AH (1992) Molecular cloning of a complimentary deoxyribonucleic acid encoding the thyrotropin-releasing hormone receptor and regulation of its messenger ribonucleic acid in rat GH cells. Endocrinol. 130:3529-3536.

Zohar J and Insel THR (1987) Obsessive compulsive disorder :psychobiological approaches to diagnosis, treatment and pathophysiology. Biol. Psych. 22:667-687.

Zohar J, Mueller EA, Insel TR, Zohar-Kadouch RC and Murphy DL (1987) Serotonergic responsivity in obsessive-compulsive disorder: comparison of patients and healthy controls. Arch. Gen. Psych. 44:946-951.