MODULATION OF CERTAIN 5-HT-RELATED BEHAVIOURS BY SEROTONERGIC AND
NORADRENERGIC SYSTEMS.

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Summary.

The modulation of 5-hydroxytryptamine (5-HT)-related head-twitch behaviour by antimigraine drugs and migraine triggers was examined in mice. The antimigraine drugs examined produced either inhibition or no effect on 5-HT-related head-twitching. On the basis of these results it is suggested that 5-HT-related head-twitching is unlikely to be useful in the preclinical screening and discovery of systemically-active antimigraine agents. The migraine triggers examined, tyramine and beta-PEA initially produced a repeatable complex time-related effect on 5-HT-related head-twitching, with both inhibition and potentiation of this behaviour being observed, however, when further examination of the effect of the migraine triggers on 5-HT-related head-twitching was attempted some time later the effects seen initially were no longer produced.

The effect of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, ((±)DOI), on on-going behaviour of mice and rats was examined. Shaking behaviour was observed in both species. In mice, excessive scratching behaviour was also present. (±)DOI-induced scratching and shaking behaviour were found to be differentially modulated by noradrenergic and serotonergic agents, however, the fact that both behaviours were blocked by ritanserin (5-HT2/5-HT1c receptor antagonist) and inhibited by FLA-63 (a dopamine-beta-oxidase inhibitor which depletes noradrenaline), suggests the pathways mediating these behaviours must be convergent in some manner, and that both behaviours require intact 5-HT receptors, probably 5-HT2 receptors, for their production. In general, the behavioural profile of (±)DOI was as expected for an agent which exhibits high affinity binding to 5-HT2/5-HT1c receptors. Little sign of the 5-HT1-related '5-HT syndrome' was seen in either mice or rats.

The effect of a variety of noradrenergic agents on head-twitching induced by a variety of shake-inducing agents was examined. A pattern of modulatory effect was seen whereby the modulatory effect of the noradrenergic agents on 5-hydroxytryptophan (5-HTP) (and in some cases, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT)) was found to be the opposite of that observed with quipazine and (±)DOI.

The relationship between these effects, and their implications for understanding the pharmacology of centrally acting drugs is discussed.

Key words : 5-hydroxytryptamine, migraine, head-twitch, scratching, noradrenaline.

ACKNOWLEDGEMENTS

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GENERAL INTRODUCTION.

1. General Introduction.

The work reported in this thesis examined the effect of drugs which modulate 5-HT-mediated behaviour in mice and rats by altering (in the main) 5-HT and noradrenaline (NA) neurotransmission. In an attempt to understand how drugs acting at noradrenergic receptors and serotonergic receptors modulate the various 5-HT-mediated behaviours examined in this thesis, the biochemistry of these neurotransmitters is outlined. In the later stages of this Introduction the pharmacology of 5-HT-mediated shaking behaviour, scratching and grooming behaviour induced by a variety of agents and the "5-HT syndrome" is discussed.

2. The anatomical distribution of 5-HT in the central nervous system.

Significant amounts of 5-HT have been found in the mammalian brain (Twarog and Page 1953), especially in the mesencephalon (midbrain) and diencephalon (hypothalamus and thalamus), the highest concentration being found in the hypothalamus and the caudate nucleus, the lowest in the cerebellum (Amin et al 1954 & Bertler and Rosengren 1959).

The advent of fluorescence histochemistry (Falck et al 1962) enabled mapping of 5-HT containing neurones. New techniques have led to a more detailed visualisation of the fine structure and organisation of 5-HT neurones in the central nervous system (see Steinbusch 1981 & Steinbusch and Nieuwenhuys 1983). The 5-HT neurones are largely confined to the nine defined raphe nuclei lying in or near the midline of the pons and upper brain stem (B1-B9 by the classification of Dahlstrom and Fuxe 1964). There are also some cells in the area postrema, the caudal locus coeruleus and around the interpenduncular nucleus (Dahlstrom and Fuxe 1964 & Azmitia 1978).

The more caudal B1-B3 groups project descending pathways to the medulla and spinal cord. The more rostral B7-B9 groups (raphe dorsalis, raphe medianus and centralis superior) are thought to provide the extensive innervation of the telencephalon and

diencephalon. The B8 group (median raphe) provides a major component of the 5-HT innervation of the limbic system while B7 (dorsal raphe), which contains the greatest number of 5-HT cell bodies, projects to the neostriatum, cerebral and cerebellar cortices and thalamus (Breese 1975).

3. 5-HT receptors.

Gaddum and Picarelli (1957) first reported that the direct action of 5-HT on 5-HT receptors located on smooth muscle cells in the guinea pig ileum could be blocked by dibenzyline (D), whereas the indirect contractile response to 5-HT, mediated via the release of acetylcholine from intramural cholinergic nerves in the myenteric plexus, could be antagonised by morphine (M). This was the first demonstration of multiple 5-HT receptors and led to the adoption of the 5-HT D- and M-receptor classification.

The advent of receptor binding studies also revealed the existence of multiple 5-HT binding sites. Peroutka and Snyder (1979) published data showing that [3H]-5-HT and [3H]-spiperone bind in nanomolar concentration to two different sites in rat brain cortex membranes. They defined the binding site with high affinity for [3H]-5-HT as the 5-HT1 site, and that with high affinity for [3H]-spiperone as the 5-HT2 site. Later, using a variety of antagonist drugs, Peroutka et al (1981) suggested that stimulation of the 5-HT1 site is associated with increased adenylate cyclase activity whereas 5-HT2 sites are involved in mediating central behavioural effects of 5-HT in rats. Recent studies with a large number of 5-HT agonist and antagonist drugs indicate that the affinities of these compounds for the 5-HT2 site in rat cerebral cortex correlate closely with those obtained in functional studies of D-receptor in vascular and intestinal smooth muscle (Maayani et al 1984 & Engel et al 1985). Thus cortical 5-HT2 binding sites are probably identical with the 5-HT D-receptors of Gaddum and Picarelli. No such correlation exists between M-receptors and 5-HT1 binding sites and this led to the suggestion that M-receptors are pharmacologically distinct from either 5-HT1 binding

sites or 5-HT2 binding sites/D-receptors. Accordingly, M-receptors were designated 5-HT3 receptors in the now generally accepted classification of Bradley et al (1986).

Evidence suggests that both 5-HT1 (Pedigo et al 1981 & Monroe and Smith 1983) and 5-HT2 (Kendall and Nahorski 1983 & Pierce and Peroutka 1989a) binding sites are heterogeneous.

Autoradiographic techniques revealed three sub-populations of 5-HT1 sites characterised on their anatomical localisation and drug sensitivity. These three sites have been referred to as 5-HT1a, 5-HT1b and 5-HT1c (Cortes et al 1984). 5-HT1a sites were concentrated in the hippocampus, cerebral cortex and dorsal raphe. These sites were labelled with nanomolar concentrations of [3H]-5-HT, of [3H]-LSD and of [3H]-8-OH-DPAT (8-hydroxy-2-[di-n-propylamino]tetralin)) (Hjorth et al 1982). The 5-HT1b sites were labelled by [3H]-5-HT and highly concentrated in the globus pallidus, subiculum and substantia nigra and were sensitive to nanomolar quantities of RU 24969 (5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl] 1H-indole) Euvrard and Boissier 1980) and some beta-adrenergic blockers (Middlemiss et al 1977). The 5-HT1c sites were extremely enriched in the choroid plexus of the brain ventricles and labelled with nanomolar affinities by [3H]-mesulergine, [3H]-5-HT and [3H]-LSD. However, RU 24969 and 8-OH-DPAT, the beta-adrenergic blockers and 5-HT2 receptor blockers such as ketanserin and pirenpirone had very low affinities at these sites (Cortes et al 1984 & Pazos et al 1985b). Interestingly, the pharmacological profile of the 5-HT1c site more closely resembles that of the 5-HT2 site. For instance, it has a nanomolar affinity for many putative 5-HT antagonists. In general, the 5-HT2 site has micromolar affinity for 5-HT agonists and nanomolar affinity for 5-HT antagonists, whereas the 5-HT1 sites have nanomolar affinity for 5-HT agonists and little or no affinity for 5-HT antagonists. Also, the 5-HT1c site can be labelled with many of the same radioligands that are used to label the 5-HT2 site (Hoyer 1988b).

The present situation with regard to the radioligands used to study the subtypes of 5-HT1 receptor is that [3H]-8-OH-DPAT is the agent of choice for 5-HT1a receptor; for 5-HT1b, [125I]-iodocyanopindolol is used; and for 5-HT1c, [3H]-mesulergine, [125I]-LSD and 1-methyl [125I]-LSD (Gozlan et al 1983; Hoyer et al 1985a&b; Pazos et al 1985a; Yagaloff and Hartig 1985; Hoyer et al 1986 & Yagaloff and Hartig 1986).

Heuring and Peroutka (1987) have recently reported that another site (5-HT1d) present in the bovine brain was labelled by [3H]-5-HT. The 5-HT1d site displayed an affinity profile which was different from that of 5-HT1a, 5-HT1b or 5-HT1c sites. No selective ligands are at present available for the 5-HT1d site. The 5-HT1b receptor exists only in the rat and the mouse (Hoyer and Middlemiss 1989), whereas the 5-HT1d receptor appears to be the analog of the 5-HT1b receptor in all other species including humans (Peroutka 1990).

The existence of subtypes of the 5-HT2 receptor is controversial. Many putative 5-HT2 agonists display shallow competition curves for 5-HT2 sites labelled by [3H]-ketanserin in rat cortex (Titeler et al 1985; Lyon et al 1987 & Titeler et al 1987). Titeler and colleagues suggested that the rat cortex contains both "high affinity" and "low affinity" states of the 5-HT2 receptor. Moreover, these authors have suggested that agonist radioligands such as [3H]-4-bromo-2,5-dimethoxyamphetamine ([3H]-DOB) selectively label a high affinity state of the 5-HT2 receptor, whereas antagonist radioligands such as [3H]-ketanserin label both high and low affinity states of this receptor. Pierce and Peroutka (1989a) proposed an alternative explanation of these data based on an analysis of R(-)[77BR]-DOB binding in rat cortex i.e. that putative 5-HT2 agonist ligands label a distinct 5-HT2 receptor subtype (as opposed to "state" of the 5-HT2 receptor (Wang et al 1988 & Peroutka et al 1988)). Pierce and Peroutka (1989a) examined drug interactions with 5-HT2 binding sites labelled by [3H]-ketanserin in rat, human and bovine cortex in an attempt to identify differences in the presence of putative 5-HT2 binding site subtypes. They found that [3H]-ketanserin appeared to label an

homogeneous population of 5-HT2 binding sites in bovine cortical membranes, as opposed to an heterogeneous population of 5-HT2 sites in rat cortex. Analysis of the data suggested that the "high affinity" component of [3H]-ketanserin binding, which can be identified and analysed in rat and human brain, does not exist in bovine cortical membranes. This conclusion being in agreement with the finding that specific binding cannot be detected in bovine cortex using (±)[3H]-DOB, a radioligand that can be used to label the high affinity component in rat brain (Titeler et al 1985; Peroutka et al 1988 & Wang et al 1988). They suggest that (±) [3H]-DOB may label not a high-affinity state of the 5-HT2 binding site but rather a distinct sub-population of 5-HT2 binding site. They suggest that states of a receptor by definition should be interconverting confirmations of a single molecular structure. The ability of a molecule to change conformations should not be dependent on the species analysed under the same experimental conditions. They propose that sites labelled by agonist radioligands such as (±)[3H]-DOB should be tentatively designated 5-HT2a site (this site has also been referred to as the "DOB binding site" - Pierce and Peroutka 1989b). These can be identified in rat and human brain (Titeler et al 1985; Peroutka et al 1988 & Wang et al 1988), but not in bovine cortical membranes, whereas, [3H]-ketanserin appears to label not only the 5-HT2a site but also a second sub-population which are tentatively designated 5-HT2b site. These sites can be identified in all three species. While most of the work towards elucidating the physiology and clinical significance of the 5-HT2 receptor has been done under conditions that do not distinguish between the 2a and 2b sites, it appears that the 5-HT2b site corresponds to the 'classic' 5-HT2 receptor as their affinities for a variety of ligands are similar (McKenna and Peroutka 1989).

It remains to be seen which of these two views with regard to the 5-HT2 receptor subtypes is proved correct.

Looking at the above data in terms of its implications for the neurochemical analysis of 5-HT in the CNS, some studies have

demonstrated heterogeneous patterns in 5-HT2 receptor-mediated
responses e. g. Conn and Sanders-Bush (1985) reported differences in
the ability of 5-HT to stimulate phosphoinositide turnover in the
cortical as opposed to sub-cortical regions in the rat brain. If 5-HT2
receptor subtypes do exist then conceivably these differences could be
explained on the basis of an heterogeneous 5-HT2 receptor population.

As mentioned above 5-HT3 receptors were initially characterised in the
periphery where they mediate the excitatory effects of 5-HT
(Richardson and Engel 1986), 5-HT3 receptors have also recently been
labelled in the central nervous system (Kilpatrick et al 1987;
Peroutka and Hamik 1988 & Watling et al 1988) using a variety of
radioligands including [3H]-GR 65630, [3H]-ICS 205-930, [3H]-
quipazine, [3H]-zacopride, [3H]-BRL 43694 and [3H]-QICS 205-930. 5-HT,
2-methyl 5-HT and phenylbiguanide, which are 5-HT3 agonists, have
moderate affinity (Ki value approximately 150nM) for this site whereas
granisetron (formerly called BRL 43694), zacopride and ICS 205-930
display sub-nanomolar affinity (Ki values = 0. 1 - 0. 8nM) for this
site. 8-OH-DPAT, mesulergine, and ketanserin have low affinity for 5-
HT3 binding sites in rat cortical membranes (see Schmidt and Peroutka
1989). The highest density of 5-HT3 binding sites in the rat brain is
found in cortical areas (particularly the entorhinal cortex) and in
the area postrema (see Schmidt and Peroutka 1989). In the CNS,
activation of the 5-HT3 receptor appears to cause a release of
dopamine from rat striatal sites (Blandina et al 1988) and an
inhibition of acetylcholine release from rat cortical slices (Barnes
et al 1989). These results are consistent with the observation that
ondansetron (formerly GR 38032F), a 5-HT3 antagonist, inhibits
dopamine-mediated hyperactivity in rats (Costall et al 1987).

Significant differences in the potency of selective 5-HT3 antagonists
in various physiological systems suggest that heterogeneity may exist
within the 5-HT3 class of receptors. At least three distinct subtypes
of 5-HT3 receptors have been hypothesised (see Richardson and Engel
1986).

4. Cellular localisation of 5-HT1 and 5-HT2 receptors in the CNS.

Lesion studies have provided information regarding the cellular
localisation of 5-HT receptors in the rat brain. The data published on
5-HT1 receptors have depended on the [3H]-ligand used (Gozlan et al
1983). Following the destruction of noradrenaline and dopamine
neurones by local injections of 6-hydroxydopamine (6-OHDA), the [3H]-
5-HT binding in forebrain was unchanged. This suggests that these
sites do not occur on presynaptic catecholamine neurones or do so in
very small amounts which are undetectable by means of binding studies.
The destruction of cell bodies in the striatum with local injection of
kainic acid resulted in a reduction of [3H]-5-HT binding, but did not
affect the striatal [3H]-8-OH-DPAT binding. However, [3H]-8-OH-DPAT
binding was reduced in the hippocampus following local application of
the kainic acid. On destruction of 5-HT neurones following 5,6- or
5,7-dihydroxytryptamine (DHT), neither the forebrain [3H]-5-HT binding
nor the hippocampal [3H]-8-OH-DPAT binding was altered, but [3H]-8-OH-
DPAT binding in the striatum was decreased. This lesion data indicates
that some of the 5-HT1 receptors are located postsynaptically (those
affected by kainic acid lesions), whereas the striatal [3H]-8-OH-DPAT
sites were localised on presynaptic 5-HT neurones. Blackburn et al
(1984) reported an increase of [3H]-5-HT binding in the substantia
nigra following 5,7-DHT lesions of the dorsal raphe nucleus,
suggesting a postsynaptic location of 5-HT1 sites in this region of
the brain.

Following lesions of catecholamines and 5-HT neurones, the 5-HT2
receptors, as labelled by [3H]-ketanserin in frontal cortex and
striatum, were unaffected (Leysen et al 1983). However, local
injections of high doses of kainic acid into the frontal cortex caused
a reduction of 5-HT2 receptors; this reduction was accompanied by a
decrease in glutamic acid decarboxylase activity, suggesting the
prescence of 5-HT2 receptors on the GABA neurones in frontal cortex
(Leysen et al 1983). Interestingly, electrophysiological data
indicates the 5-HT2 receptors are not located on dorsal raphe nucleus
(Lakosi and Aghajanian 1985).

Fischette et al (1987) used autoradiography to examine the effects of 5,7-DHT on 5-HT1 and 5-HT2 receptors in the rat CNS. They found 5,7-DHT treatment resulted in a decrease in 5-HT1 binding in the dentate gyrus and CA3c/4 of the anterior hippocampus and in the dorsal raphe nucleus, whereas no changes were observed in the posterior hippocampus nor in many other brain structures. 5-HT2 receptors exhibited no change in any brain area in response to 5,7-DHT treatment despite over 90% 5-HT depletion in most of the forebrain nuclei examined. These results, they suggest, indicate that at least some of the 5-HT1 sites labelled by [3H]-5-HT in the hippocampus and dorsal raphe nucleus are presynaptic, whereas 5-HT2 receptors labelled with [3H]-ketanserin, are probably postsynaptic.

With respect to subtypes of the 5-HT1 receptor, several studies have established that the terminal 5-HT autoreceptors of the rat cortex are of the 5-HT1b subtype (e.g. Middlemiss 1984a&b; Middlemiss 1985a&b; Engel et al 1986). A significant correlation between the potencies of drugs (or their affinities) at rat autoreceptors (measured as inhibition of electrically evoked [3H]-5-HT (5-HT agonists), or antagonism of the effect of unlabelled 5-HT on evoked [3H]-5-HT overflow (5-HT antagonists)) and their affinities at 5-HT1a and 5-HT1b sites was found by Engel et al (1986). However, they suggested that the best correlations were obtained with the 5-HT1b binding site. Recent research has suggested that the 5-HT autoreceptors from species other than rat, e.g. rabbit, guinea pig, pig and humans, are not of the 5-HT1b subtype, but the 5-HT1d subtype (see Hoyer and Middlemiss 1989).

Electrophysiological studies have suggested that 5-HT1a receptor sites are located on presynaptic (dorsal raphe nuclei) as well as postsynaptic (hippocampal formation) elements (Traber and Glaser 1987) and that the 5-HT autoreceptor located on serotonergic cell bodies in the dorsal raphe nucleus may be of the 5-HT1a subtype (Sprouse and Aghajanian 1985).

5. Distribution of 5-HT1 and 5-HT2 receptors in the CNS.

Autoradiographic studies have revealed a regional distribution of 5-HT1 subtype binding sites (Marcinkiewicz et al 1984 & Pazos and Palacios 1985). In general, 5-HT1a sites are concentrated in the hippocampus and dorsal raphe nucleus, 5-HT1b sites in extrapyramidal structures and 5-HT1c sites almost exclusively in the choroid plexus. The 5-HT2 sites show a similar distribution in brain of various mammalian species including man (Leysen et al 1983 & Schotte et al 1983). 5-HT2 receptors have been found in the highest density in the frontal parts of the cortex with a gradual decrease towards the occipital parts. In subcortical areas, the nucleus accumbens, tuberculum olfactorium and striatum contain about half the density of the prefrontal cortex and less than 10% is found in other brain areas and the spinal cord (see Glennon 1987).

Fischette et al (1987) examined the distribution of 5-HT1 (5-HT1 binding sites were identified using [3H]-5-HT, they did not use selective ligands to examine the 5-HT1 receptor subtypes) and 5-HT2 receptors (5-HT2 binding sites were identified using [3H]-ketanserin) throughout the rat CNS. They observed that both receptor subtypes displayed distinctly different localisation patterns, which in most cases was the inverse of the other pattern.

Interestingly, in the brainstem 5-HT2 receptors were found to be concentrated in the facial nucleus and the motor nucleus of the trigeminal neurone, areas known to influence head and facial movement. The 5-HT-related shaking behaviour is mediated through 5-HT2 receptors (see Introduction section 15). In contrast, 5-HT1 receptors are distributed throughout the brainstem and in specific portions of the spinal cord. These areas are thought to control the "5-HT syndrome" (Jacobs and Kelmfuss 1975 & Deakin and Green 1978). The syndrome is thought to be principally mediated through 5-HT1 receptors (see Introduction section 14). All raphe nuclei were devoid of 5-HT2 receptors; only 5-HT1 receptors were found in these nuclei.

6. Regulation of central 5-HT receptors.

For the most part, studies of the regulation of central 5-HT receptor sites have depended on radioligand binding data and were done prior to the recognition of the 5-HT1 receptor subtypes. [3H]-5-HT binding, which labels all of the 5-HT1 subtypes, responds as might be expected to in vivo manipulations that alter 5-HT availability, although the magnitude of the response is less than is found in other neurotransmitter systems. Chemical denervation induces an increase in the density of [3H]-5-HT binding sites in some, but not all, brain areas (Nelson et al 1978; Seeman et al 1980 & Fischette et al 1987); conversely, inhibition of 5-HT inactivation with monoamine oxidase inhibitors (Savage et al 1980b) or chronic reuptake blockade (Wong and Bymaster 1981 & Dumbrille-Ross and Tang 1983) reduces the number of 5-HT1 binding sites. Furthermore, chronic treatment with 5-HT receptor agonists or antagonists decreases or increases the density of 5-HT1 binding sites, respectively (Samanin et al 1980 & Savage et al 1980a).

The 5-HT2 site, on the other hand, does not respond predictably to in vivo manipulations. For instance, it does not develop increased density after denervation (Seeman et al 1980; Blackshear et al 1981 & Quick and Azmita 1983) and paradoxically, chronic treatment with putative 5-HT2 antagonists results in an apparent down-regulation of the 5-HT2 binding site (Blackshear and Sanders-Bush 1982; Blackshear et al 1983 & Leysen et al 1986). Although the 5-HT2 site is down-regulated by chronic administration of some 5-HT uptake inhibitors (Peroutka and Snyder 1981), this effect is not universal (Dumbrille-Ross and Lang 1983), and it correlates more closely with affinity for the 5-HT2 site than with affinity for the 5-HT uptake carrier (Leysen et al 1986). The effects of agonist treatment have been less extensively investigated and findings are difficult to interpret because of the mixed antagonistic properties of the drugs (ergot derivatives), the lack of selectivity for the 5-HT2 receptor (phenylpiperazine) or too low an affinity of drugs (mescaline). However, a recent study by Leysen et al (1989) examined the effect of repeated injections of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane

(DOM) (2.5mg/kg s.c.), a drug which has been described as a relatively selective 5-HT2 receptor agonist (Shannon et al 1984), on 5-HT2 receptor-mediated head-twitching and on 5-HT2 binding in the frontal cortex. Four injections in 24 hours produced large reductions in the head-twitch response (-70%), and in the Bmax for [3H]-ketanserin binding [-41%]. The Kd values tended to increase slightly. These results suggest that DOM treatment results in reduction of the total number of 5-HT2 sites and that although 5-HT2 receptors show an anomolous response to receptor denervation or blockade, when a relatively selective 5-HT2 agonist is used, a normal response to agonist treatment is elicited with 5-HT2 receptors. The authors propose a refinement in the interpretation of the receptor regulation theory to explain these differences. They suggest that under normal conditions in vivo, a receptor could exist in either a subsensitive or supersensitive state. A receptor that receives a tonic stimulation under physiological conditions such as the dopamine receptor, would exist in a desensitized state. Chronic blockade of such a receptor would rapidly lead to receptor supersensitivity. In the case of a receptor that receives little input under normal conditions the receptor might exist in a supersensitive state. Chronic antagonism of such a receptor would not disrupt the normal situation as further supersensitivity could not develop, however, the receptor would be very sensitive to agonists and rapidly develop desensitisation. There is evidence from electrophysiological studies (Trulson and Jacobs 1979 & Yen and Blum 1984) in support of a large population of 5-HT neurones which show little activity under normal conditions.

Another possible explanation of the anomolous regulation of the 5-HT2 receptor is that 5-HT receptor sensitivity responds predictably to in vivo manipulation even though the 5-HT2 recognition site does not. Studies of 5-HT-stimulated phosphoinositide hydrolysis, a cellular response that is linked to the 5-HT2 recognition, allow a direct examination of 5-HT2 receptor responsiveness. In such studies, chronic administration of both mianserin (a 5-HT2 antagonist) and antidepressants has been shown to cause both down regulation of 5-HT2 binding sites and a decrease in the maximum phosphoinositide response

(Kendall and Nahorski 1985 & Conn and Sanders-Bush 1986). Such results indicate that it is unlikely that 5-HT receptor sensitivity responds predictably to in vivo manipulation even though the 5-HT2 recognition site does not, since in both cases mentioned (mianserin and the antidepressants) both the receptor number and a cellular response that is linked to the 5-HT recognition site were decreased by chronic treatment with these agents.

Chemical lesioning of 5-HT neurones with 5,7-DHT or the administration of para-chlorophenylalanine (PCPA) results in profound, selective depletion of 5-HT and its metabolite, but has no effect on either the density of 5-HT binding sites (Seeman et al 1980; Blackshear et al 1981 & Quik and Azmita 1983) or 5-HT-stimulated phosphoinositide response in the cortex i.e. on the transduction mechanism of 5-HT2 receptors (Conn and Sanders-Bush 1986).

There are some reports of 5-HT-mediated behavioural responses where the 5-HT2 receptor responds predictably to manipulation. Heal et al (1985) found that lesioning of 5-HT neurones with 5,7-DHT produced enhanced 5-MeODMT-induced head-twitching only when there was an increase in 5-HT2 receptor number. Other authors have also reported that lesioning of 5-HT neurones with 5,7-DHT (Nakamura and Fukushima 1978 & Yamamoto and Ueki 1981) resulted in a supersensitive 5-HT2 mediated behavioural response; as did chronic administration of 5-HT2 antagonists (Mogilnicka and Klimek 1979; Friedman et al 1983 & Stolz et al 1983). This raises the possibility that behavioural supersensitivity to activation of 5-HT2 receptors occurs distal to the receptor/effector complex since, as outlined above, chemical lesioning of 5-HT neurones has no effect on 5-HT2 receptor density or on the transduction mechanism, but causes supersensitivity of some 5-HT-mediated behavioral responses. An example of such regulation exists in 1321N1 astrocytoma cells where sensitivity to muscarinic agonists is regulated by changes in sensitivity of the cell to IP3, a second messenger released upon stimulation of phosphoinositide hydrolysis. This is not accompanied by alterations in muscarinic binding properties or in carbachol-induced phosphoinositide hydrolysis

(Masters et al 1985). Another possible mechanism of regulation that exists in the CNS is an alteration of the activity of a neuronal system that is antagonistic to the 5-HT system involved in these 5-HT2-mediated behavioural responses. If activity of such a system is decreased following 5-HT denervation, it could result in supersensitive behavioural responses to 5-HT.

7. Biochemistry of the 5-HT receptors.

5-HT receptors are coupled to several major effector systems: adenylate cyclase; phospolipase C-mediated phosphoinositide (PI) hydrolysis; and ion channels (K^+ and Ca^{2+}) (Roth and Chuang 1987).

5-HT1a receptors are linked to adenylate cyclase. This is reflected by the ability of guanine nucleotides to alter the binding affinities of 5-HT1a agonists as well as by the ability of these agonists to inhibit the adenylate cyclase activity stimulated by forskolin in hippocampal membrane preparations (Gozlan et al 1983; De Vivo and Maayani 1986 & Bockaert et al 1987). 5-HT1a receptors are coupled, either positively in some tissues, or negatively in others, to adenylate cyclase activation, depending on the pre-existing state of activation (Conn and Sanders-Bush 1987 & Sanders-Bush and Conn 1987). 5-HT1a receptors are negatively coupled to the adenylate cyclase system in rat, guinea pig and calf hippocampus (De Vivo and Maayani 1986; Bockaert et al 1987 and Schoeffter and Hoyer 1988). Electrophysiological studies suggest that the 5-HT autoreceptor located on 5-HT cell bodies in the dorsal raphe may be a 5-HT1a site. Thus, this receptor may partially mediate the inhibitory effect of 5-HT on its own release (Conn and Sanders-Bush 1987).

5-HT1b receptors are negatively coupled to the adenylate cyclase system. For example, 5-HT1b receptor mediates the inhibition of forskolin-stimulated adenylate cyclase in rat substantia nigra. 5-HT, 5-CT, RU 24969 and CGS 12066B, reportedly a selective 5-HT1b agonist (Neale et al 1987), inhibit forskolin-stimulated adenylate cyclase, whereas 8-OH-DPAT and ipsapirone are ineffective (Bouhelal et al

1988). Cyanopindolol, propranolol and metergoline reverse 5-HT
inhibition of forskolin-stimulated adenylate cyclase, whereas
spiperone, mesulergine and ketanserin are ineffective. This
pharmacological profile is consistent with that of a 5-HT1b receptor.

5-HT1d receptors appear to be coupled negatively to the adenylate
cyclase system. In the presence of GTP, 5-HT, 5-CT and 5-
methoxytryptamine inhibit forskolin-stimulated adenylate cyclase in
the calf substantia nigra, whereas 8-OH-DPAT has no effect (Schoeffter
et al 1988). Cyanopindolol, spiperone, and mianserin do not antagonise
the effect of 5-HT. The non-selective 5-HT antagonist methiothepin
antagonises 5-HT inhibition of forskolin-stimulated adenylate cyclase.
This pharmacological profile is consistent with that of the 5-HT1d
receptor (Schmidt and Peroutka 1989).

Since the 5-HT1b receptor is either not present or is barely
detectable in the CNS of those species in which the 5-HT1d subtype
occurs (Heuring and Peroutka 1987 & Waeber et al 1988) as previously
mentioned, it has been hypothesized that in such species e.g. calf,
guinea pig, pig, human, the 5-HT1d receptor subtype subserves
functions mediated by the 5-HT1b subtype in other species, such as
being the 5-HT terminal field auto receptor (Hoyer and Middlemiss
1989).

In contrast to the other 5-HT1 receptor subtypes, the 5-HT1c receptor
is not linked to the modulation of adenylate cyclase but rather to
phospoinositide (PI) hydrolysis for signal transduction, with
stimulation leading to activation of phospholipase C, accumulation of
inositol phosphates and mobilisation of intracellular Ca^{2+} (Conn and
Sanders-Bush 1987). 5-HT stimulates PI turnover in choroid plexus, a
brain area rich in 5-HT1c receptors (Sanders-Bush and Conn 1987).
Mianserin, ketanserin and spiperone inhibit the response. The
potencies of these antagonists correlate with their binding affinities
at the 5-HT1c receptor. Thus 5-HT1c receptors in choroid plexus
mediate 5-HT-induced PI turnover (Sanders-Bush and Conn 1987).

Interestingly, DOI, a 5-HT agonist whose effect on on-going behaviour was examined in this study, appears to act as a partial agonist at the 5-HT1c receptor, in terms of the modulation of PI turnover. It was amongst the most potent agents (of the agents examined by Hoyer et al 1989) in eliciting the PI response, along with alpha-methyl-5-HT and 1-methyl-5-HT, being more potent than 5-HT in this respect.

5-HT2 receptors are also reported to be linked to PI hydrolysis for signal transduction. 5-HT-induced PI hydrolysis in rat cerebral cortex appears to occur as a result of 5-HT receptor activation (Sanders-Bush and Conn 1987). 5-HT stimulates PI turnover with an EC50 of 1 micromolar. The response to 5-HT is potently blocked by ketanserin and other 5-HT2 antagonists. The effects of 5-HT2 antagonists on PI turnover in the rat cerebral cortex correspond well with their binding affinities at [3H]-labelled 5-HT2 sites. Many other tissues such as rat thoracic aorta, cultured bovine aortic smooth muscle cells, and platelets contain a 5-HT receptor that appears to modulate PI turnover via the 5-HT2 binding site.

5-HT3 receptors are positively coupled with adenyl cyclase (Dumuis et al 1988) and increase cyclic GMP levels (Reiser and Hamprecht 1989). These receptors have also been reported to be ligand-gated to ion channels (Hartig 1989).

Three 5-HT receptor subtypes, 5-HT1a, 5-HT1c, and 5-HT2 have been cloned (Hartig 1989). All three are single subunit proteins and members of the G protein superfamily (Hartig 1989).

8. Central noradrenergic receptors.

Binding studies have shown that receptors, which resemble pharmacologically the peripheral alpha1- and alpha2-adrenoceptors, are also present in the brain (U'Prichard et al 1978 & Tanka and Starke 1980). The presence of central beta-adrenoceptors has been demonstrated by means of various techniques, including radioligand binding (see Maguire et al 1977 & Minneman et al 1979b),

electrophysiological methods (Bloom et al 1975) and studies of cyclic
adenosine monophosphate (cAMP) generating systems (see Daly 1977 &
Iversen 1977).

8.1 Subtypes of beta-adrenoceptors in the CNS.

Physiological evidence suggests that two types of beta-adrenoceptors
exist (Lands et al 1967). Radioligand binding and autoradiographic
techniques have identified these subtypes of beta-adrenoceptors in the
CNS. In the cerebral cortex, limbic forebrain and striatum of the rat,
beta1-adrenoceptors are predominant while the cerebellum contains
exclusively the beta2-subtype (Minneman et al 1979a & Nahorski 1981).

Evidence suggests that beta1- and beta2-adrenoceptors in the CNS are
independently regulated. In rat cerebellum, an increase in beta1-
adrenoceptors accompanied by a decrease in beta2-adrenoceptors,
occurred with ageing from 3 to 12 months (Pittman et al 1980). Chronic
administration of desmethylimipramine (DMI) to adult rats caused a
substantial reduction in the density of beta1-adrenoceptors in the
cortex but had no effect on the beta2-adrenoceptors; conversely,
destruction of NA neurones by administration of 6-OHDA to neonatal
rats which selectively destroys noradrenergic neurones originating
from the locus coeruleus (Clark et al 1972), caused a significant
increase in the density of beta1-adrenoceptors in the adult cerebral
cortex with no change in the density of beta2-adrenoceptors (Minneman
et al 1979a). The changes in beta1-adrenoceptor density produced by
chronic DMI treatment (to increase brain NA levels) and the 6-OHDA
lesion (to decrease NA levels) indicate that beta1-adrenoceptors are
located postsynaptically. Conversely, receptors of the beta2-subtype
are present on noradrenergic terminals and appear to be involved in
the facilitation of noradrenaline release (Westfall 1977 & see Misu
and Kubu 1983). Furthermore, the firing activity of noradrenergic
neurones originating from the locus coeruleus is reduced by beta2-
adrenoceptor blockade (Dhaloff et al 1981).

Compared with radioligand binding studies, there are relatively few
functional studies that have examined whether or not differential
regulation of beta-adrenoceptor subtypes occurs. These studies have
measured beta-adrenoceptor agonist-induced responses of isolated
tissues and/or activation of adenylate cyclase, and the data supports
the concept that beta-adrenoceptor subtypes can be differentially
regulated. With few exceptions these studies have involved peripheral
tissues.

8.2 Biochemistry of beta-adrenoceptors in the CNS.

As in peripheral tissue, central beta-adrenoceptors are coupled to
adenylate cyclase in a stimulatory manner (Mobley and Sulser 1979).
Both beta1- and beta2-adrenoceptors are coupled to adenylate cyclase
(Harden 1983 & Harden et al 1983) and receptor occupancy leads to an
increase in cAMP production. This is mediated by GTP binding proteins
(that is G or N proteins) which consist of dissociable subunits
(alpha, beta & gamma).

8.3 Up-regulation of central beta-adrenoceptors.

Several studies have indicated enhanced beta-adrenoceptor reactivity
to intraventricular noradrenaline following depletion of the stores by
reserpine or chemical sympathectomy with 6-OHDA (Geyer and Segal 1973
& Mandell 1974). An increased responsiveness of the beta-adrenoceptor
coupled adenylate cyclase system has been reported following 6-OHDA in
brain tissues (Palmer 1972 & Vetulani et al 1976). This up-regulation
of adenylate cyclase is characterised by supersensitivity to
noradrenaline and isoprenaline but not to adenosine (Vetulani et al
1976), thus indicating "homologous" sensitivity changes (changes
exclusive to beta-adrenoceptor stimulation). Lack of agonist can
induce supersensitivity by enhancing beta-adrenoceptor coupling to the
regulatory subunit of cAMP in a short period of time (see Harden
1983).

8.4 Subtypes of alpha-adrenoceptors in the CNS.

Alpha-adrenoceptors were subdivided into alpha1- and alpha2-
adrenoceptor subtypes in the periphery (Langer et al 1974) such that
alpha1-adrenoceptors were thought to exist predominantly
postsynaptically to mediate the end-organ response, whereas alpha2-
adrenoceptors were proposed to exist predominantly presynaptically to
mediate the inhibition of neurotransmitter release. This
classification was decided on the basis of showing differences in the
rank order of potency to a series of agonists.

Subsequently, it was suggested that alpha1- and alpha2-adrenoceptors
should not be characterised on the basis of their anatomical
distribution within the synapse, but rather on their pharmacological
specificity to a series of drugs (Berthelsen and Pettinger 1977).
Thus, alpha1-adrenoceptors were found to have high affinity for
agonists such as phenylephrine, methoxamine and 6-fluoronoradrenaline
whereas alpha2-adrenoceptors had higher selectivity for clonidine,
para-aminoclonidine and iodoclonidine. The subdivision of alpha-
adrenoceptors was further substantiated by the development of
selective antagonists for each type, with alpha1-adrenoceptors being
antagonised by prazosin, WB-4101, phenoxybenzamine, benoxathian and
benextramine, and alpha2-adrenoceptors being selectively inhibited by
rauwolscine, yohimbine, idazoxan, efaroxan and RX 821002.

Binding data has shown that receptors which resemble pharmacologically
alpha1- and alpha2-adrenoceptors found peripherally are present in
brain tissue (U'Prichard et al 1978 & Tanaka and Starke 1980). Alpha2-
adrenoceptors are located both pre- and postsynaptically, whereas
alpha1-adrenoceptors appear to be exclusively located post
synaptically (Janowsky and Sulser 1987).

Recently, it has become apparent that alpha2-adrenoceptors may be
subdivided into as may as four different subtypes. Detailed analysis
of radioligand binding studies in a variety of tissues led Bylund
(1988) to propose the existence of two populations of alpha2-

adrenoceptors, which were termed alpha2a- and alpha2b-adrenoceptors. Both alpha2-adrenoceptor subtypes have high affinity for yohimbine and rauwolscine, but surprisingly the alpha1-adrenoceptor antagonist prazosin, and several structural analogues also have high affinity for the alpha2b-adrenoceptor subtype. Two additional subtypes, alpha2c- and alpha2d- have most recently been proposed, again on the basis of detailed analysis of binding data (Ruffolo 1990).

It has also been recently suggested that pre- and postjunctional alpha2-adrenoceptors are not an homologous population of receptors on the basis of studies utilising a series of alpha2-adrenoceptor antagonists of the 3-benzazepine class. Thus, the alpha2-adrenoceptor antagonist, SK&F104078 has been shown to have at least 100-fold higher affinity for postjunctional alpha2-adrenoceptors in canine saphenous vein relative to prejunctional alpha2-adrenoceptors in guinea pig atrium (Ruffolo 1987).

Presynaptic alpha2-adrenoceptor-mediated modulation of NA release in the CNS has been clearly demonstrated (Starke and Montel 1973 & Langer 1979). The physiological role of postsynaptic alpha2-adrenoceptors in the brain remains to be elucidated.

Electrophysiological studies have shown that alpha2-adrenoceptors are present on noradrenergic neuronal cell bodies in the locus coeruleus (LC) (Cedarbaum and Aghajanian 1976) and activation of these receptors by microiontophoretic application of alpha2-adrenoceptor agonists or by increase in endogenous NA caused by administration of monoamine oxidase inhibitors (MAOIs), decrease neuronal firing, and alpha2-adrenoceptor antagonists cause an increase (Svensson and Usdin 1978 & Campbell et al 1986). These alpha2-adrenoceptors on noradrenergic cell bodies are believed to function to control the firing rate of these neurones and maintain a tonic inhibition. Lesioning of noradrenergic neurones with 6-OHDA does not produce a significant decrease in the number of alpha2-adrenoceptors in the rat cerebral cortex (the area containing noradrenergic neurone endings) suggesting that presynaptic autoreceptors on the noradrenergic nerve endings represent a very

small fraction of the cortical alpha2-adrenoceptor population (Bylund and U'Prichard 1983). Studies on K+-evoked or electrically stimulated release of other monoamines such as [3H]-5-HT from brain slices or synaptosomes (Ellison and Campbell 1986), indicate that many alpha2-adrenoceptors exist on neurones postsynaptic to noradrenergic ones (heteroreceptors). It should be noted, however, that the idea of presynaptic alpha2-adrenoceptors having an important role under normal physiological conditions is not without its critics (Kalsner 1985a&b).

8.5 Biochemistry of alpha-adrenoceptors in the CNS.

8.5a. alpha1-adrenoceptor.

Responses to alpha1-adrenoceptor stimulation are rapid in onset, short-lived and are mediated by phosphatidyl inositol (PI) breakdown to diacylglycerol (DG) and inositol-1,4,5-triphosphate (IP3) (Soukup and Schambergs 1978; Kendall et al 1985 and Pearce et al 1985). This occurs via an N-protein transmembrane coupling system which is apparently distinct from those N-proteins controlling adenylate cyclase systems (Houslay et al 1986).

8.5b. alpha2-adrenoceptor.

Alpha2-adrenoceptor function has been linked to inhibition of adenylate cyclase activity in a number of tissue preparations (Limbird 1983). In brain preparations, however, the effects of alpha2-adrenoceptor agonists such as clonidine on adenylate cyclase activity are controversial. Kitamura et al (1985), using rat brain membrane preparations, were unable to demonstrate inhibition of "basal" adenylate cyclase activity but demonstrated a clonidine-induced inhibition of forskolin/GTP-stimulated activity. Treatment of the membrane preparations with pertussis toxin attenuated the clonidine-induced inhibition. ADP-ribosylation of Ni reduced the clonidine binding affinity to alpha2-adrenoceptors in cortical membranes (Nomura et al 1985). Thus, the guanine nucleotide regulatory protein, Ni, may be involved in alpha2-adrenoceptor-mediated inhibition of adenylate

cyclase activity in the brain (Kawahara and Bylund 1985). At present
it is unknown how the alpha2-adrenoceptor-mediated inhibition of
adenylate cyclase is linked to the physiological effects of alpha2-
adrenoceptor stimulation.

8.6 Interaction of 5-HT and beta-adrenoceptors.

A discussion of the interaction of non-selective beta-adrenoceptor
antagonists (with 5-HT1a binding affinity) and 5-HT2-mediated
behaviour occurs in Introduction section 17.1.

Clenbuterol, dobutamine and other beta-adrenoceptor agonists stimulate
central 5-HT turnover (Waldmeier 1981) and enhance 5-HT-mediated
hyperactivity, head-twitch (see Introduction section 17.1; Ortmann et
al 1981; Cowen et al 1982 & Handley and Singh 1984; 1986b&c),
hypothermia (Green et al 1986) and tremor (Hallberg 1986). This
potentiation appears to be mediated by beta-adrenoceptor mechanisms
rather than by direct effects on 5-HT receptors since these compounds
do not bind to 5-HT receptors (Green et al 1983) and their modulation
of 5-HT effects is blocked by beta1- and beta2-selective antagonists
(Green et al 1986 & Hallberg 1986) which neither bind to 5-HT
receptors (Middlemiss et al 1977; Green et al 1983 & Nahorski and
Willocks 1983) nor affect 5-HT-mediated behaviour (e.g. Handley and
Singh 1986c).

8.7 Interaction of 5-HT and alpha-adrenoceptors.

The 5-HT1a agonist 8-OH-DPAT has been shown to have alpha2-
adrenoceptor antagonist properties both in electrophysiological
studies (Crist and Suprenant 1987) and in drug discrimination studies
(Winter 1988). It is therefore likely that some of the actions
attributed to its agonist effects at 5-HT receptors may be due to
alpha2-adrenoceptor blockade.

However, 8-OH-DPAT also facilitates NA release hence indirectly producing postsynaptic alpha2-adrenoceptor agonist effects such as mydriasis (Heal et al 1989a&b).

In addition, the alpha2-adrenoceptor antagonists yohombine, rauwolscine and idazoxan bind to 5-HT1a receptors (pKi yohimbine = 7.3, idazoxan = 7.4 and rauwolscine = 7.5 (Armah 1989)).

8.8 Interaction of 5-HT and dopamine receptors.

It has recently been demonstrated that the 5-HT1a agonist 8-OH-DPAT stimulates dopamine2 receptors located presynaptically in mouse vas deferens (Smith and Cutts 1989). Bull et al (1990) have shown that 8-OH-DPAT can act as a partial agonist at dopamine2 receptors in the CNS (study looked at the effect of 8-OH-DPAT on central dopamine2 receptors located somatodentritically in the zona compacta of the substantia nigra). The concentration of 8-OH-DPAT necessary to activate dopamine2 receptors was only 10-20 times its EC50 at 5-HT1a receptors in septal neurones (Joëls et al 1987) and it is thus possible that some of 8-OH-DPAT's behavioural effects are a result of this additional dopaminergic action.

Ritanserin, an antagonist at 5-HT2/5-HT1c receptors (Hoyer 1988a&b) with low affinity for dopamine (D2) receptors (Leysen et al 1985) has been shown to dose-dependently increase both the burst firing and firing rate of midbrain DA neurones. This effect was prevented by endogenous 5-HT depletion through pretreatment with para-chlorophenylalanine which did not significantly alter the firing characteristics of the midbrain DA cells when given alone. It is suggested that these results indicate that 5-HT exerts an inhibitory control over midbrain DA cell activity mediated by 5-HT2 receptors (Ugedo et al 1989).

9. Spontaneous shaking.

A wide variety of tic-like spontaneous shakes occur in virtually all furred and feathered species (Wei 1981). These range from the brisk head-twitch characteristic of the mice consisting of rapid intermittent side to side movements of the head, with little or no involvement of the shoulders, to 'wet-dog shakes' (WDS) seen in a dog emerging from water involving multiple rotatory movements of head and shoulders about the long axis. In the rat, there is a continuum between the single head-twitch and the multiple wet-dog shake. Functionally these behaviours may be important for the removal of irritants from pinna or the coat. Indeed, studies with rodents indicate that the frequency of the spontaneous shaking can be increased by tactile stimulation, especially of pinna (which involves a multineuronal pinna reflex) (Wei 1981). Hence, water immersion or application of irritants to the external auditory meatus increase, while local anaesthesia of the pinna region decrease shaking in mice and rats (Boulton and Handley 1973 & Wei 1981).

10. Drug-induced shaking behaviour.

The primary interest of shaking behaviour to pharmacologists is that, in addition to physical stimulation e.g. irritation of pinna or water immersion, a wide variety of drugs, including large doses of L-5-hydroxytryptophan (5-HTP), precursor of the neurotransmitter, 5-hydroxytryptamine (5-HT), 5-HT itself, 5-HT receptor agonists and hallucinogens (mostly 5-HT-related but some which are not)(see Corne et al 1963 & Corne and Pickering 1967), gamma-aminobutyric acid (GABA)-related agents, opiates and opiate withdrawl, cholinergic agonists and antagonists, some hormones e.g. thyrotropin releasing hormone (TRH) can induce shaking behaviour in rodents identical with the spontaneous variety but at frequencies many orders of magnitude above the spontaneous rate (Handley and Singh 1986b). Shaking behaviour is easy to quantitate, either as frequency (number of shakes per unit time) or incidence (proportion of animals exhibiting any shake behaviour at all). The former measure appears to be more

sensitive. For instance, a series of 5-HT2 antagonists were assessed for their potency in antagonising 5-HTP-induced shakes in the rat (see Tricklebank 1985). The results were analysed by both methods and it was found that the ID50 values (i.e. the dose to halve shake frequency) were 1-2 orders of magnitude below the ED50s (the dose to abolish shaking in 50% of rats). In addition, barbiturates have potent effects on 5-HTP shake frequency but seem unable to abolish it, so that they appear inactive on an incidence measure (Handley and Singh 1985).

The amenability of shaking behaviour to quantative evaluation has resulted in its use as probes of receptor function, particularly for 5-HT receptors. In addition, because so many transmitters have been implicated in their control, they have started to prove useful in pinpointing a number of potentially interesting transmitter interactions.

11. Sensory component of drug-induced shaking behaviour.

Although the drug-induced shaking behaviour occurs in the absence of any obvious external stimulation, studies with 5-HTP-induced head-twitching in mice indicate that sensory input from the pinna is a necessary precondition for the head-twitch to occur since local anaesthesia of the pinna region virtually abolishes the response (Boulton and Handley 1973). Suggestion was made by these authors that 5-HTP might allow the perception of a previously subliminal stimuli from the pinna region. Boulton and Handley (1973) further demonstrated that the rate of 5-HTP-induced head-twitching could be reduced by concurrent variable frequency sound or following complete isolation of the mice, indicating that drug-induced shaking can be modified by environmental conditions. This highlights the importance of maintaining a controlled environment in experiments utilising the drug-induced animal model of shaking.

It should be noted, however, that Lucki et al (1987) found that local anaesthesia of external auditory meatus (bilateral subcutaneous

irrigation of the local anaesthetic procaine hydrochloride (10% with 0.5% epinephrine)) had no effect on head-twitching induced by either the 5-HT agonist, quipazine or 5-HTP. Injection of procaine into three lateral sites (in the tragus, post-tragus and lateral concha) of the rat ear failed to reduce 5-HT-elicited shaking behaviour. These results appear to differ from those of Boulton and Handley (1973) who found that local anaesthesia of the three lateral sites at homologous areas on the mouse ear completely blocked head-twitching behaviour caused by 5-HTP. As Lucki et al (1987) suggested, such differences could point to different controls of head-twitching behaviour between rats and mice. However, modulation of the two behaviours has been found to be identical in all other respects (see Handley and Singh 1986b).

12. Anatomy of shaking.

In view of the similarities between the spontaneous and the drug-induced shaking, it would appear likely that they share a common organisational area in the brain. Corne et al (1963) examined whole brain and brain stem 5-HT concentrations at various times following 5-HTP administration to mice. They found that head-twitches are related to brain stem concentrations of 5-HT indicating the importance of this area in the mediation of shaking behaviour. Possibly this area is the common organisational area for shaking suggested above.

Brain transection studies are in agreement with this suggestion, indicating such an area lies between the colliculi and the anterior commissure. Cuts in this area reduce responses to 5-HTP, morphine withdrawal and water immersion, while mid-collicular transection abolishes them (Wei et al 1973; Bedard and Pycock 1977 & Wei 1981). The sites at which local injection of TRH can induce shaking and at which naloxone precipitates the behaviour in morphine-dependent rats fall within these these limits (Wei et al 1973 & Wei 1981). Although this area corresponds with the motor areas controlling shivering (Wei et al 1973), hypothermia is not a necessary stimulus for shaking.

It is likely that more rostral regions also play a role in mediating head-twitching. Although cuts at the level of the anterior commissure did not modify the frequency of shaking due to morphine withdrawal, 5-HTP or ice-cold water, wet-dog shakes can be induced in rats by electrical stimulation of the hippocampus and hippocampal lesions abolished 5-HTP- as well as enkephalin- and kainic acid-induced shaking (Yamada et al 1983).

Recent lesion experiments suggest that the receptors mediating WDS behaviour are not located in the cortex (Lucki and Minugh-Purvis 1987) which is interesting as the cortex contains the greatest density of central 5-HT2 receptors (Leysen et al 1982), the receptor which is thought to mediate shaking behaviour (Peroutka et al 1981; Lucki et al 1984 & see Green and Heal 1985).

Lesion experiments suggest that, in addition to the caudal brainstem, receptors mediating WDS are located in the spinal cord (Marley and Wozniak 1984).

13. 5-HT-component of shaking.

The exact mechanism for the induction of the shaking behaviour following a variety of substances and procedures is not yet clear. However, prior treatment with classical 5-HT antagonists or depletors can inhibit shaking behaviour in most of these cases, with a few exceptions. For example, shaking behaviour following the administration of opiates, opiate withdrawal, thyrotrophin releasing hormone (TRH), and stimulus-induced shaking are not blocked by older 5-HT antagonists (Handley and Singh 1986b). This immunity to older antagonists, however, may be more apparent than real since inhibition of morphine-withdrawal shakes has been recently observed following a more selective 5-HT antagonist, ritanserin (Handley et al 1986). Thus the possibility of a vital 5-HT link in the mediation of shaking behaviour is raised.

Indeed, intracerebroventricular (i.c.v.) administration of 5-HT itself produces shaking (Suchowsky et al 1969 & Handley and Miskin 1976). However, most work has been carried out on this behaviour following systemic administration of 5-HT agonists, for example 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) and quipazine or following large doses of the 5-HT precursor, 5-HTP, which can cross the blood-brain barrier. Considerable evidence has accumulated to suggest that the head-twitch to 5-HTP in mice and rats results from the formation of 5-HT in the brain (Corne et al 1963; Corne and Pickering 1967 & Matthews and Smith 1980).

14. Shaking and other behaviours of the 5-HT behavioural syndrome.

Following the administration to rats of compounds which activate central 5-HT receptors either indirectly by increasing synaptic 5-HT (e.g. momoamine oxidase inhibitors, 5-HT releasing agents or 5-HT precursors) or directly by 5-HT receptor agonists, in addition to shaking behaviour, a stereotyped behavioural syndrome, generally termed 'the 5-HT syndrome' (see Green and Heal 1985) is produced. The components of this syndrome include resting tremor, hyperlocomotion, reciprocal forepaw treading (rhythmic dorso-ventral movements of the hindlimbs), lateral head weaving (slow side to side movements), hindlimb abduction (splaying out of the hindlimbs), tremor, increased sniffing and grooming (especially involving the stereotyped rubbing of face and nose region with forepaws), Straub tail, retropulsion (backward motion), salivation and hyperreactivity. To add to the complexity, these behaviours show differences in onset, intensity of peak effect and duration. Also interactions between the component behaviours is not uncommmon with one behaviour altering the intensity, duration and manefestation of another. For example, development of profound hindlimb abduction prevents hyperlocomotion.

A similar syndrome is also seen in other species following increases in central 5-HT function (see Jacobs 1976).

As with head-shaking in mice and rats, the behaviours of the syndrome
(including forepaw treading and hindlimb abduction) are hindbrain-
mediated with a spinal component (see Introduction section 4; Jacobs
and Klemfuss 1975 & Deakin and Green 1978).

5-HT-related head-shaking appears to be mediated exclusively by 5-HT2
receptors (see Introduction section 15) whereas the component
behaviours of the 5-HT syndrome involve 5-HT1a and 5-HT1b receptors
(see Lucki et al 1984). However, this view is controversial with some
authors suggesting that some components of the 5-HT syndrome are
mediated by 5-HT2 receptor activation (see Tricklebank 1985).

15. Head-shaking as a functional correlate of 5-HT2 receptor activation.

Following the demonstration of two subtypes of 5-HT binding sites (5-
HT1 and 5-HT2) by Peroutka and Snyder (1979) these authors suggested
that the head-twitch response may be mediated by activation of the 5-
HT2 receptor subtype (Peroutka et al 1981). They reported that there
was a good correlation between the ability of various drugs to inhibit
spiperone binding to 5-HT2 receptors and inhibit head-twitch
behaviour. These observations were strengthened by the observations of
Ortmann et al (1982). The 5-HT2 receptor antagonists pirenperone and
ketanserin blocked WDS behaviour in rats (Colpaert and Janssen 1983 &
Yap and Taylor 1983) and pirenperone also reduced head-twitching in
mice (Green et al 1983). However, neither of these agents could
provide firm evidence for the mediation of shaking behaviour by 5-HT2
receptors as both ketanserin and pirenperone are not selective
specifically for the 5-HT2 receptor. Ketanserin displays an
appreciable affinity for dopaminergic, histaminergic (H1 receptor) and
noradrenegic (alpha1-receptor) binding sites (Leysen et al 1981).
Ketanserin also displays an appreciable affinity for 5-HT1c sites
(Hoyer et al 1985b) although its affinity for this site is at least 50
times less than for the 5-HT2 site. Pirenperone is structurally
related to ketanserin with a binding profile similar to that of
ketanserin (Janssen 1983). Since prazosin, an alpha1-adrenoceptor

antagonist, devoid of affinity for 5-HT2 receptors (see Tricklebank 1985), can also antagonise 5-HT-related head-twitching (Handley and Brown 1982), some caution might be applied in attributing the inhibitory effect of ketanserin solely to blockade of 5-HT2 receptors.

Further evidence that shaking behaviour is mediated via 5-HT2 receptors was provided by the work of Arnt et al (1984) who found that ability of an antagonist to inhibit the shaking response correlated significantly with their affinity for the 5-HT2 binding site. Also the selective 5-HT1 agonists, 8-OH-DPAT and RU 24969 do not induce head-twitching in mice (Goodwin and Green 1985).

However, probably the two most convincing pieces of evidence to date suggesting the involvement of 5-HT2 receptors in the mediation of shaking behaviour are :

1) ritanserin, which was the first selective 5-HT2 antagonist (although it has since been shown to possess appreciable affinity for 5-HT1c receptors (Hoyer 1988a&b)). This agent has a somewhat lower affinity for alpha1-adrenoceptors and about a 5-10 fold higher affinity for 5-HT2 binding site than ketanserin (Laduron 1985 & Leysen et al 1985). The ED50 for the displacement of [3H]-spiperone (5-HT2) binding in the rat frontal cortex by ritanserin in vivo (0.1mg/kg) is essentially identical to the ED50 for the antagonism of head-shaking induced by mescaline (Laduron 1985).

2) The recent finding that after lesioning with the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), 5-MeODMT-induced head-twitch responses of mice were only enhanced when there was an increase in 5-HT2 receptor number (Heal et al 1985).

Overall, evidence indicates that both head-twitching in mice and WDS in rats are 5-HT2-mediated.

16. Modulation of 5-HT-induced shaking behaviour by drugs acting at the 5-HT1a receptor.

8-OH-DPAT, buspirone, gepirone and ipsapirone have been reported to bind selectively to the 5-HT1a subtype of the 5-HT receptor (see Hoyer 1988a) and to have agonist/partial agonist activity at this receptor (e.g. Hamon et al 1984; Yocca et al 1986 & Traber and Glaser 1987 and see below). 8-OH-DPAT is the prototypic 5-HT1a agonist, it is selective for 5-HT1 vs 5-HT2 receptors and displays a high affinity and 500-fold selectivity for 5-HT1a vs 5-HT1b sites (Middlemiss and Fozard 1983; Glennon 1987 & Hoyer 1988a). Buspirone and ipsapirone display a high affinity for 5-HT1a sites; both compounds are essentially inactive at 5-HT1b (IC50 = 26000 nM for both) and at 5-HT2 (IC50 = 2100 and 10,000 respectively) sites (Peroutka 1985).

There is evidence that these 5-HT agents can produce both agonist and antagonist effects; whether they act as mixed agonist-antagonists (partial agonists) or whether they act as agonists at one site and antagonists at another is unknown. For example 8-OH-DPAT is active in the licking conflict model for anxiolytic activity, but it reverses a similar effect produced by p-chlorophenylalanine in the same procedure (Engel et al 1984). With the use of rats trained to discriminate the 5-HT1a agents from saline, 8-OH-DPAT, buspirone & ipsapirone substitute for each other but not with 5-MeODMT, quipazine, RU 24969, LSD or TFMPP). This work suggests that all of these 5-HT1a agents act in a similar manner at the 5-HT1a receptor. However, 8-OH-DPAT produces the "5-HT syndrome" in rodents whereas buspirone and ipsapirone have produced conflicting results. Ipsapirone has shown very little ability to induce the syndrome, as has buspirone in some studies (Spencer et al 1984; Eison et al 1986; Goodwin et al 1986; Lucki 1986 & Smith and Peroutka 1986), however in others buspirone has produced either the full syndrome (Hjorth and Carlsson 1982) or parts of it (Smith and Peroutka 1986). Ipsapirone produces a dose-dependent inhibition of 8-OH-DPAT-induced hypothermia in mice suggesting that it may be an antagonist of presynaptic (possibly somatodendritic) 5-HT1a sites (Goodwin et al 1986). On the other hand ipsapirone only

partially antagonised 8-OH-DPAT-induced hypothermia in rats and had no effect on "5-HT syndrome" induced by 8-OH-DPAT (Goodwin et al 1986). However, there have been contrasting reports suggesting that both buspirone and ipsapirone can antagonise 8-OH-DPAT- and 5-MeODMT-induced "5-HT syndrome" (Lucki 1986).

These differences in modulatory activity of 5-HT1a agents extend to their effect on 5-HT-related shaking behaviour. Thus 8-OH-DPAT inhibits 5-HTP- but not 5-MeODMT-induced head-twitching in mice (Goodwin and Green 1985). Ipsapirone, however, has been reported to potentiate 5-MeODMT- but not 5-HTP-induced head-twitching in mice (Goodwin et al 1986). Moser (1990) also found that ipsapirone potentiated 5-MeODMT- but not 5-HTP-induced head-twitching. They also reported that buspirone and MDL 73005EF (a 5-HT1a partial agonist (Moser et al 1990)) had the same effect. 8-OH-DPAT was found to have no effect on 5-MeODMT-induced head-twitching in agreement with Goodwin and Green (1985). Yocca et al (1990) looked at the effect of these 5-HT1a agents on the quipazine-induced head-shake response in rats. They found that, in contrast to the non-uniformity of the effects observed with this group of 5-HT1a agents (8-OH-DPAT, buspirone, and ipsapirone) on 5-HTP- and 5-MeODMT-induced head-twitching in mice, all three of these compounds inhibited quipazine-induced head-shaking in rats. In addition, gepirone was also found to inhibit quipazine-induced head-shaking in rats. Interestingly, Yocca et al (1990) found that pretreatment with (±)pindolol, a beta-adrenoceptor antagonist with partial agonist/antagonist activity (see Introduction section 17.1 for more details) at the 5-HT1a receptor blocked the inhibitory effect of 8-OH-DPAT on quipazine-induced head-shaking to the level of inhibition that (±)pindolol produced itself suggesting that stimulation of central 5-HT1a receptors can modulate the expression of a central 5-HT2-mediated behaviour. They postulated that the effect may be mediated by presynaptic 5-HT1a receptors (autoreceptors) as the doses of 8-OH-DPAT and the buspirone analogues required to inhibit the quipazine-induced head-shaking are similar to those required for the inhibition of central 5-HT synthesis (Galloway et al 1985 & Torrente et al 1988), an effect thought to be mediated by presynaptic 5-HT1a

receptors (Hjorth and Magnusson 1988). They further suggested that
stimulation of presynaptic 5-HT1a autoreceptors by the 5-HT1a agents
reduces 5-HT impulse flow and synthesis which may alter the degree of
postsynaptic receptor stimulation by 5-HT in the presence of
quipazine. Alternatively, they suggest, if stimulation of postsynaptic
5-HT1a receptors is responsible for the inhibition of this 5-HT2-
mediated behaviour, then putative cross-talk between the receptors may
be initiated by low concentrations of the 5-HT1a agent that cannot
elicit a behavioural response i.e. the "5-HT syndrome" in rats.

It should be mentioned, however, that not all authors have
demonstrated inhibition of 5-HT synthesis with these 5-HT1a agents.
For example, Goodwin and Green (1985) found that 8-OH-DPAT decreased
5-HT synthesis in mice, and Hjorth et al (1982) the same effect in
rats. However, Goodwin et al (1986) found that rather than reduce 5-HT
synthesis ipsapirone increased it.

For the effect of the beta-adrenoceptor/5-HT1a/5-HT1b antagonists on
5-HT-related head-shaking behaviour, see Introduction section 17.1.

These results above from behavioural experiments looking at the
modulation of a 5-HT2-mediated behaviour by 5-HT1a agents are
complimented by several other lines of evidence which suggest an
interaction between central 5-HT1a and 5-HT2 receptors. Thus chronic
administration of gepirone was shown to decrease by 20% cortical d-
bromo-LSD-sensitive [3H]-spiperone binding sites, while attenuating
ketanserin-sensitive quipazine-induced behaviour (Eison and Yocca
1985). Also Pericic and Manev (1988) found that imipramine which dose-
dependently inhibited 5-HT2-mediated behaviours in mice, elicited the
"5-HT syndrome" in rats, a behaviour thought to be mediated, at least
in part, by the 5-HT1a receptor (see Introduction section 14). Also,
and interestingly, the "5-HT syndrome" induced by 8-OH-DPAT has been
reported to be potentiated by both the 5-HT2/5-HT1c agonist, (±)DOI
(Arnt and Hyttel 1989) and the 5-HT2/5-HT1c antagonist, ritanserin
(Backus et al 1989; 1990). Physiological and biochemical studies
provide corroborating evidence for an interaction between central 5-

HT1a and 5-HT2 receptors. Thus, Araneda and Andrade (1988) reported that when 5-HT1a and 5-HT2 receptors coexisted on the same cell in rat prefrontal cortex, the activation of 5-HT2 receptors reduced the ability of activation of 5-HT1a receptors to hyperpolarise these cells. Similarly, treatment in humans with ritanserin potentiated the prolactin response to L-tryptophan (Charig et al 1986), an effect that is thought to be mediated by 5-HT1 receptors.

17. Modulation of 5-HT-induced shaking behaviour by other transmitters.

17.1 Noradrenaline.

The administration of alpha2-adrenoceptor agonists e.g. clonidine and guanabenz inhibited the head-twitch response in mice induced by 5-HT (i.c.v.), whereas the alpha2-adrenoceptor antagonists, yohimbine and piperoxane, potentiated the response (Handley and Brown 1982). Clonidine also reduced the 5-HTP-, 5-methoxytryptamine-, LSD- and quipazine-induced WDS in the rat (Bednarczyk and Vetulani 1978 & Vetulani et al 1980) and 5-MeODMT- and 5-HTP-head-twitching in mice (Heal et al 1986).

In the above studies, clonidine was administered before 5-HTP, 5-HT or the 5-HT agonists. However, clonidine appeared to have no effect on 5-HTP-induced WDS in rats, when administered 90 minutes after 5-HTP (Bedard and Pycock 1977). This parallels the situation with the 5-HT hyperactivity syndrome : the beta-adrenoceptor agonists, salbutamol and clenbuterol potentiated the 5-HT hyperactivity syndrome in rats when administered before 5-HTP or quipazine but were ineffective if administered after the inducing agent (Ortmann et al 1981 & Nimgaonkar et al 1983).

The mechanism of action of clonidine was investigated by Bednarczyk and Vetulani (1978). These authors destroyed NA and 5-HT neurones by administering 6-OHDA and 5,6-DHT respectively. Since the effect of clonidine was not altered by these lesions, they concluded that the

action of this alpha2-adrenoceptor agonist was not dependent on pre-
or postsynaptic noradrenergic receptors or presynaptic 5-HT neurones.
However, the data of Bednarczyk and Vetulani (1978) is difficult to
interpret, because they did not measure the extent of noradrenaline
and 5-HT depletion following the chemical lesions i.e. some
presynaptic NA and 5-HT neurones may still be intact following
administration of the neurotoxins.

More recently Heal et al (1986) again looked at the effect of NA
lesions induced by 6-OHDA on 5-HT-related shaking behaviour (5-HTP-
induced head-twitching in mice) and found, as did Bednarczyk and
Vetulani (1978), that the lesion did not affect the inhibitory
response induced by clonidine. They concluded that the alpha2-
adrenoceptors mediating the inhibitory effect were not located on
presynaptic noradrenergic terminals. Unlike Bednarczyk and Vetulani
(1978), Heal et al (1986) measured central NA and DA levels in the
lesioned animals and both were highly significantly decreased, in the
case of NA a depletion of approximately 80% was seen, in the case of
DA, the depletion was approximately 65%.

Handley and Brown (1982) looked at the effect of alpha1-agonists and
antagonists on 5-HT (i.c.v.)-induced head-twitching in mice. The
effect found depended on the route of administration used for these
agents. The alpha1-agonists, methoxamine and phenylephrine (i.c.v.)
inhibited 5-HT-induced head-twitching (except for the lowest dose of
phenylephrine used (0.625 micrograms) which produced potentiation).
When given peripherally (s.c.) phenylephrine produced a biphasic
effect, doses of less than 2.0mg/kg produced potentiation while higher
doses produced inhibition. The corresponding antagonists (prazosin and
thymoxamine) were given s.c. and produced inhibition of 5-HT-induced
head-twitching.

Heal et al (1986) also looked at the effect of alpha1-agonists and
antagonists on 5-HT-related head-twitching in mice. In general their
results are in agreement with those of Handley and Brown (1982). Two
alpha1-agonists, methoxamine and phenylephrine (i.c.v.) produced no

effect at moderate doses (2 micrograms phenylephrine and 10 micrograms methoxamine), but inhibition of 5-MeODMT-induced head-twitching was seen at higher doses (25 micrograms methoxamine and 10 micrograms phenylephrine). They did not see potentiation with the lower dose of phenylephrine (0.625 micrograms), a possible reason for this is that different injection routes and inducers were employed for producing head-twitching in the two studies. The alpha1-antagonist prazosin (i.p.) produced no effect on 5-MeODMT at a dose level of 0.3mg/kg whereas a higher dose (3mg/kg i.p.) markedly reduced this behaviour. The 0.3mg/kg dose is at the bottom of the dose range tested by Handley and Brown (1982), their ID50 for prazosin inhibition of 5-HT-induced head-twitching being approximately 1mg/kg.

In contrast, Matthews and Smith (1980) reported that WB401 showed no effect on 5-HTP-induced wet-dog shakes in rats. This discrepancy may be due to a lower selectivity of this agent for alpha1-adrenoceptor compared with the drugs used by Handley and Brown (1982) (Massingham et al 1981).

It has been reported that beta1- (metoprolol; Ablad et al 1973) and beta2-selective antagonists (ICI 118,551; O'Donnell and Wanstall 1980) have no effect on head-twitching in mice induced by 5-HTP (Handley and Singh 1986c) and 5-MeODMT (Singh and Handley 1987). These antagonists have been demonstrated by binding studies to have negligible affinity for 5-HT2 receptors (Green et al 1983). Other (non-selective) beta-antagonists have also been shown to have no effect on 5-HT-related shaking behaviour. Thus (-) propranolol has no effect on 5-HTP-induced head-twitching in mice (Goodwin and Green 1985), 5-HTP-induced WDS behaviour in rats (Bedard and Pycock 1977), 5-MeODMT-induced head-twitching in mice (Heal et al 1986). (±)Propranolol has no effect on quipazine-induced head-shaking in rats (Eison et al 1988).

In contrast, Weinstock et al (1977) reported that 5-HTP-induced head-twitching in mice was inhibited by a variety of non-selective beta-antagonists ((±)pindolol, (±)propranolol and (±)oxprenolol)). Martin et al (1986) reported that DL-propranolol and L-penbutolol inhibited

5-HTP-induced head-twitching in mice but caused potentiation of head-twitches produced by 5-MeODMT. Yocca et al (1990) reported that quipazine-induced head shake behaviour in rats was inhibited by (±)pindolol. Interestingly, there are reports that the non-selective beta-antagonists, unlike the selective ones, have shown affinity in binding studies for 5-HT receptors. In older studies such as that of Green et al (1983) it was suggested that such agents ((-)-alprenolol and (-)-propranolol) had affinity for 5-HT2 receptors. They found that these compounds inhibited [3H]-spiperone binding with a similar micromolar IC50 value to 5-HT. Methysergide and pirenperone, compounds with 5-HT2 antagonist activity, had IC50 values in the low nanomolar range. Middlemiss et al (1977) looked at the inhibition of [3H]-5-HT binding by a selection of beta-adrenoceptor antagonists ((-)-propranolol, (±)propranolol, (±)alprenolol, (±)oxprenolol and (±)pindolol) and found that they all were potent inhibitors of this binding (IC50's in the micromolar range) indicating substantial affinity for the 5-HT1 receptor. More recent studies (such as Hoyer 1988a) using more selective radioligands ([3H]-8-OH-DPAT for 5-HT1a binding, [125I]-(±)pindolol for 5-HT1b binding and [3H]-ketanserin for 5-HT2 binding) indicate that it is the 5-HT1a and 5-HT1b receptor subtypes for which the non-selective beta-antagonists such as (±)pindolol and (-)propranolol show greatest binding affinity with very little affinity for the 5-HT2 site. Spiperone has been shown to bind with high affinity to 5-HT1a receptors (Pedigo et al 1981; Engel et al 1986 & Hamon et al 1986) in addition to 5-HT2 and dopamine2-receptors (Leysen et al 1978) which puts a different interpretation on the binding data of Green et al (1983). It is possible that it was the 5-HT1a binding affinity of these agents which was responsible for the binding seen by Green et al (1983).

It is difficult to say what component of the pharmacology of the non-selective beta-adrenoceptor antagonists is responsible for their activity in modulating 5-HT-related shaking behaviour, although the lack of effect of the selective beta-antagonists on this behaviour perhaps points to the 5-HT-component rather than the beta-adrenoceptor antagonistic component. The lack of affinity for the 5-HT2 receptor

seen, in recent binding studies, would indicate the effect is not mediated via an interaction with this 5-HT receptor. It seems more likely that it is interaction with the 5-HT1a and/or 5-HT1b receptors where high affinity binding has been reported. Whether these beta-adrenoceptor antagonists would be acting as 5-HT1a/5-HT1b antagonists, or partial agonists, in this interaction with this 5-HT2-mediated behaviour, is a matter of debate as authors have reported both partial agonist and antagonist activity for these compounds. Thus for example, Maura et al (1987) looking at the effect of (-)propranolol, (+)propranolol and (±)pindolol on the release of (3H]-5-HT from hippocampal synaptosomes and suggested that (-)propranolol and (±)pindolol behaved in this model as mixed agonist-antagonists at the 5-HT1b autoreceptor. Divo and Maayani (unpublished results - see Yocca et al 1990) found that (±)pindolol weakly inhibited the forskolin stimulation of rat hippocampal adenyl cyclase. They suggested that this was indicative of partial agonist activity of this agent at the 5-HT1a receptor. There have, however, been reports, both behavioural and biochemical (Oksenberg and Peroutka 1988 & Tricklebank et al 1985a) that in contrast to racemic (±)pindolol, (-)pindolol shows full antagonist activity at 5-HT1a receptor, which might suggest that the differences observed between authors in the modulatory effect of the non-selective beta-antagonists on the 5-HT-related head-twitch might have been a product of the choice of beta-antagonist used in a particular study. This is unlikely, however, since, some of the studies where no effect was seen ((±)propranolol - Eison et al 1988) used the same antagonist as those which found inhibition ((±)propranolol - Weinstock et al 1977)).

There is a variety of behavioural evidence which suggests that the · non-selective beta-antagonists act as 5-HT1a antagonists (Gardner and Guy 1983; Middlemiss 1984b; Tricklebank 1984a&b; Goodwin and Green 1985 & see Green and Heal 1985). Also propranolol blocks the 5-HT1a-mediated inhibition of firing of dorsal raphe neurones (Sprouse and Aghajanian 1986).

Comparison of the doses used (of the same drug) between those studies
where an effect of beta-adrenoceptor on 5-HT-related shaking was seen
and those where there was no effect indicates that, in general, where
no effect was seen much higher doses of the beta-adrenoceptor
antagonists were used, it is difficult to say whether this observation
is of any significance, especially, since the different studies
utilise different 5-HT-related inducing agents or the same one in
different doses, and different methods of quantitating the data e.g.
Weinstock et al (1977) used an incidence measure (proportion of
animals exhibiting any shake behaviour at all) whereas the other
studies used a frequency measure (number of shakes per unit time) (see
Introduction section 10 for a discussion of these different methods).
In addition, differing experimental conditions and protocol i.e.
differing pretreatment times, injection routes etc. may also be
involved in the differing results achieved by different groups for the
effect of non-selective beta-antagonists on 5-HT-related shaking
behaviour.

Beta2-adrenoceptor agonists potentiate the 5-HTP-induced head-twitch
response in mice (Delini-Stula et al 1979; Ortmann et al 1981;
Nimgaonkar et al 1983; Handley and Singh 1986b&C; Heal et al 1986 &
Martin et al 1986). Beta1-adrenoceptor agonists also potentiate 5-HTP-
induced head-twitching (Handley and Singh 1986b&c).

Beta2-adrenoceptor agonists have been reported to produce both
potentiation and inhibition with other 5-HT-related inducers. Thus
both Heal et al (1986) and Martin et al (1986) found beta2-agonist-
induced inhibition of 5-MeODMT-induced head-twitching (in mice),
whereas Singh and Handley (1987) reported beta2-agonist-induced
potentiation of 5-MeODMT-induced head-twitching (in mice). Using
quipazine as the head-twitch inducing agent, Heal et al (1986) have
reported beta2-agonist-induced inhibition in mice whereas Handley and
Singh (1986) reported beta2-agonist potentiation of quipazine-induced
WDS in rats.

Beta1-agonists have been reported to induce potentiation of both 5-HTP- (Handley and Singh 1986c) and quipazine- (Handley and Singh 1986a) induced head-twitching in mice. Interestingly, Handley and Singh (1986c) looked at the effect of selective beta1- and beta2-antagonists on the potentiation of 5-HTP-induced head-twitching by beta1- and beta2-agonists and they found that the potentiatory effect of beta1-agonists could be blocked by selective beta1-antagonists but not beta2-antagonists, whereas beta2-agonist-induced potentiation of 5-HTP-induced head-twitching was blocked by both beta1- and beta2-selective antagonists. They interpreted these effects as indicating that the potentiatory effect of beta2-adrenoceptor activation is exerted ultimately by releasing noradrenaline onto beta1-adrenoceptors. A possibility that is not without support, since, as was previously mentioned, receptors of the beta2-subtype have been found on noradrenergic terminals and appear to be involved in facilitation of noradrenaline release (Westfall 1977; Dhalof et al 1981 & see Misu and Kubo, 1983).

This possibility was further investigated by Handley and Singh (1986a) using biochemically verified bilateral lesions of the locus coeruleus (L.C.). Beta1-agonist-induced potentiation of quipazine-induced WDS in rats was enhanced by such lesions, an effect typical of the up-regulation shown by postsynaptic beta1-adrenoceptors when deprived of their agonist (Minneman et al 1979a). In contrast, beta2-induced potentiation of quipazine-induced WDS was prevented by the L.C. lesion, suggesting a presynaptic location for the relevant beta2-adrenoceptors.

In addition to their findings with respect to the effect of L.C. lesions on beta1- and beta2-agonist potentiation of quipazine-induced WDS, Handley and Singh (1986a) also reported that the L.C. lesions in their own right significantly reduced the frequency of quipazine-induced WDS. These lesions were found to produce a 90% depletion of forebrain NA. Although, as previously mentioned, alpha1-adrenoceptor activity appears to be essential for head-twitching to occur in mice (Handley and Brown 1982), work with NA depletion has failed to detect

any inhibitory effect of reserpine (Nakamura and Fukushima 1978), intraventricular 6-OHDA (Bednarczyk and Vetulani 1978 and Heal et al 1986) or dopamine beta-hydroxylase inhibition with FLA-63 (Singh et al 1986) on 5-HT-mediated head-twitching. It might be suggested that only minimal noradrenaline release is necessary onto alpha1-adrenoceptors at critical sites and whatever the receptor involved, the reduction in twitch-rate in lesioned rats suggests a net tonic facilitatory role for noradrenergic L.C. neurones under the conditions of this work.

In contrast, Heal et al (1986) reported that lesioning of central NA neurones with 6-OHDA or DSP-4 resulted in enhanced 5-MeODMT-induced head-twitch behaviour and that alpha2-agonists inhibit and alpha2-antagonists potentiate such behaviour. On the basis of these results they suggested that the 5-HT-related head-twitch is normally under tonic inhibitory noradrenergic control. They proposed that alpha2-adrenoceptors control the head-twitch response, that lesioning removes this tonic inhibitory control and this results in the enhancement of the 5-HT2-mediated behaviour.

As mentioned above, in addition to Heal et al (1986), other authors have examined the effect of NA depletion on 5-HT-related head-twitching and failed to find an inhibitory effect of such treatments (Bednarczyk and Vetulani 1978; Nakamura and Fukushima 1978; Heal et al 1986; Singh et al 1986 & Eison et al 1988). With the exception of Bednarczyk and Vetulani (1978), all these authors reported that NA depletion led to potentiation of 5-HT-related head-twitching. This effect may be mediated by beta-receptors, as Eison et al (1988) found that on lesioning of central NA neurones with DSP-4, no effect was seen on either quipazine-induced head-twitching or beta-receptor number in rats 3 days after DSP-4 treatment, whereas 10 days after the treatment an enhancement of quipazine-head-twitching and an increase in beta-adrenoceptor was present. In the case of Singh et al (1986) who found that NA-depletion by FLA-63 produced potentiation of 5-MeODMT-induced head-twitching in mice, it is unlikely that the effect is associated with an increase in beta-adrenoceptor number, as the time scale is too short (4hr). Since the potentiation produced by FLA-

63 could be blocked by the selective beta1-adrenoceptor antagonist, metoprolol (Ablad et al 1973), it was postulated that the potentiation was mediated via beta-adrenoceptors, possibly by changes in the degree of coupling of the beta-adrenoceptor to the regulatory sub-unit of adenylate cyclase (see Harden 1983).

The above results, although often conflicting, indicate that NA has a major role in the mediation and modulation of 5-HT-related shaking behaviour in mice and rats.

17.2 Dopamine.

Drugs such as methylamphetamine, amphetamine and apomorphine which stimulate dopamine receptors directly or indirectly attenuate 5-HT-mediated head-twitching in mice (Corne et al 1963) and WDS in the rat (Bedard and Pycock 1977). Neuroleptics also block these behaviours in both rats and mice (Corne et al 1963; Corne and Pickering 1967; Maj et al 1978 & Matthews and Smith 1980). Arnt et al (1984) looked at the correlation between the antagonist effect of 26 neuroleptic drugs on the 5-HTP (plus citalopram)-induced head-shake in rats. These authors found that there was no correlation between dopamine-D2 receptor affinity of these compounds and their inhibitory potency on 5-HTP-induced head-shaking. The inhibitory potency of the neuroleptics tested was closely correlated with their 5-HT2 receptor affinity. There was a slightly weaker correlation to alpha1-adrenoceptor affinity. The role of dopamine in modulating 5-HT-mediated shaking behaviour is thus unclear.

18. Migraine.

Migraine has been defined by the research group of the World
Federation of Neurology as : "A familial disorder characterised by
recurrent attacks of headache widely variable in intensity, frequency
and duration. The attacks are commonly unilateral and are usually
associated with anorexia, nausea and vomiting. In some cases they are
preceded by, or associated with, neurological and mood disturbances.
All of the above characteristics are not necessarily present in each
attack nor in each patient". This rather wide definition can be
usefully subdivided into specific forms of migraine differing in their
clinical presentation (Lance 1982) :

(A) In 'Classical' migraine, an episode of visual (e.g. fortification
spectra) or neurological disturbance precedes the headache by a period
of time varying from minutes to an hour or more. Occasionally the
headache and neurological disturbance occur together (migraine
accompagnee). The period of neurological or visual disturbance prior
to the headache is called the prodrome. In some patients the prodrome
may be preceded by a period lasting a few hours to a day during which
mild psychological or autonomic symptoms may be experienced e.g. mood
changes (commonly a feeling of elation and hyperactivity), thirst,
increased appetite (craving for sweet foods), drowsiness - the
premonitory period (Lance 1982). The terms above are not always used
consistently; some authors refer to the neurological disturbance as
'aura' and the preceding disturbances as the 'prodrome' (Blau 1980).

(B) 'Common' migraine comprises the features of 'classical' migraine
but without the neurological or visual component. The headache is
unilateral or bilateral and is usually severe. There is
hypersensitivity to light and sound, acccompanied by nausea and often
vomiting.

18.1 The role of food.

It is not uncommon for migraineurs to relate their attacks to certain foods or drinks, most commonly chocolate, dairy produce, citrus fruit, fried foods, tea, coffee or alcohol (Waters 1974). Tyramine is a constituent of a number of these precipitating agents (cheese, citrus fruit, red wine) and oral tyramine has been shown to produce headaches more frequently in dietary migraine sufferers than in non-migrainous subjects (Hannington and Harper 1968). However, other controlled studies have failed to demonstrate a significant increase in migraines (Forsythe and Redmond 1974 & Boisen et al 1978). Phenylethylamine (PEA) may be the agent responsible in chocolate which does not contain tyramine. Patients with chocolate-induced migraine frequently have headaches after administration of PEA (Sandler et al 1974). Both tyramine and PEA are vasoactive amines which may act directly on the cranial vasculature to initiate migraine. Alternatively they may exert their effect by altering NA metabolism or causing the release of 5-HT from platelets. Patients with dietary migraine have been shown to exhibit abnormal responses to vasoactive amines between attacks, suggesting that they might be abnormally sensitive to dietary factors (Ghose et al 1977 & Muck-Seler et al 1979).

Although there are clinical and laboratory reasons for implicating food as an important precipitant of migraine attacks, there have been few adequately controlled trials of elimination diets. However, an impressive double blind trial of oligoantigenic diet has demonstrated recovery in 98% of 88 subjects (Egger et al 1983). Children with severe migraine were studied and the findings may not be applicable to adults or subjects with milder forms of migraine.

18.2 Migraine Treatments.

The pharmacotherapeutic regimen for migraine can be separated into agents used for acute attack and those employed in the prophylaxis of migraine for patients experiencing two or more serious attacks a month.

<u>18.2a</u> <u>Acute</u> <u>treatments.</u>

The vast majority of mild to moderate migraine attacks can be treated with analgesics such as paracetamol, paracetamol and codeine etc. Aspirin has been used in migraine for almost 100 years and remains the drug used most frequently in abortive therapy (Peatfield et al 1986). Other non-steroidal anti-inflammatory drugs (NSAIDs) are also effective. It is not clear if the analgesic and/or anti-inflammatory effects of these drugs are the primary basis for their effects in migraine (see discussion in Chapter 1 for further details).

<u>Ergots.</u>

Extensive literature has arisen in the last sixty years regarding the effectiveness of ergots in the acute treatment of migraine headache (Rothlin 1955). Indeed, ergot compounds are the most common class of agents used in the acute treatment of severe migraine. Taking dihydroergotamine as an example, studies indicate that this agent is effective in approximately 70% of patients (Callaham and Raskin 1986 & Raskin 1986). However, ergot compounds have significant side efects e.g. nausea, which frequently exacerbates the underlying nausea and vomiting of a migraine attack. Chronic use (and abuse) of ergots may be associated with vasoconstrictor disorders (ergotism).

The pharmacology of ergots will be covered in greater depth in the discussion of Chapter 1, however, a binding profile of a representative compound of this group is presented below in table 0.1.

The mechanism of action of these compounds in relieving acute migraine attacks is unknown.

Table 0.1

Dihydroergotamine and sumatriptan interactions with neurotransmitter
receptor sites.

Receptor   Ki values (nM)

Taken from Peroutka (1990).

5-HT3-receptor antagonists.

At present there is little published data on the effectiveness of 5-
HT3-antagonists in treating migraine. Loisy et al (1985) found that
MDL 72,222 could abort acute attacks. The mechanism of action of these
compounds in treatment of acute migraine attack is unknown. Although
the 5-HT3 receptor has been implicated in mediating the painful effect
of 5-HT in humans e.g. the pain produced when 5-HT is applied to a
blister on the human forearm can be inhibited by low concentrations of
ICS 205-930 (another 5-HT3 antagonist) (10nM) in an apparently
competitive manner whereas methysergide, a 5-HT1 and 5-HT2 receptor

antagonist, produces no such inhibitory effect (Richardson et al 1985).

According to Blau (1978), headache results from activation of afferent sensory nerves in the walls and perivascular areas of cranial microvasculature, presumably by substances released during the hypothetical "sterile inflammatory reaction" (Dallessio 1978). Fozard (1982) proposed that 5-HT, which is present in the cerebral microvessels (Reinhard et al 1979), would almost certainly be involved and would, by activating 5-HT3 receptors on pain transmitting fibres, generate pain (Keele and Armstrong 1964). Also the presence of 5-HT would sensitize the nerves to other nociceptive stimuli, such as kinins, inorganic cations, and possibly the mechanical stimulation arising from pulsatile flow (Sicuteri et al 1965). Fozard (1982) observed that the 5-HT3 receptor antagonists might be expected to block the 5-HT component of the sensory afferent neuronal stimulant response and diminish the pain of migraine.

Sumatriptan.

Recently, sumatriptan (formerly GR 43175) has been found to be extremely effective in the acute treatment of migraine. Doenicke et al (1988) reported that 2mg sumatriptan i.v. completely abolished headache in 71% of 24 migraine attacks and significantly reduced headache in the remaining patients. Only minor side-effects (transient pressure in the head, feeling of warmth or tingling) were observed. Although the mechanism of action of sumatriptan in migraine remains unclear, it has been suggested that the drug may selectively stimulate a subpopulation of 5-HT receptors (5-HT1-like receptors) (Humphrey et al 1988). Peroutka (1990) has suggested that it may be the affinity for the 5-HT1d receptor of sumatriptan which is significant with respect to the mechanism of action of this agent as an anti-migraine drug.

18.2b Prophylactic Treatments.

5-HT-receptor antagonists.

5-HT antagonists such as methysergide represent the first class of drugs that were shown to be effective in migraine prophylaxis (Sicuteri 1959). The three main 5-HT antagonists used in migraine prophylaxis are cyproheptadine, methysergide and pitzotifen. These drugs have complex effects on 5-HT and other neurotransmitter systems but share the ability to potently block 5-HT2 receptors (see Peroutka 1990). Binding studies indicate these compounds have high affinity for the 5-HT2 & 5-HT1c receptor, with lower affinity for 5-HT1a and 5-HT1b receptors (see Hoyer 1988a). The mechanism of action of these agents in treating migraine is unknown and a comparison of the binding profile of the 5-HT antagonists and other anti-migraine drugs led Peroutka (1988) to suggest that antimigraine efficacy cannot be explained by drug interactions with a single 5-HT receptor subtype. Indeed, a role in the pathogenesis of migraine has been ascribed for several of the 5-HT receptor subtypes - 5-HT1a receptors (Hiner et al 1986), 5-HT2 receptors (Fozard 1982 and Peatfield et al 1986) and 5-HT1c receptors (Fozard and Gray 1989).

Beta-adrenoceptor antagonists.

Table 0.2

Effectiveness of various beta-adrenoceptor antagonists in migraine prophylaxis.
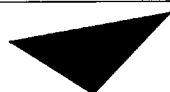
Aston University

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Taken from Weerasuriya et al (1982).

A review of multiple clinical studies shows that approximately 50-70% of patients will derive some benefit from prophylactic propranolol therapy (Peatfield et al 1986). A variety of other beta-adrenoceptor antagonists have been used in treatment of migraine. Atenolol, metoprolol, nadolol and timolol appear to be at least as effective as propranolol in migraine prophylaxis. More variable results have been obtained with pindolol (Antony et al 1972; Ekborn and Lundberg 1972 & Sjaastad and Stensrud 1972). By contrast a number of other beta-adrenergic antagonists e.g. acebutolol, oxprenolol and alprenolol do not appear to be effective in migraine therapy. From the pattern of activity in migraine prophylaxis of beta-adrenoceptor antagonists (see Table 0.2), it might be suggested that only 'pure' beta-adrenoceptor antagonists (without intrinsic sympathomimetic activity) are effective agents in migraine prophylaxis. This is interesting as tyramine and

PEA two agents suggested to act as migraine triggers possess sympathomimetic activity.

Some of the beta-adrenoceptor antagonists that are active migraine prophylactic agents do interact with 5-HT receptors, principally the 5-HT1a and 5-HT1b subtypes (they have, in general, similar affinities for these two receptors e.g. (±) pindolol has pKD of 8.27 for 5-HT1a and 8.28 for 5-HT1b receptors, (-)propranolol has pKD of 6.81 for 5-HT1a and 7.33 for 5-HT1b (Hoyer 1988a). However, the beta-adrenoceptor antagonists vary widely in their affinity for these receptors e.g. in the case of affinity for the 5-HT1a receptor, pindolol is the most potent drug studied with a Ki value of 4.5nM. Alprenolol and propranolol are 21- and 36-fold, respectively, less potent than pindolol at this receptor, while atenolol is inactive at concentrations below 1000nm (Peroutka 1990). It is difficult to see how the activity shown by some of the beta-adrenoceptor antagonists at 5-HT receptors is responsible for their anti-migraine actions, as those beta-adrenoceptor blockers with ISA e.g. oxprenolol, alprenolol and acebutolol show as potent an affinity for the 5-HT1 receptor as those without this activity e.g. propranolol, but are ineffective in migraine prophylaxis (see Middlemiss et al 1977; Weerasuriya et al 1982; Green et al 1983 & Nahorski and Willcocks 1983). In addition, there are several beta-adrenoceptor blockers with effectively no affinity for 5-HT1 receptors which are effective prophylactic anti-migraine agents e.g. metoprolol and atenolol (see Weerasuriya et al 1982; Green et al 1983 & Peroutka 1990).

As a general statement, the mechanism of action of beta-adrenoceptor antagonist drugs in migraine is at present unknown. It seems unlikely that it is derived solely from antagonism of the 5-HT receptor.

Calcium antagonists.

Beginning in 1981, a variety of clinical studies have demonstrated that calcium antagonists may be effective in migraine prophylaxis. More recent data, however, suggest that these drugs might not be as

efficacious as initially reported (e.g. Albers et al 1989). Although, a preliminary report (which was not double-blind) of the effectiveness of sublingual formulation of the calcium antagonist, flunarizine in the treatment of acute migraine attacks suggests that as well as being effective as prophylactic anti-migraine agents, the calcium antagonists may possibly have a role in the treatment of acute attacks (Takeshima et al 1988).

Of the calcium antagonists available at present, the following have been demonstrated to have prophylactic action in migraine : verapamil, nimodipine, nifedipine, flunarazine, cinnarazine, diltiazem , cyproheptadine and amitriptyline (see Meyer and Hardenberg 1983; Peroutka 1983 & Peroutka et al 1984). The latter two also have a variety of other properties which have been linked with their anti-migraine properties.

Previously the anti-migraine efficacy of the calcium antagonists was attributed to their protective effects during anoxia (Amery 1982) and/or their ability to block intracranial vasoconstriction, irrespective of the constricting agent (Peroutka 1983). However, more recently certain calcium antagonists (e.g verapamil (Adachi and Shoji 1986; Defeudis 1987; Peroutka 1988 & Green et al 1990) & nicardipine (Green et al 1990)) have been shown to possess affinity (in the micromolar range) for the 5-HT2 receptor. Other calcium antagonists have little or no affinity (nitrendipine (Cohen et al 1986)) for the 5-HT2 receptor. In the case of nifedipine and diltiazem differing results have been observed by different authors, thus Peroutka (1988) found high affinity binding of nifedipine (Ki of 0.3mM) at the 5-HT2 binding site, whereas Adachi and Shoji (1986) a 100-fold lower affinity of Ki 29 mM, which they suggest indicates that nifedipine has practically no ability to inhibit ligand binding to 5-HT2 receptors i.e. nifedipine has no affinity for said receptors; a finding in agreement with the results of Green et al (1990). In the case of diltiazem, Cohen et al (1986) found a Ki value binding to the 5-HT2 receptor of > 10,000nM suggesting that diltiazem does not bind to 5-

HT2 receptors whereas Peroutka (1988) found a Ki value of 2,400nM suggesting some affinity of this agent for the 5-HT2 site.

As far as the significance of the 5-HT2 receptor binding affinity of these agents to their anti-migraine activity is concerned, it seems unlikely that their anti-migraine activity is derived solely from this antagonism of 5-HT2 sites as not all of the calcium antagonists that are effective migraine prophylactic agents share this ability to bind at 5-HT2 receptors.

Other Agents.

A variety of other medications have been reported to be effective in migraine prophylaxis including clonidine, the alpha2-adrenoceptor agonist, especially in preventing dietary migraine (Wilkinson et al 1971), although the BNF No. 19 (March 1990) suggests that clonidine may be "little better than placebo" in migraine prophylaxis; tricyclic antidepressants e.g. amitriptyline (Peatfield et al 1986), its principal pharmacological property is generally considered to be blockade of monoamine reuptake with claimed selectivity towards 5-HT (it has been shown to bind with high affinity to 5-HT2 receptors with low affinity for 5-HT1 receptors (Peroutka 1988)), it also has significant antagonist action at cholinergic, alpha-adrenergic and histamine receptors; chlorpromazine (Peroutka 1990), a wide variety of pharmacological actions including dopamine receptor blockade, alpha-adrenoceptor blockade, muscarinic cholinergic receptor blockade, it can also inhibit monoamine reuptake and is a powerful 5-HT2 antagonist (see Fozard 1982); some monoamine oxidase inhibitors such as phenelzine may be of value in migraine prophylaxis (Anthony and Lance 1969). Interestingly, indoramin (an alpha-adrenoceptor antagonist and vasodilator which, in larger doses, also blocks NA reuptake and exerts antihistamine and local anaesthetic activity) has been shown to reduce the frequency of migraine attacks in some patients and to inhibit the precipitation of migraine attacks by i.v. tyramine (Bowman and Rand 1980).

18.2c Precipitating Agents.

As has been previously mentioned dietary factors (tyramine, PEA) may trigger migraine attacks. Disulfiram (a dopamine beta-hydroxylase inhibitor which reduces central NA levels (Goldstein et al 1964 & Musacchio et al 1964; 1966)) has been shown to increase migraine frequency, an effect abolished by beta-adrenoceptor antagonists (Ghose and Carroll 1984). This group also found that propranolol, a beta-adrenoceptor antagonist used in migraine prophylaxis (see Weerasuriya et al 1982) reduced the number of migraine attacks after tyramine administration (Ghose and Carroll 1984). Drugs that release 5-HT from tissue stores are known to precipitate or exacerbate migraine such as reserpine (Nappi et al 1979) (this agent will also deplete other monoamines e.g. NA (Dahlström and Häggendal 1966)), fenfluramine (Sicuteri et al 1976), viloxazine (Barnes et al 1979) and zimeldine (Syralahti et al 1979). Interestingly, in the case of reserpine-induced headaches, they can be prevented by pretreatment with the non-selective 5-HT antagonist methysergide which is used in migraine prophylaxis (Carroll and Hilton 1974).

19 A theory of migraine pathogenesis.

Migraine has long been viewed as a primarily vascular disease, neurological disturbances being secondary to ischaemia. Recent evidence has, however, resulted in a reverse hypothesis i.e. that neuronal abnormalities could lead to the observed vascular changes, since cerebral NA and 5-HT pathways have been shown to affect the cranial circulation. The known pathology of these pathways suggests that abnormalities of these pathways could also lead directly to certain perceptual changes, or augment them when caused by vascular effects.

<u>19.1 A 'neural' hypothesis of migraine.</u>

The hypothesis that migraine is primarily a vascular disease is open
to question, not least because of inconsistencies observed between the
distribution of symptoms and changes in regional blood flow (Pearce
1984 & Olesen 1985). Moreover, it is now known that cerebral bloodflow
is unchanged in common migraine (Olesen 1985). The possibility that
the observed vascular changes could be secondary to neural effects of
central origin has recently received more attention (Lance et al 1983
& Pearce 1984), and it is possible, that certain perceptual changes
which can accompany migraine could be neural in origin (see below).

It is now known that noradrenergic and serotonergic neurones of
central origin can affect the cerebral microcirculation.

<u>NA.</u>

Cerebral blood vessels are richly innervated by NA neurones whose cell
bodies are located within the superior cervical ganglia (Edvinsson et
al 1978) and in the locus coeruleus (L.C.) and related cell groups in
the lower brain stem (Rennels and Nelson 1975; de la Torre 1976 &
Edvinsson 1982). NA, whether applied directly or released by tyramine
or nerve stimulation, produces vasoconstriction of cerebral arteries
leading to reduction in local blood flow; these contractile responses
are mediated by alpha-adrenoceptors; beta-adrenoceptor-mediated
vasodilation of isolated brain vessels has also been demonstrated
(Edvinsson 1982). Interestingly, a recent detailed study has shown
that electrical stimulation of the L.C. results in ipsilateral
intracranial vasoconstriction at low frequencies while extracranial
blood vessels are dilated in a frequency-dependent manner (Lance et al
1983).

<u>5-HT.</u>

Direct immunohistological evidence for 5-HT in perivascular cerebral
nerves has been reported (Griffith et al 1982 & Griffith and Burnstock

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1983). It seems likely that the neurones containing 5-HT arise in the mid-brain raphe nuclei (n. raphe dorsalis and n. raphe medianus) and project to microvessels within the brain (Chan-Palay 1976; 1977; Reinhard et al 1979; Napoleone et al 1982). Reports of the action of 5-HT on cerebral vessels are conflicting. 5-HT has been reported to constrict large cerebral arteries of the cat (Lee et al 1978) but dilate arterioles (Pickard et al 1978). It has been suggested that 5-HT from nerves acts on smooth muscle directly to produce vasoconstriction, while 5-HT released from platelets and possibly endothelial cells acts via EDRF to produce vasodilation (Vanhoutte 1984).

These two monoamine systems (NA and 5-HT) are closely interrelated and have reciprocal neural connections (Foote et al 1983 & Jacobs 1983; 1985). They are thus capable of modifying each other's activity. In the case of fibres from L.C. to n. raphe dorsalis, a tonic input activating alpha-adrenoceptors has been identified which appears to be necessary to maintain firing of the 5-HT neurones (Baraban and Aghajanian 1980).

Lance et al (1983) have proposed that changes in the activity of these systems could be involved in the vascular changes of migraine. In addition they have suggested that the pain of migraine could be due to a disruption of descending pain control pathways in which both 5-HT and NA participate. In order to incorporate the latter, they invoke the possibility that there is 'fatigue' of L.C., this nucleus becoming inactive during the headache phase of migraine. The descending 5-HT systems apparently control only noxious input but the descending L.C. neurones modulate non-noxious signals as well (Hodge et al 1981).

19.2 Perceptual changes in migraine in relation to monoamine systems.

A traditional view that perceptual disturbances in migraine are due solely to cerebral ischaemia or the controversial 'spreading depression' has also been questioned (Pearce 1984). If, as proposed by the neural hypothesis of migraine, the vascular changes are due to

abnormalities in central NA and 5-HT pathways, it is unlikely that these would exist in isolation. The majority of L.C. neurones project to more than one brain area (Foote et al 1983), so that more generalised disturbances may exist which could give rise to perceptual changes directly and augment effects of vascular origin.

Both NA and 5-HT have been implicated in the control of sensory information processing. Both are able to enhance neuronal responses to sensory input, despite a tendency to inhibit spontaneous firing (Foote et al 1983 & Jacobs 1983; 1985). The NA effects particularly involve L.C. which has been suggested to produce a state of "quiet readiness to respond" and improved signal/noise ratio in central neurones (see Madison & Nicoll 1982 & Foote et al 1983). Both alpha- and beta-adrenoceptors have been implicated and the beta-adrenoceptor effects involve postsynaptic modulation of GABA-mediated inhibition (see Foote et al 1983). Sensory effects of 5-HT are, perhaps, less well understood but Jacobs (1983; 1985) has reviewed the mechanism by which activation of postsynaptic 5-HT receptors can give rise to the perceptual disturbances caused by hallucinogens.

In addition to perceptual disturbances during an attack, migraineurs show several other interesting differences such as low pain threshold (Dalessio 1980) and intolerance of noise and bright lights (Debney and Harding 1971). They are also more likely to be 'augmentors' (Klein 1983) i.e they overestimate the intensity of stimuli, perhaps in an attempt to reduce the effects of incoming sensory stimuli (Sicuteri 1983). Abnormalities of visual evoked potentials have also been recorded (Gawel et al 1983). Such findings have led to proposals that migraine sufferers may have a deficiency of some 'sensation modulating substance' (Sicuteri et al 1978) or other transmitter abnormality (Gawel et al 1983). It may be important that these effects could also be predicted from overactive NA or 5-HT systems as described above. In line with this, migraineurs have also been shown to be significantly more sensitive than normal to the induction of perceptual distortions by the hallucinogens, LSD and pentazocine (Sicuteri 1983). LSD at the doses used is a 5-HT agonist (see Jacobs 1983; 1985). Interestingly,

induction of tolerance to the effects of LSD coincides with a reduction in migraine attacks. The L.C. and raphe nuclei thus have three identified functions which may be relevant to migraine, viz constriction of vessels of the cerebral microcirculation, enhancement of sensory input and the control of pain. The first two effects are consistent with the symptoms of migraine, particularly some aspects of the prodromal phase of classic migraine. For the last, however, a secondary reduction in L.C. firing must be postulated.

The nature of abnormalities in central monoamine pathways of migraineurs is a matter for speculation but the proposal of Willoughby (1981) might provide an answer. In seeking to account for the vegetative symptoms she postulated that amines of dietary origin or peripheral origin would gain access to the circumventricular organs which lack a functional blood-brain barrier. It is known that the L.C. also lacks a functional blood-brain barrier (Foote et al 1983 & Felten and Crutcher 1979). It may be that these substances can also reach L.C. in certain circumstances, resulting in the prodrome of classical migraine. Although speculative, this idea links peripheral and neural hypotheses and suggested that the effect of migraine triggers on L.C. function should be evaluated. This work was undertaken in the present study; a study of the effect of antimigraine agents on central 5-HT systems was also undertaken (see Chapter 3).

On the basis of the information outlined above, it became apparent that an animal model sensitive to changes in NA and 5-HT, which is capable of showing up interactions between NA and 5-HT systems, in which sensory mechanisms play a role and which is modulated by the L.C. could be invaluable in investigating a neural hypothesis.

Findings from this and other laboratories would suggest that rodent head-twitch is such a model. As previously outlined, this behaviour occurs in several species in response to 5-HT agonists and the majority of hallucinogens including LSD. Their origin appears to be in the brain stem but frequency is modulated by the higher centres. This behaviour is modulated by many other neurotransmitters including NA,

acetylcholine, GABA and opiates, as well as, perhaps dopamine (see Handley and Singh 1986b). Its relevance lies in the fact that it is also modulated by sensory input, by L.C. and by drugs which trigger or relieve migraine.

This relationship is summarised in the table below (based on work previously outlined in this Introduction, (with the exception of fenfluramine, a reference for its activity in inducing head-shaking behaviour is Joshi et al 1982)) :

Table 0.3

Correlation between the pharmacology of shaking behaviour and migraine.

Modulatory factor	effect on 5-HT-induced rodent shaking.
1) Sensory input	rodent shake is abolished by local anaesthesia of the pinna, showing that it is dependent on sensory input from this region (N.B. this is contoversial see Introduction section 11). It is reduced by novel sounds, flashing lights and isolation (Boulton and Handley 1973).
2) L.C.	bilateral 6-OHDA lesions of L.C. reduce shaking to quipazine and modify the actions of shake potentiators (Handley and Singh 1986a).

Table 0.3 continued :

3) Correspondance between drug effects in migraine and on shaking
   behaviour.
----
                             anti-migraine drugs.   shake antagonists.
                             --------------------   ------------------

alpha1-antagonists   +   +
alpha2-agonists   +   +
beta-antagonists   some   + (see *)
5-HT antagonists   +   +
antidepressants (tricyclic)   +   (chronic)
dopamine antagonists   +   +
calcium antagonists   +   +
prostaglandin inhibitors are effective against migraine (prophylactic
and acute attack) but have not been tested for their effect on shaking
behaviour.

       worsen or trigger migraine.   induce or potentiate shake response.
       ---------------------------   -------------------------------------
reserpine   +   +
other NA depletors   +   +(FLA-63)
e.g. disulfiram
sympathomimetics   +   +
(e.g. tyramine)
fenfluramine   +   +
(5-HT releasing agent)

* - when potentiated by NA depletion ± beta-agonist.

20. Scratching and grooming behaviour.

Scratching is part of the normal repertoire of behaviour of rodents. However, the form of scratching produced by peptidergic compounds that will be discussed below is not one normally seen in on-going behaviour in rodents i.e. reciprocal forepaw scratching.

Most of the work that has looked at the production of scratching and grooming behaviour in rodents has involved the assessment of the effects of various peptide compounds on on-going behaviour. These compounds include substance P and other tachykinins, bombesin and related agents, somatostatin, ACTH, melatonin, oxytocin, arginine vasopressin and arginin vasotocin.

20.1 Scratching.

One of the substances which has been investigated most thoroughly with respect to the production of scratching behaviour is substance P (and related compounds).

Substance P undecapeptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met) is unevenly distributed in the mammalian CNS (Ljungdahl et al 1978 & Kanazawa et al 1982). It is found in high concentrations in areas of brain rich in DA-containing neurones and closely associated with motor behaviour (Ljungdahl et al 1978); the substantia nigra and ventral tegmental area of the mesencephalon. It has been reported to be active neurophysiologically and behaviourally. Behavioural effects include disruption of memory, motivation, pain perception (see section 11 below) and motor activity, and prominent increases in grooming (e.g. Goldstein and Malick 1977; Frederickson et al 1978; Huston and Staubli 1978; Kelly and Iversen 1978; Rondeau et al 1978 & Strauss et al 1978).

20.1a <u>Substance P.</u>

Intraventricular (i.c.v.) (Rackham and Share 1979; in mice) and
intrathecal adminstration (i.t.) (Hylden and Wilcox 1981; Fasmer et al
1983; in mice / Rackham et al 1981; in rats) of substance P have both
been reported to produce dose-dependent reciprocal (alternating from
one hind limb to the other) forepaw scratching directed immediately
rostral to the hindlimb (at the side of the abdomen). This behviour
was not seen on peripheral administration i.e. i.p., i.v. or s.c.
(Dobry et al 1981). In addition to scratching behaviour, i.t.
substance P also produced biting or licking of the hind legs and the
lower parts of the abdomen. A detailed description of the sequence of
effects produced by substance P (i.c.v.) was given by Share and
Rackman (1981), "within 60-90 sec of injection, the mice seemed
agitated, exhibited excessive grooming, and engaged in rapid
reciprocal hindlimb scratching movements. The hindlimb scratching
directed towards the sides of the upper body and jaw areas, alternated
from one hindlimb to the other and occasionally was sufficiently
severe as to cause an animal to rock from side to side. These
responses were paroxysmal, being interspersed with periods of
exaggerated preening and biting at the abdomen and hindquarters." They
observe that of the behaviours produced by substance P, the reciprocal
scratching was the most consistent and reproducible, being present on
every occasion 2-4min after i.c.v. injection.

20.1b <u>Pharmacology of substance P-induced scratching.</u>

Peripherally administered morphine (s.c.) has been shown to inhibit
scratching induced by substance P (given i.t.), however, i.t.
administered morphine had no effect on scratching induced by i.t.
substance P. Hylden and Wilcox (1981) suggested that this was in
agreement with the idea that morphine is not a substance P receptor
antagonist (Piercey et al 1980). The action of opiates at the spinal
level is thought to be presynaptic i.e. their action is to reduce
substance P, therefore spinal morphine would not be expected to
antagonise exogenously applied substance P, whereas systemic morphine

might be expected to block the response to substance P, as was found to be the case, also the block was antagonised by naloxone. It is suggested the stimulus produced by substance P is perceived as noxious, since mice have been shown to respond similarly to subcutaneous injection of 0.1N acetic acid, an obviously noxious stimulus (Hylden and Wilcox 1981). The fact that an opiate analgesic (morphine) blocked the response supports the proposition that the stimulus is percieved as noxious.

Dobry et al (1981) looked at the effect of a variety of agents on substance P (i.c.v.)-induced scratching. They found that the effect was not antagonised by sodium cromolyn nor mimicked by i.v. or i.c.v. histamine or compound 40/80 (which releases histamine from mast cells) suggesting that histamine does not seem to be involved in mediating this response to substance P. 5-HT, NA, acetylcholine, DOPA, glutamic acid, neurotensin and bradykinin did not produce reciprocal scratching when administered i.c.v. Morphine, methadone, amphetamine, baclofen, chlorpromazine, cyproheptadine, diazepam and imipramine all antagonised the behaviour, although the ED50's of these compounds, were, in general, quite high suggesting possibly that the inhibition was related to a motor inhibitory effect (which these compounds possess) rather than a specific effect on the mechanism of mediation of substance P-induced scratching.

Dobry et al (1981) suggested that reciprocal scratching induced by i.c.v. substance P appears to be caused by a specific substance P receptor similar to receptors in other tissue. The relative potencies of substance P and its C-terminal hexapeptide and pentapeptide were found to be similar to their activities in the guinea pig ileum (Bury and Mashford 1976 & Piercey et al 1979), cat dorsal horn neurones (Piercey et al 1979 & Piercey and Einspahr 1980), rat spinal motorneurones (Otsuka and Konishi 1976; 1977), frog spinal motorneurones (Otsuka et al 1975) and blood flow in dog femoral artery and rabbit ear vein (Bury and Mashford 1976). However, while the receptors in the guinea pig ileum (Lembeck and Fischer 1967 & Piercey et al 1979) and on substantia nigra neurones (Davies and Dray 1976)

exhibit tachyphylaxis to repeated applications of substance P, the receptor for reciprocal scratching does not. There is also no tachyphylaxis for the guinea pig vas deferens (Zetler 1977) and there are mixed reports for canine blood vesels (Pernow and Rosell 1975 & Losay et al 1977).

20.1c Involvement of 5-HT in scratching behaviour.

As previously mentioned 5-HT does not induce scratching when administered i.c.v. (Dobry et al 1981). However, Fasmer et al (1983) report that if 5-HT is given i.t. a behavioural syndrome consisting of reciprocal hindlimb scratching and biting and licking of the hind legs and the lower parts of the abdomen is observed. A syndrome very similar to that which they observed when substance P was administered i.t., although substance P elicited a consistently more intense biting or licking behaviour, whereas 5-HT and 5,6-DHT produced a stronger scratching response. 5,6-DHT is a compound known to stimulate postsynaptic 5-HT receptors (Barzaghi et al 1973). The non-selective 5-HT antagonist, metergoline (binds with high affinity to 5-HT1c > 5-HT1d = 5-HT2 >> 5-HT1a binding sites (Hoyer 1988a)) completely inhibited the effect of i.t. 5-HT suggesting that the behaviour observed was produced by stimulation of 5-HT receptors. These authors did not examine whether metergoline antagonised the substance P-induced response, however, as previously mentioned Dobry et al (1981) found that the non-selective 5-HT antagonist, cyproheptadine inhibited scratching induced by i.c.v. substance P, in this case, however, the ED50 of cyproheptadine was high (10mg/kg) making it difficult to be confident that inhibition of 5-HT receptors was the mechanism for this effect (see above). Another 5-HT related compound which been reported to induce scratching is mescaline (i.c.v.) (Meisenberg 1982). This agent is a non-selective 5-HT agonist with 5-HT2 activity (Leysen et al 1982).

Interestingly, a recent study (Vaught and Scott 1988) examined the effect of 5-HT and substance P antagonists on scratching induced by i.t. administration of 5-HT and substance P. They found that that some

but not all putative substance P antagonists e.g. [D-Pro^2,D-Try^{7,9}]-substance P (DPDT) blocked both substance P- and 5-HT-induced scratching whereas methysergide, a non-selective 5-HT antagonist blocked only 5-HT-induced scratching with no effect on substance P-induced scratching. All the substance P antagonists tested blocked substance P-induced scratching. DPDT did not block bombesin-, somatostatin-, glycine- or glutamate-induced scratching. These effects are consistent with the idea that the pathways mediating these agonist behaviours may be convergent in some manner. The fact that phenoxybenzamine blocked both 5-HT- and substance P-induced scratching also supports this idea as this compound has previously been used to delineate potential differences in receptor populations (Growcott et al 1983; Lin and Mussachio 1983 & Vaught et al 1986). It was, however, noted that the block of 5-HT-induced scratching by phenoxybenzamine was considerably more rapid than that of substance P-induced behaviour suggesting differences in the behaviours mediating this particular event (Growcott et al 1983; Lin and Mussachio 1983 & Vaught et al 1986). It would be expected that if 5-HT was producing scratching, in part, via the release of substance P or vice versa, then all substance P antagonists ought to be able to block 5-HT-induced scratching and vice versa. Vaught and Scott (1988) indicate that this is not the case, thus the mechanism by which some substance P-antagonists block both substance P- and 5-HT-induced scratching is unknown.

A behavioural interaction between substance P and 5-HT is interesting, as 5-HT and substance P co-exist in some raphe-spinal neurones projecting predominantly to the ventral horn (Hökfelt et al 1975; 1978) and both appear to be involved in pain perception.

There is strong evidence implicating 5-HT in central pain modulation. Increasing 5-HT neurotransmission in raphe-spinal neurones by pharmacological or electrophysiological manipulation reduces sensitivity to noxious stimulation (Messing and Lytle 1977; Berge 1982), and injection of 5-HT into the lumbar subarachnoid space produces a behaviourally-defined analgesia in rats, cats and rabbits (Yaksh and Wilson 1979). However, dependent on dose i.t. 5-HT produces

behavioural responses indicative of stimulation of sensory pathways (i.e. nociception), in addition to scratching behaviour (Nakano and Taira 1976; Fasmer et al 1983 & Hylden and Wilcox 1983). Also, peripheral application of 5-HT is reported to cause pain in humans (Armstrong et al 1952) and sensitise nociceptors in the dog (Nakano and Taira 1976). It is also known from electrophysiological studies that 5-HT depolarises and excites afferent nerve fibres and sensory ganglion cells (Simonds and DeGroat 1980). It is thus apparent that central and peripheral administration can have different effects on pain sensitivity.

Substance P seems to be the neurotransmitter in some nociceptive primary afferent neurones (Nicoll et al 1980) and has been implicated in the modulation of nociceptive information (Henry 1980). When injected i.t., substance P, like 5-HT, causes behavioural effects indicative of activation of sensory pathways (in addition to scratching behaviour) (Frederickson et al 1978; Hylden and Wilcox 1981; Fasmer et al 1983 & Vaught et al 1984) and has been demonstrated to have biphasic effects on nociception (Frederickson et al 1978; Mohrland and Gebhart 1979 & Naranjo et al 1982).

Given the pharmacological similarities between intraspinal substance P and 5-HT as well as their similar anatomical localisation in the spinal cord, evidence of an interaction between the two systems is not surprising. In addition to the interaction with respect to the modulation of reciprocal scratching behaviour reported above, several other laboratories have suggested functional interactions (Mitchell and Fleetwood-Walker 1981; Hylden and Wilcox 1983; Tremblay et al 1986; Murphy and Zemlan 1987). A neurochemical interaction between these two agents has also been reported. Agnati et al (1980; 1983) suggested that in the mouse spinal cord "substance P can be a rapidly acting modulator of isoreceptor interconversion". The suggestion being based on the ability of substance P to reduce the affinity and increase the density of [3H]-5-HT(LSD) binding sites. This group also demonstrated that a substance P antagonist (D-Pro^2, D-Phe^7, D-Trp^9)-

substance P) could partially prevent the effects of substance P on 5-HT binding.

20.2 Involvement of 5-HT in excessive grooming behaviour.

The involvement of NA and 5-HT systems in excessive grooming induced by mild stress (two i.p. injections of physiological saline) in rats has been investigated by Rodriguez Echandia et al (1983). This group found that two non-selective 5-HT antagonists, methysergide and pitzotifen (principal high affinity binding to 5-HT2 and 5-HT1c sites > 5-HT1a binding (Hoyer 1988a)) blocked the appearance of excessive grooming. This they suggested was not due to depression of general behaviour as the 5-HT antagonists did not affect locomotion. These results indicate that 5-HT systems are involved in the mediation of grooming behaviour response to mild stressors. Haloperidol only blocked the grooming behaviour at doses which decreased locomotion, suggesting that its effect on grooming was mediated via an overall depressive effect on behaviour. High doses of phentolamine and l-propranolol had no effect on the excessive grooming response suggesting that alpha- and beta-adrenoceptors are not involved in mediating this response. A role for NA systems in the production of this behaviour thus seems unlikely.

Aim of the project.

The initial aim of this project was to examine the effects of migraine-related agents (both migraine triggers and antimigraine drugs) on 5-HT-related head-twitching to extend our knowledge with respect to the parallels between head-twitch behaviour and migraine. This work is covered in Chapter 1.

During the project, an agonist with high selectivity for 5-HT2 in comparison with 5-HT1 receptors was described (Glennon et al 1986) and became available for study, (±)DOI. As related in the Introduction, such an agent might be expected to produce 5-HT2 receptor mediated shaking behaviour, but not the 5-HT1a/b receptor mediated "5-HT

syndrome" unlike other non-selective shake-inducing agents such as 5-MeODMT. Its selectivity would be valuable in pinpointing interactions between 5-HT2 and other neurotransmitter systems. It was therefore decided to look at the effects of this compound on ongoing behaviour to determine if (±)DOI did induce shaking behaviour and/or any other behaviours and to investigate any such behaviours produced. In terms of shaking behaviour, experiments were carried out to determine whether this behaviour was modulated by neurotransmitter systems, such as NA systems, in a similar manner to other shake inducers e.g. 5-MeODMT.

More recent binding data has suggested that this compound is not as specific for 5-HT2 receptors as was initially thought, having high affinity for 5-HT1c receptors (Hoyer 1988b) and the 'DOB binding site' (Pierce and Peroutka 1989b) which must therefore be taken into account when interpreting results produced by this compound.

The final section of this thesis concerns the modulation of a variety of shake-inducing agents i.e. (±)DOI, 5-HTP, 5-MeODMT and quipazine by NA-related compounds. The work was done to highlight any differences in modulation by the NA-related compounds between the various shake-inducing agents, a wide selection of NA-related compounds were chosen to provide a fairly comprehensive analysis of the interaction of NA systems with 5-HT-related shaking behaviour.

EXPERIMENTAL METHODS.

1. ANIMALS, ANIMAL HUSBANDRY AND LABORATORY CONDITIONS.

1.1 Mice

Experiments reported in this thesis were carried out on Aston bred male mice of MF1 strain (unless otherwise stated) 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 of 21 ± 2°C under normal lighting conditions. These animals were fed 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 5 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 (light - 08.00-20.00 hours).

1.2 Rats

The experiments involving rats in this thesis were performed on male hooded PVG rats (supplied by Bantin & Kingman Ltd., Hull) weighing between 200 and 290g. These animals were fed a conventional 41B cube diet (Pilsbury's Ltd., Birmingham) and received tap water ad libitum. The rats were kept in groups of 6 in polypropylene cages in the quiet experimental room (for at least 14 days prior to experimentation) which was maintained under the same environmental conditions as those described for mice above.

All behavioural experiments were carried out between 09.00 and 19.00hrs.

2. INJECTION TECHNIQUES.

2.1 Subcutaneous (s.c.) injection.

Injection was made into the loose skin at the back of the neck of both mice and rats. Where animals received more than one s.c. injection, the second injection was made by inserting the needle under the skin in the flank of the animal. The injection volume was 10.0ml/kg for mice and 1.0 ml/kg for rats.

2.2 Intraperitoneal (i.p.) injection.

Injection was made by inserting the hypodermic needle into the abdominal wall 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 rats.

3. Behavioural tests.

3.1 Induction of head-twitch behaviour in mice.

1hr prior to experimentation, mice from the same stock cage were placed in small sawdust-lined polypropylene cages in groups of 3. The third mouse was included only because head-twitching is reduced when there are only 1-2 mice per cage (Boulton and Handley 1973). This mouse formed no further part in the experiment. The remaining pair both received either carbidopa (9mg/kg s.c.) followed 15 min later by 5-hydroxytryptophan (L-5-HTP; 200mg/kg i.p.), 5-methoxy-N,N-dimethyltyramine (5-MeODMT; 5mg/kg i.p.), (±) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)DOI; 0.25/0.5mg/kg i.p.) or quipazine (2.0mg/kg i.p.).

3.2 Analysis of drug effects.

One mouse from each pair received the test drug and the other the injection vehicle. Twitches from the two mice were counted on alternate minutes for 10min starting 20min post L-5-HTP, 12min starting 2min after 5-MeODMT, 12min starting 3min after (±)DOI or 10min starting 2min after quipazine. This procedure compensates for between run variability (Handley and Brown 1982). There were at least 5 pairs per experimental group. Where it was possible to examine 3 or more doses of the test drug potency was expressed as ID_{50} (dose producing 50% inhibition relative to contol) or ED_{200} (dose producing a response frequency twice that of controls) from log dose-response regression analysis, where response = test mouse head-twitch frequency as a % of that in paired control mouse (Handley and Brown 1982).

The results (head-twitch counts) of the majority of experiments on head-twitching were recorded at the time of the experiments i.e. by an observer in the room where the experiment was being performed. However, a small number of experiments were recorded on videotape, and the videotapes analysed at a later date. The experimental protocol was identical for experiments using these two different methods of result collection.

3.3 Induction of shaking behaviour in rats.

1hr prior to experimentation, rats from the same stock cage were placed in large sawdust-lined polypropylene cages in groups of three. As in the case of mice, the third rat formed no further part in the experiment. The remaining pair received quipazine (2.5mg/kg i.p.) or (±)DOI 0.5mg/kg i.p. There were at least 5 pairs of rats per experimental group.

3.4 Analysis of drug effects.

As outlined in the Introduction, in mice only the head is involved in the twitching response, but in the rat the upper trunk may move as well resulting in the so called "wet dog shake" (WDS) (Bedard and Pycock 1977). In the work outlined in this thesis both head-twitches (Matthews and Smith 1980) and WDS were counted and are collectively refered to as the "twitch response".

One rat from each pair received the test drug and the other the injection vehicle. Wet dog shakes from the two rats were counted simultaneously for two rats for 30min starting 30min post quipazine or for 30min starting immediately after (±)DOI injection. There were at least 5 pairs per experimental group.

None of the experiments on shaking behaviour in rats were recorded on videotape. Results (twitch counts) were observed and recorded at the time of the experiment i.e. by an observer present in the room where the experiment was being performed.

3.5 Induction of scratching behaviour in mice

The experimental set up used for the induction of scratching in mice is identical to that used for twitching, except in this case the two experimental mice received (±)DOI 2.5mg/kg i.p.

3.6 Analysis of drug effects

As with the head-twitching experiments, one mouse from each pair received the test drug and the other the injection of vehicle. The 3 mice were then videotaped over the next 10min. The videotapes were analysed for the number of scratching bouts exhibited by the two experimental mice in the 10min starting immediately after (±)DOI. There were at least 5 pairs per experimental group.

3.7 Analysis of the effect of (±)DOI on the locomotor activity of mice.

The apparatus used to measure locomotor activity was an Animex activity meter type SE (LKB Farad, Sweden). This consists of 6 inductance coils forming part of a resonant circuit. Movement over any of the coils results in a change in current within the circuits which is then amplified and registered.

The apparatus was placed in a quiet room, with a piece of A3 paper placed on top, and, with a polypropylene animal cage (with no bottom), providing containment, on top of this. The cage was placed carefully so that all the coils were within its confines. The activity meter was tuned to 40 microamperes and the sensitivity was set at 25 microamperes. This sensitivity has previously been shown to detect locomotion, but not small movements such as tremor and grooming (Thomas 1975).

Mice were injected with (±)DOI or saline and placed back in their home cage for 10min, their locomotor activity was then assessed for 10min using the Animex. Mice were placed individually in the apparatus, and the paper replaced after each mouse, so that the enviroment presented to each animal was essentially identical. In the dose-response experiments assessing DOI-induced increases in locomotor activity there were at least 5 mice per experimental group.

3.8 Analysis of drug effects.

Each mouse received either the injection vehicle or the test drug according to a latin squares system, 30min later they received (±)DOI 1mg/kg i.p. and were assessed as above.

3.9 Analysis of the effect of (±)DOI on grooming behaviour in mice.

The videotapes of the dose-response experiment (Fig. 2.16 and Table 2.3 2)) were reanalysed by an observer blind to the treatments each mouse had received. Three forms of grooming – face/paw, penile and lick elsewhere – were scored for time spent grooming and no. of grooming bouts for three 5 min periods (0-5, 5-10 and 10-15min after DOI or vehicle administration). Total scores for the 15 min period of observation were obtained by adding together the individual scores for the three 5min periods. Lick elsewhere grooming refers to grooming that was not face/paw or penile in nature and involved licking by the mice. There were at least 6 mice per experimental group.

3.10 Gross behavioural assessment of (±)DOI in mice.

This was done in mice using a modified form of the scoring scheme described by Irwin (1968). Three mice were placed in the cage for 10min to allow for initial exploration of the novel environment. One mouse then received saline s.c. and the other two varying doses of (±)DOI.

Behavioural ratings were made over 10 minute periods, beginning 5 minutes after injection and subsequently every 20 minutes until there was no difference between the mice. This method of scoring was standardised by using a 0-6 scale, a score of 0 being allocated for absence of effect, 6 for maximal effects, 2 or 4 for progressively increasing effect and odd numbers where the degree of effect could not be allocated otherwise. Each mouse was compared to the mean of the control mice. The ratings were made using a standardised procedure, observations on unrestrained mice being first, followed by testing which involved approaching the mice and observations which required some degree of handling as follows :

Observations on unrestrained animals

The following were scored by degree when present: Straub tail, exophthalamous/ptosis, head-twitches, convulsions, writhing, tremor, abnormal gait, limb splay, piloerection, lachrymation, salivation, vasodilation or cyanosis, vocalisation and diarrhoea. Increases in total motor activity, respiration rate and depth, grooming and stereotyped activity, the nature of which was also noted, and the intensity and incidence of abnormal posturing were also scored.

Observations which required minimal handling.

The following were scored as increases or decreases over controls :

fearfulness - the freezing or withdrawal on approach of a pen towards the mouse's head.

alertness - assessed simultaneously as amount of interest.

startle response - response to puff of air directed at the mouse's head.

touch response - escape response to the application of pressure to the flanks with the finger and thumb.

tail pinch - reaction to the application of pressure to a point 1cm from the base of the forceps

flexor/reflexor - limb withdrawal on pinching the toe of the hindlimb with forceps

pinna reflex - degree of twitch response to stimulation of either external auditory meatus with a fine wire.

Observations which required removal of the mice from the box.

The degree of pupil dilation and hypothermia were assessed when holding the animal in a scruff grip. The following were assessed as increases or decreases over control :

passivity - point at which the mouse struggled to escape when held first by the scruff, then the hind limb, then the forelimb.

grip strength - ability to hang from a wire grid.

body position - the height of the ventral abdomen above a raised bar.

The animals were also assessed for catalepsy by placing the forepaws on a 4cm high bar, and for loss of righting reflex when flipped over to land in the cage from a height of 20cm.

3.11 Effect of (±)DOI on rat gross behaviour.

Male PVG rats were given (±)DOI 5mg/kg i.p. and put in an open topped cage and their behaviour observed and scored 5, 15 and 30min after injection, either by two observers sitting quietly in the room or monitored via a video camera. Behaviours were scored from the method of Deakin and Green (1978), as follows : 0-absent, 1-just present, 2-definite, 3-severe. Behaviours observed were wet-dog shakes, flat body posture, reciprocal forepaw treading, forelimb tremor, chewing and gnawing.

3.12 5-HT syndrome.

Male PVG rats were given their pre-treatments (saline for controls) at the required time prior to administration of the agent inducing the 'syndrome'. The 'syndrome' was induced by either 8-OH-DPAT (5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.). Rats were then put in an open topped cage and their behaviour observed for a period of 20min either by two observers sitting quietly in the room or monitored via a video camera.

Behaviours were scored at 5min intervals using the method of Deakin and Green (1978), as stated above. Behaviours observed and scored were flat body posture, reciprocal forepaw treading, abducted hindlimbs, head weaving, and tremor. Straub tail was observed but not scored. Where possible experiments were done blind and each run included a control animal.

Scores for each 5min period were added together to give an overall score for each individual behavioural component of the syndrome e.g. flat body posture, forepaw treading etc, for the 20min observation period, then a total '5-HT syndrome' score was produced by adding the scores for each individual behavioural component, for the 20min observation period, together.

3.13 Statistical analysis.

The statistical tests used in evaluation of the data presented in this thesis are paired Student t-test, one-way analysis of variance followed by Duncan's multiple-range test (Walpole and Myers 1972), two-way (2 x 2) factorial analysis of variance blocked by run followed by Tukey's U test (for unconfounded means) (Linton and Gallo 1975) and the Mann Whitney U test (Haber and Runyon 1977).

4. Drug sources and vehicles used.

DRUG	SOURCE
* BAY K8644	Bayer.
* buspirone	Bristol Myers Ltd.
* BRL 24924 (+)-(endo)-4-amino-5-chloro-2-methoxy-N(1-azabicyclo (3,3,1) non-4-yl)benzamide	Beecham Pharmaceuticals Ltd.
* carbidopa	Merck, Sharp and Dohme Ltd.

* clenbuterol hydrochloride — Dr Karl Thomas GMBH.

* cyproheptadine hydrochloride — Merck, Sharp and Dohme Ltd.

diethyldithiocarbamate (DDC) — Sigma Ltd.

disulfiram — Sigma Ltd.

(±)DOI   1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride — Research Biochemicals Inc.

dobutamine hydrochloride — Eli Lilly and Co. Ltd.

ergotamine tartrate — Sigma Ltd.

* FLA-63   bis-(4-methyl-1-homopiperazinyl-thiocarbonyl) disulphide — Astra Pharmaceuticals Ltd.

* GR38032F   1,2,3,9-tetrahydro-9-methyl -3((C2-methyl-1H-imidazol-1-yl)methyl)- 4H-carbazol-4-one (Ondansetron). — Glaxo Group Research.

1-5-hydroxytryptophan (L-5-HTP) — Sigma Ltd.

8-hydroxy-2-(di-n-propyl-amino) tetralin (8-OH-DPAT) — Research Biochemicals Inc.

* ICI 118,551   erythro-DL-1- (7-methylindan-4-yloxy)-3- isopropylamino-2-butan-2-ol — Imperial Chemical Industries plc.

* ICI 169,369   2-(2-dimethylaminoethyl thio)-3-phenylquinoline — Imperial Chemical Industries plc.

* ICS 205-930 (3-alpha-tropanyl)-1H-indole-3-carboxylic acid ester   Sandoz AG/SA.

* idazoxan (RX781094)   Reckitt and Colman.

* ipsapirone (TVXQ7821)   Troponwerke.

5-methoxy-N,N-dimethyltryptamine (5-MeODMT)   Sigma Ltd.

* metoprolol tartrate   Geigy Pharmaceuticals.

mescaline hydrochloride   Sigma Ltd.

* methysergide   Sandoz Products Ltd.

* MDL 72,222 (tropanyl-3-yl)3,5-dichlorobenzoate   Merrell Dow Research Institute (Strasbourg, France)

* nadolol   E.R. Squibb & Sons, Inc.

naloxone   Endo Laboratories.

* nitrendipine   Bayer.

L-phenylephrine hydrochloride   Sigma Ltd.

beta-phenylethylamine hydrochloride (beta-PEA)   Sigma Ltd.

pindolol   Sigma Ltd.

* pitzotifen hydrogen malate   Sandoz Products Ltd.

prazosin hydrochloride   Sigma Ltd.

* prenalterol hydrochloride   Astra Pharmaceuticals Ltd.

* procaterol hydrochloride   Warner-Lambert Co.

  quipazine maleate   Miles Laboratories Inc.

* ritanserin   Janssen Pharmaceutica.

  salbutamol base   Sigma Ltd.

* terfenadine   Merrell Dow Research
                                    Institute (Berks., U.K.)

  tyramine hydrochloride   Sigma Ltd.

* verapamil hydrochloride   Knoll AG.

* xylamidine tosylate   Wellcome Research
                                    Laboratories.

* - Gift gratefully acknowledged.

Drugs were dissolved in saline (0.9% w/v sodium chloride solution)
except the following : -

L-5-HTP   - dissolved in saline by the addition of a few
                          drops of concentrated hydrochloric acid (HCL)
                          and the pH brought back to 7.0 with
                          concentrated sodium hydroxide solution
                          (NaOH).

FLA-63   - dissolved in saline by the addition of a few
                          drops of HCL and the pH brought back to 7.0
                          with NaOH.

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

terfenadine   – suspended in saline by ultrasonication.

ritanserin   – suspended in saline by ultrasonication.

aspirin   – suspended in saline by ultrasonication.

nitrendipine   – suspended in saline by ultrasonication.

BAY K8644   – suspended in saline by ultrasonication.

In the case of both nitrendipine and BAY K8644, preparation of
suspensions took place in the absence of light as much as possible.

prazosin   – suspended in saline by ultrasonication.

diethyldithiocarbamate – suspended in saline by ultrasonication.

disulfiram   – suspended in saline by adding 2 drops of
                              Tween 80.

cyproheptadine   – suspended in 1% w/v acacia in saline.

All doses of drugs shown refer to the weight of the salt used.

CHAPTER 1

THE EFFECT OF MIGRAINE TRIGGERS AND ANTIMIGRAINE TREATMENTS ON 5-MeODMT-INDUCED HEAD-TWITCHING.

NB In the results sections throughout this thesis, where it is stated that an agent has produced an effect – either inhibition or potentiation – it implies this effect is statistically significant (i.e. $p < 0.05$), unless otherwise stated.

CHAPTER 1.

Introduction.

As previously described in the Introduction, the pharmacology of 5-HT-
related head-twitching has elements in common with that of migraine. A
neural hypothesis for the pathogenesis of migraine was outlined in
which it was postulated that central NA (e.g. n. locus coeruleus) and
5-HT (n. raphe dorsalis and medianus) systems might play a role in the
symptomatology of migraine - both pain and visual disturbances. Thus
an experimental model for migraine might be expected, on the basis of
this theory, to be sensitive to changes in NA and 5-HT function. As
previously outlined, the 5-HT-related head-twitch is such a model. In
addition, it has also been shown to be modulated by locus coeruleus
(Handley and Singh 1986a).

The experiments described in this chapter were carried out to
determine the effect of migraine triggers and migraine treatments on
the 5-HT-related head-twitch, in order to further evaluate the
similarities between the pharmacology of this behaviour and migraine,
and to assess the usefulness of the 5-HT-related head-twitch as a
model for pre-clinical screening of potential antimigraine agents.

Results.

For the results described below, mice received 5-MeODMT (5mg/kg i.p.).
This dose was chosen on the basis of previous work in our laboratory
(unpublished data & Singh and Handley 1987) which suggested that such
a dose would produce head-twitch frequency in the centre of the linear
part of the dose-response curve for head-twitching induced by 5-
MeODMT, thus allowing both inhibitory and potentiatory effects of
agents modulating such head-twitching behaviour to be observed. The
pretreatment times refer to the time before injection of the head-
twitch inducing agent, 5-MeODMT, that the migraine triggers and
antimigraine drugs were injected. All agents were injected i.p. unless
otherwise stated.

1. Effect of antimigraine treatments on 5-MeODMT-induced head-twitch.

As previously mentioned there are two main forms of antimigraine drug usage :- a) acute - those agents used only when an attack is imminent or has begun.

b) prophylactic - those agents taken continuously in order to reduce or abolish attack occurence.

In general, the approach taken was that a single high dose of each compound was tested against 5-MeOMDT-induced head-twitching i.e. in the manner a primary screening might test for antimigraine activity. If activity was shown, then a range of doses was tested.

1.1 Acute.

Aspirin has been used in migraine treatment for almost 100 years and remains the drug most frequently used in abortive therapy (Peatfield et al 1986). It is also effective in migraine prophylaxis (Pradalier et al 1988). Other NSAID's with demonstrated efficacy in treatment of migraine attacks are naproxen, tolfenamic acid, flurbiprofen & ibuprofen. Ketoprofen, indomethacin, tolfenamic acid, naproxen and fenoprofen have also been shown to be effective in migraine prophylaxis (see Pradalier et al 1988).

It was decided to examine one example of this class - aspirin.

Ergotamine and dihydroergotamine are the two other main agents used in the treatment of migraine attacks (Rothlin 1955).

It was decided to examine one example of this class - ergotamine.

Neither ergotamine (0.1 & 1mg/kg 30min pretreatment) nor aspirin (10 & 50mg/kg 30min pretreatment) produced any effect against 5-MeODMT-induced head-twitching at the doses examined (Fig. 1.1 and 1.2 & Table 1.1).

1.2 Prophylactic.

The experiments carried out with these agents were to examine their acute effects on 5-MeODMT-induced head-twitching, it was decided to concentrate on agents whose effect on 5-MeODMT-related head-twitch had not previously been examined

Cyproheptadine (0.05-5mg/kg 30min pretreatment), methysergide (0.1-5mg/kg 30min pretreatment), pizotifen (0.01-2mg/kg 30min pretreatment), verapamil (10-50mg/kg 15min pretreatment) and nitrendipine (25-50mg/kg 15min pretreatment) produced dose-dependent inhibition of 5-MeODMT-induced head-twitching with ID50's of 2.05 x 10^{-3} (5.82 x 10^{-7} to 1.88 x 10^{-1}), 7.02 x 10^{-2} (3.45 x 10^{-4} to 2.15), 1.18 x 10^{-1} (3.45 x 10^{-4} to 3.57 x 10^{1}), 3.07 x 10^{1} (2.34 x 10^{-1} to 7.44 x 10^{4}) and 4.4 x 10^{1} (1.56 x 10^{1} to 8.55 x 10^{4}) mg/kg respectively (Fig. 1.4, 1.3, 1.5, 1.7 and 1.6 (respectively) & Table 1.2). BAYK8664 (15min pretreatment) produced no effect at 1mg/kg and inhibition at 5mg/kg (Fig. 1.8 and Table 1.2c). This latter effect, however, was associated with behavioural toxicity e.g. mice were very slow moving, eyes semi-closed, flat body posture with paws outstretched. When being injected with 5-MeODMT the mice struggled excessively, then when placed back in their cage they were totally immobile, an effect which seemed almost like a form of catalepsy since they stayed in exactly the position they were put down into their cage in. After several minutes they appeared to recover and moved around again. Some, but not all, of the animals showed an abnormal lifting of the head up and then bringing it down, and jumping around, not seen in the control animals . Nadolol (5mg/kg 20min pretreatment) (Fig. 1.9 & Table 1.2b), metoprolol (1.5mg/kg 15min pretreatment) and pindolol (0.25-10mg/kg 30min pretreatment) (Fig. 1.10 & Table 1.2b) had no effect at the doses tested.

1.3 Acute - experimental antimigraine agents.

GR38032F (0.001-1mg/kg) and ICS 205-930 (0.001-10mg/kg, both compounds 20min pretreatment) produced no effect on 5-MeODMT-induced head-

twitching over the dose ranges tested (Fig 1.11 and 1.12 (respectively) and Table 1.3a).

MDL 72,222 produced complex effects. On initial examination, 0.01 and 0.1mg/kg MDL 72,222 produced potentiation of 5-MeODMT-induced head-twitching, 1mg/kg produced inhibition, 0.5mg/kg and 2mg/kg no effect and 10mg/kg inhibition (all 20min pretreatment) (Fig. 1.13 and Table 1.3a). The 10mg/kg result is questionable since the variability in the control group of the 10mg/kg dose was greater than in the other dose-response experiments and the frequency of head-twitching in the control group was greater than any potentiatory effect produced by other doses of MDL 72,222 and more than twice that of all but one other control group in this series of experiments. A time course study using 2mg/kg (Fig. 1.14 and Table 1.3b) suggested that potentiation could occur with this dose. However this effect was not reliable (Fig. 1.14 and Table 1.3a). On repetition 1mg/kg produced potentiation (Fig. 1.13 and Table 1.3a), an effect which was repeated in the experiment shown in Fig. 1.16, where metoprolol (1.5mg/kg 15min pretreatment) was found to inhibit MDL 72,222-induced potentiation of 5-MeODMT-induced head-twitching (the interaction term, F(AB), from the two-way between/within subjects analysis of variance = 12.08; df 1,10; p <0.01)) whilst metoprolol itself had no significant effect on 5-MeODMT-induced head-twitching (assessed using Tukey's test for unconfounded means, p >0.05) (Fig. 1.16 & Table 1.4). In the initial experiment where the effect of 1mg/kg MDL 72,222 on 5-MeODMT-induced head-twitching was tested, a high control head-twitch frequency was observed which may explain the inhibitory effect of this dose of MDL 72,222 in the light of subsequent potentiation, although, the variability in effect seen with both 1mg/kg and 2mg/kg may reflect a position at the top of an inverted U-shape dose-response curve which was shifting around leading to changing effects (Fig. 1.13 and Table 1.3a).

On re-examination, 3 and 29 months later MDL 72,222 1mg/kg (20min pretreatment) produced no effect on 5-MeODMT-induced head-twitching (Fig. 1.15 & Table 1.5), indicating that although potentiation induced

by this dose of MDL 72,222 had been repeatable it was not a reliable effect. In retrospect the dose of MDL 72,222 chosen for these experiments (1mg/kg) might have been wrong if, as suggested above, this dose is at the top of a shifting inverted U-shaped dose-response curve. It is interesting that the control responses in Fig. 1.13, 1.15 and 1.16 are all similar, indicating that variability in the effect of MDL 72,222 on 5-MeODMT-induced head-twitching was unlikely to be due to changes in the control response.

2. Effect of migraine triggers on 5-MeODMT-induced head-twitching.

The aim of these experiments was to explore the effects produced by the migraine triggers, beta-PEA and tyramine, and their time course. Since this was essentially a pilot study, each time of the time-course was a separate experiment done on a separate occasion with its own controls, and therefore there is the influence of time to be taken into account when interpreting this data.

2.1 Time-course - tyramine.

Tyramine (20mg/kg s/c) produced significant inhibition of twitching at 0.5 and 1hr pretreatment times and potentiation at 15 and 24hr. Peak inhibitory and potentiatory effects were at 1hr and 15hr respectively after injection (Fig. 1.17 & Table 1.6). Interpretation of these results is complicated by the changing control response, however, the inhibition produced by tyramine at 0.5 and 1hr pretreatment times was to below the lowest control response and also below the general range of control responses. In contrast, the potentiation seen with 15 and 24hr pretreatment was only present in comparison with paired controls.

2.2 Time-course - beta-PEA.

Beta-PEA (20mg/kg s/c) produced significant inhibition of twitching at 0.5 and 1hr pretreatment times and potentiation at 15 and 24hr. Peak inhibitory and potentiatory effects were at 1hr and 24hr respectively after injection (Fig. 1.18 and Table 1.6). In contrast to the effects of tyramine on 5-MeOMDT-induced head-twitching, the pattern of inhibition and potentiation seen with beta-PEA was only revealed in comparison with paired controls. Nevertheless it was the same pattern as that produced by tyramine.

2.3 Effect of metoprolol on tyramine and beta-PEA-induced potentiation of 5-MeODMT-induced head-twitching.

Metoprolol (1.5mg/kg s.c. 15min pretreatment) was found to significantly inhibit both tyramine and beta-PEA (both 20mg/kg s.c. 24hr pretreatment)-induced potentiation of 5-MeODMT-induced head-twitching (the interaction term, $F(AB)$, from the two-way between/within subjects analysis of variance for beta-PEA = 10.05; df 1,14; $p <0.01$, and for tyramine = 14.86; df 1,10; $p <0.01$), whilst having no significant effect on 5-MeODMT-induced head-twitching itself (assessed using Tukey's test for unconfounded means, in both cases (beta-PEA and tyramine), $p >0.05$) (Fig. 1.19 and 1.20 & Table 1.7).

2.4 Effect of ICI 118,551 on tyramine and beta-PEA-induced effects on 5-MeODMT-induced head-twitching.

The interaction between ICI 118,551 (2.5mg/kg s.c. 30min pretreatment) and beta-PEA (20mg/kg s/c 24hr pretreatment) was found to be significant (the interaction term, $F(AB)$, from the two-way between/within subjects analysis of variance = 7.96; df 1,12; $p <0.05$), however, Tukey's test for unconfounded means revealed that beta-PEA did not significantly potentiate 5-MeODMT-induced head-twitching ($p >0.05$) (although a paired t-test on this data was significant - $p <0.05$). ICI 118,551 had no significant effect on 5-

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MeODMT-induced head-twitching itself (assessed using Tukey's test for unconfounded means - p >0.05) (Fig. 1.21 and Table 1.8).

Tyramine (20mg/kg s/c 24hr pretreatment) produced a significant potentiation of 5-MeODMT-induced head-twitching (assessed using Tukey's test for unconfounded means - p <0.05) which was not significantly inhibited by ICI 118,551 (the interaction term, F(AB), from the two-way between/within subjects analysis of variance = 0.75; df 1,30; p >0.05). Again ICI 118,551 had no significant effect on 5-MeODMT-induced twitching itself (assessed using Tukey's test for unconfounded means - p >0.05) (Fig. 1.22 and Table 1.8).

2.5 <u>Repeat experiments investigating the loss of effect of the migraine triggers, beta-PEA and tyramine, on 5-MeODMT-induced head-twitching.</u>

<u>Tyramine.</u>

a) <u>Effect of tyramine on 5-MeODMT-, 5-HTP- and (±)DOI-induced head-twitching.</u>

Tyramine (20mg/kg s/c 24hr pretreatment) produced no effect on twitching induced by the three inducers examined, although there was a trend towards potentiation seen with 5-MeODMT as the inducer (Fig. 1.23 and Table 1.9a). This experiment was performed 5 months after the original work on the effect of tyramine on 5-MeODMT-induced head-twitching (sections 2.1-2.4).

b) <u>Effect of tyramine on 5-MeODMT-induced head-twitching in Tuk MF1 mice.</u>

Tyramine (20mg/kg s/c 24hr pretreatment) produced no effect on 5-MeODMT-induced head-twitching in Tuk MF1 mice (Fig. 1.25 and Table 1.9b). This experiment was performed 11 months after the original work on the effect of tyramine on 5-MeODMT-induced head-twitching (sections 2.1-2.4).

c) Effect of tyramine on 5-MeODMT-induced head-twitching in Olac MF1 mice.

Tyramine (20mg/kg s/c 24hr pretreatment) produced no effect on 5-MeODMT-induced head-twitching in Olac MF1 mice (Fig. 1.26 and Table 1.9b). This experiment was performed 6 months after the original work on the effect of tyramine on 5-MeODMT-induced head-twitching (sections 2.1-2.4).

d) Effect of sub-chronic dosing of tyramine on 5-MeODMT-induced head-twitching.

Tyramine (10mg/kg s/c daily for 4 days followed 24hr later by 5-MeODMT) produced no effect on 5-MeODMT-induced head-twitching (Fig. 1.24 and Table 1.9d). This experiment was performed 11 months after the original work on the effect of tyramine on 5-MeODMT-induced head-twitching (sections 2.1-2.4).

e) Effect of tyramine on 5-MeODMT-induced head-twitching.

Two repetitions (10 months and 23 months) after the original work was performed failed to repeat the potentiatory effect of tyramine (20mg/kg s/c 24hr pretreatment) on 5-MeODMT-induced head-twitching (section 2.1-2.4). One repetition (23 months after the original experiments) of tyramine (20mg/kg s/c 30 min pretreatment) failed to reproduce the inhibitory effect originally seen (section 2.1-2.4), although there was a slight trend towards inhibition (see Table 1.9e).

f) Multidose/pretreatment time study.

The effect of varying dose/pretreatment time of tyramine on the 5-MeODMT-induced head-twitch response. This study was a small (in terms of the number of animals per experimental group used, which was 4) pilot study. If an effect, or a trend towards an effect, was seen in any of the experimental groups, a full scale experiment was then to be done using the set of conditions (dose/pretreatment time) in that

group. The dose/pretreatment time combinations used were 10mg tyramine s/c/15, 24 & 30hr pretreatment times, and 20mg/kg tyramine s/c/15 & 30hr pretreatment times. With only the 20mg/kg/15hr pretreatment combination was a trend (non-significant) towards potentiation seen. On using this combination (20mg/kg dose and 15hr pretreatment) with a larger number of animals per experimental group (7), no effect was seen. When this negative result was achieved the, 20mg/kg tyramine with a 24hr pretreatment time combination, previously used was also tried, no effect was seen (see Table 1.9c, part I and II). This study was performed 10 months after the original work on the effect of tyramine on 5-MeODMT-induced head-twitching (sections 2.1-2.4).

Beta-PEA.

g) Effect of beta-PEA on 5-MeODMT-induced head-twitching.

Two repetitions, at 5 months and 23 months, after the original work was performed failed to repeat (Table 1.10a) the potentiatory effect of beta-PEA (20mg/kg s/c 24hr pretreatment) on 5-MeODMT-induced head-twitching (section 2.1-2.4).

In one experiment the effect of 40mg/kg s/c 24hr pretreatment on 5-MeODMT-induced head-twitching was examined (6 months after the original work), again no effect was seen (Table 1.10b).

In the case of some of these experiments a tendency towards the original results were seen, but the effects were not significant.

Three repetitions (at 5 months and 24 months) after the original work failed to repeat the inhibitory effect of beta-PEA (20mg/kg s/c 30min pretreatment) (section 2.1-2.4). In fact, in one of the experiments, significant potentiation was seen (one of two repeats done 4 days apart 5 months after the original work, no effect was seen in the other experiment) (see Table 1.10a).

3. Clonidine acute and sub-chronic studies.

Clonidine (0.05mg/kg 30min pretreatment) produced inhibition of 5-MeODMT-induced head-twitching (Fig 3.19 and Table 3.15).

Neither 0.05 or 1mg/kg clonidine given daily for 4 days followed by 5-MeODMT 24hr later produced a significant effect on the 5-MeODMT-induced head-twitching, although with the higher dose there was a tendency towards a potentiatory effect being present (Fig. 1.27 and Table 1.11). This work was done 11 months after the original work on the effect of tyramine/beta-PEA on 5-MeODMT-induced head-twitching (section 2.1-2.4).

4. Effect on head-twitching frequency of repeated administration of the twitch-inducers, 5-MeODMT and (±)DOI.

5-MeODMT (5mg/kg) was given on four consecutive occasions, at the same time of day - as there is some evidence to suggest that 5-HT-related head-twitching is subject to time of day variations (Moser and Redfern 1984) - 5 days apart and the head-twitch frequency measured. The following pattern was observed : an increase in head-twitching on the 1st repeat and 2nd repeats followed by a reduction towards the twitch-rate of the original on the 3rd repeat. On repetition a similar pattern was observed, however, in this case the second increase in head-twitching frequency between the 1st and 2nd repeat was not observed. In the case of both these experiments administration of 5-MeODMT on the 1st, 2nd and 3rd repeat occasions produced significantly higher twitch frequency than the original administration (Fig. 1.28a and Fig 1.28b and Table 1.12).

Using the data from the initial experiment, correlation coefficients obtained during least square linear regression analysis showed that there were significant correlations between the twitch frequencies obtained from the mice on occasions 1 and 4, 2 and 3 & 3 and 4. The comparisons of occasions 1 and 2 & 1 and 3 were narrowly non-significant (see Table 1.12).

Significant correlations between different occasions of 5-MeODMT administration were also seen on statistical evaluation of the data from the second experiment. In this case, a significant correlation coefficient was found for the comparisons between the twitch frequencies obtained from mice on occasions 1 and 2, 2 and 3, 2 and 4 & 3 and 4. The comparison of occasion 1 and occasion 3 was narrowly non-significant (see Table 1.12).

The above experiment was repeated using (±)DOI (0.5mg/kg) as the head-twitch inducing agent. In this case the pattern observed was a significant increase in twitching on the 1st and 3rd repetitions, the head-twitch frequency observed on the 2nd repeat being higher than on the original administration of (±)DOI but not significantly so (Fig. 1.29a & Table 1.12). These results, however, were complicated by the appearance in the 2nd and 3rd repeats of the phenomenon of 'cage leaving'- whereby several of the mice spent time climbing round the edge of the cage, and despite being gently pushed back into the cage (by hand) they repeatedly climbed back up again. While they were on the sides of the cage they did not twitch at all, therefore making interpretation of the data difficult, as it requires the comparison of mice who remained in the cage (1st repeat plus the non – 'cage leaving' mice in repeats 2 and 3) and the 'cage leaving' mice of the 2nd and 3rd repeats.

The experiment was repeated using cages covered with transparent plastic which allowed viewing of mice (the reason why the cage lids were off initially) but prevented them from climbing out onto the cage's edge. Mice were allowed to adjust to this new set up in the same manner as in other experiments i.e. they were placed in the experimental cages 1hr prior to the experiment. Using this set up there was no increase in head-twitching observed with any of the repetitions (Fig. 1.29b and Table 1.12).

Statistical analysis of the two experiments on the effect of repeated (±)DOI administration on head-twitch frequency induced by this agent, revealed much less correlation between the head-twitch frequencies

induced on the various occasions of the administration of (±)DOI. In the first experiment, a significant correlation coefficient was found for the comparisons between the twitch frequencies obtained from the mice on occasions 2 and 3 & 2 and 4. However, in the case of the second experiment where the plastic sheeting was placed over the experimental cage, no significant correlations were found between any of the occasions (see Table 1.12).

5. Analysis of 5-MeODMT-induced head-twitching distribution.

On analysis using a chi square test, the distribution of head-twitching frequency induced by 5-MeODMT in the vehicle treated experimental animals (a random sample of 150 results (this was the maximum no. of data points the computer program used would accept) taken from all experiments except those using an ANOVA design) was found to be Gaussian (see Fig. 1.30). The chi square value for this data was found to be 34.67, df of 28, since the null hypothesis (Ho) was that the sample was Gaussian in distribution it can be accepted as the chi square value computed from this data is less than the chi square value in the table (0.05 value = 41.33).

A comparison of the 5-MeODMT-induced head-twitch frequencies of vehicle treated experimental animals used in experiments of a non-ANOVA design from before the loss of significant effect of tyramine/beta-PEA on 5-MeODMT-induced head-twitching (75 results chosen at random), to those used in experiments after the change (75 results chosen at random), revealed no differences in head-twitching frequency. This data was analysed using an unpaired t-test, t value for this data = -0.16, df of 148 for which p > 0.05.

6. <u>Effect</u> <u>of</u> <u>a</u> <u>variety</u> <u>of</u> <u>5-HT-receptor</u> <u>partial</u> <u>agonists</u> <u>and</u>
<u>antagonists</u> <u>on</u> <u>'5-HT syndrome'</u> <u>induced</u> <u>by</u> <u>5-MeODMT</u> <u>and</u> <u>8-OH-DPAT.</u>

<u>'5-HT syndrome'</u> <u>induced</u> <u>by</u> <u>8-OH-DPAT (5mg/kg i.p.).</u>

Ipsapirone (10mg/kg i.p.) and ICS 205-930 (1mg/kg i.p.) reduced a
total '5-HT syndrome' score, whereas, buspirone (5mg/kg i.p.),
GR38032F (0.1mg/kg i.p.) and MDL 72,222 (1mg/kg i.p.) had no effect on
total '5-HT syndrome' score (Table 1.13). All drugs were given 20min
prior to 8-OH-DPAT (5mg/kg i.p.).

Analysis of the effect of the 5-HT-receptor partial agonists and
antagonists on three individual behavioural components of the syndrome
induced by 8-OH-DPAT revealed that :

Ipsapirone reduced FBP (flat body posture) and FPT (forepaw treading),
with no effect on HLA (hindlimb abduction) (Table 1.13).

Buspirone reduced FPT, but had no effect on FBP or HLA.

ICS 205-930 reduced FBP, FPT and HLA.

MDL 72,222 reduced HLA, but had no effect on FBP and FPT.

GR38032F had no effect on any of these behavioural components.

(see Table 1.13).

<u>'5-HT syndrome'</u> <u>induced</u> <u>by</u> <u>5-MeODMT (5mg/kg i.p.).</u>

ICS 205-930 (1mg/kg i.p.), given 20min prior to 5-MeODMT (5mg/kg i.p.), had no effect on total '5-HT syndrome' score nor any of the three individual components of the syndrome examined (Table 1.14).

The effects of 5-HT partial agonists and antagonists on '5-HT syndrome' induced by 5-MeODMT and 8-OH-DPAT were statistically analysed using the Mann-Whitney U test.

Discussion.

The purpose of the experiments performed in this chapter was to
attempt to validate the head-twitch 'model' of migraine. In the
process data was generated which is of some scientific interest in
elucidating the interaction of 5-HT2 receptor mechanisms with other
neuronal and biochemical systems.

Antimigraine drugs.

Acute.

The mechanism of action of NSAID's in the relief of migraine attacks
still remains unresolved. However, measurement of prostaglandin levels
in plasma or saliva of patients suffering from migraine offers some
support for their involvement in its pathogenesis e.g. in 1981 Amroyan
studied migraine patients between attacks and during attacks and found
PGE2 levels three and six times higher than those in controls.

Recent work on the analgesic effect of aspirin have suggested there
may be a central site for its activity involving 5-HT systems (in
addition to the traditionally acknowledged peripheral one).
Displacement of tryptophan from its plasma binding sites and
acceleration of the metabolism of 5-HT occurred in aspirin-treated
rats (Tagliamonte et al 1971). Subsequently, an apparent relationship
between the degree of analgesia and the degree of change in the
turnover rate of serotonin in brain, induced by these compounds, has
been found (Bensemana and Gascon 1978). Groppetti et al (1988)
reported that intravenous administration of acetyl salicylate of
lysine, a soluble salt of aspirin, reduced the firing discharge of
thalamic neurones evoked by noxious stimuli and increased the
concentrations of 5-hydroxyindoleacetic acid in several areas of the
brain including the hypothalamus and brainstem. The antinociceptive
effect of the drug was counteracted by pretreating the animals with
the non-selective 5-HT antagonist, metergoline (Hoyer 1988a).

Despite this evidence to suggest an interaction between NSAID's and 5-HT systems, aspirin was inactive against 5-MeODMT-induced head-twitching even at high dose (50mg/kg). However, there is evidence that systems other than 5-HT are affected by salicylate treatment e.g. dopamine (DA) and NA seem to be involved (Bensamana and Gascon 1978 & Shyu and Lin 1985) which makes it difficult to predict its effect on head-twitching.

Ergotamine shows high binding affinity with Ki-values of nanomolar order, for five receptor sites (in decreasing order of affinity) 5-HT1a, b&c, dopamine2, alpha2, alpha1 and 5-HT2 (Leysen and Gommeren 1984 & Hoyer 1988a). Since 5-HT2, alpha1- and alpha2-receptors have been implicated in the modulation of 5-HT2-related head-twitching (see Introduction sections 16 and 17), it was somewhat surprising that ergotamine had no effect on 5-MeODMT-head-twitching. This binding data does not, however, indicate whether these receptor interactions are agonist, partial agonist or antagonist in nature. In peripheral tissue it has been shown to possess antagonist activity at 5-HT receptors, however, in the CNS it has been suggested to have partial agonist activity (Hardebo et al 1978 & Muller-Schweinitzer 1978). More recent work has suggested it has antagonist action at 5-HT2 receptors (see Peroutka 1990) in the CNS. It is also said to possess partial agonist affinity for alpha-receptors of vascular smooth muscle (Bowman & Rand 1980). It is possible that two or more of these receptor interactions cancelled each other out as far as an effect on the 5-MeODMT-induced head-twitch is concerned.

Prophylactic.

The acute effect of a variety of prophylactic antimigraine agents on 5-MeODMT-induced head-twitch was examined. Those agents which interact principally with 5-HT2/5-HT1c receptors (cyproheptadine, methysergide and pizotifen (Hoyer 1988a)) produced the expected inhibition.

One calcium antagonist that has 5-HT2 binding activity (verapamil -
(Adachi and Shoji 1986, Peroutka 1988 & Green et al 1990)) and one
which does not (nitrendipine - (Cohen et al 1986)) were examined. Both
antagonised 5-MeODMT-induced head-twitching (verapamil - ED50 = 30.8
& nitrendipine - ED50 = 44.0). Nitrendipine does not appear, at
present, to have been tested for anti-migraine activity. However, all
other calcium antagonists, on which there are data available, appear
to possess anti-migraine activity (amitriptyline, cinnarizine,
cyproheptadine, diltiazem, flunarizine, nifedipine and nimodipine
(see Meyer and Hardenberg 1983; Peroutka 1983 & Peroutka et al 1984)).
The mechanism by which calcium antagonists inhibit 5-MeODMT-induced
head-twitching is at present unclear. Since verapamil binds to 5-HT2
receptors, 5-HT2 receptor blockade might be such a mechanism, however,
nitrendipine has no discernable 5-HT2 receptor binding activity making
this doubtful. Green et al (1990) found that the inhibitory effect
produced by calcium antagonists on 5-MeODMT-induced head-twitching was
not affected by pretreatment with BAY K8644, the calcium channel
agonist (Schramm et al 1983), suggesting that it was also unlikely
that the calcium antagonists actually exert their effects through an
action on calcium channels. Previous studies have reported that BAY
K8644 can reverse pharmacological actions of the calcium antagonists,
for example, 1mg/kg BAY K8644 (the same dose as used by Green et al
1990) reversed the anticonvulsant activity of high doses of nifedipine
and nicardipine (100mg/kg) (Dolin et al 1988).

Green et al (1990) speculate, on the basis of the similarities of
calcium antagonists and lithium on 5-HT-induced behaviour, that it is
possible that the effect of calcium antagonists on 5-HT2-mediated
behaviour might be mediated by an effect on 5-HT function indirectly
through changes in ion flux and disposition in the CNS. Interestingly,
nimodipine and BAY K8644 have been shown to produce, respectively,
decreased and increased catecholamine (DA and NA) synthesis in mouse
brain (Pileblad and Carlsson 1987). Since NA has a major role to play
in the modulation of head-twitching (see Introduction section 17.1),

this effect might also play a role in the inhibition produced by the calcium antagonists on 5-MeODMT-induced head-twitching.

In the present study, BAY K8644 was inactive at 1mg/kg against 5-MeODMT-induced head-twitching. Inhibition was seen at 5mg/kg but was accompanied by signs of toxicity. Green et al (1990) also found no effect with 1mg/kg BAY K8644, they did not report the effect of higher doses.

It is also possible that the inhibitory action of the calcium channel antagonists on 5-HT-related head-twitching is merely an indication of toxicity since only high doses produced inhibition, both in the present study and by Green et al (1990). In fact, the doses found necessary to produce inhibitory effects on 5-MeODMT-induced head-twitching in rodents were high in comparison to those used clinically in the treatment of migraine in humans; for example, the dose of verapamil used for migraine prophylaxis is 3.4-5.1mg/kg daily (Peroutka 1990) in comparison with an ED50 of 30.7mg/kg for the inhibition of 5-MeODMT-induced head-twitching in mice in the present study. Although, it should be noted that it is difficult to equate doses used in humans and rodents due to the differences in the species.

In contrast to the inhibitory effect on 5-MeODMT-induced head-twitching of the migraine prophylactic agents mentioned above, none of the beta-adrenoceptor antagonist drugs tested (metoprolol, nadolol and (±)pindolol) had any effect on this behaviour. Nadolol (non-selective beta-antagonist) and metoprolol (beta1-selective antagonist) are active in migraine prophylaxis, whereas (±)pindolol (non-selective beta-antagonist) has produced mixed effects in migraine prophylaxis (Weerasuriya et al 1982 and see Table 0.2)).

Previous studies have been conflicting. The selective beta-adrenoceptor antagonists which have no 5-HT receptor binding affinity such as metoprolol (Green et al 1983) produced no effect on 5-HT-related head-twitching whichever shake inducer was being used. Those

beta-adrenoceptor antagonists which have high binding affinity for 5-HT1a/1b receptors such as pindolol (Hoyer 1988a) produced differing modulatory effects depending on the particular study examined e.g. Yocca et al (1990) found that (±)pindolol inhibited quipazine-induced head-shaking, whereas, in the present study, it produced no effect on 5-MeODMT-induced head-twitching. Also, in some cases, differences have been seen within a particular study and depending on the 5-HT-related shake inducer being used e.g. Martin et al (1986) found that DL-propranolol and L-penbutolol inhibited 5-HTP- but potentiated 5-MeODMT-induced head-twitching. The reason for these differences is unclear, it could be suggested that differing experimental protocols, animals and methods of drug administration might be involved. Another possibility for the differences in modulatory activity, at least between the various compounds, could be their affinity for 5-HT1a and 5-HT1b receptors (the beta-adrenoceptor antagonists show little affinity for the 5-HT2 receptor (see Hoyer 1988a)), and whether they act as partial agonists or antagonists at these receptors. Such effects might also explain the differences seen with a particular agent on two different inducers.

The present studies with migraine prophylactic agents were acute single dose experiments, however, clinically these agents are used chronically to treat migraine. Chronic studies would therefore be necessary to test the similarity of effect of the antimigraine agents on a 5-HT2-receptor-mediated response on acute and chronic administration.

Acute - experimental antimigraine agents.

The 5-HT3-receptor antagonist, MDL 72,222 has been shown to be effective in aborting migraine attacks (Loisy et al 1985). There is no evidence available about the effectiveness of the other 5-HT3 antagonists in treating migraine. MDL 72,222, GR38032F (ondansetron) and ICS 205-930 did not produce a consistent effect on 5-MeODMT-induced head-twitching over a wide range of doses indicating that 5-HT3 receptors are unlikely to modulate this 5-HT2 response. This

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agrees with Sherman and Tolcsvai (1987) who found no appreciable effect with ICS 205-930 and MDL 72,222 on 5-HTP-induced head-twitching. Intial experiments with MDL 72,222 suggested that it potentiated 5-MeODMT-induced head-twitching at low dose (1mg/kg or less). A further experiment suggested that this potentiation might involve beta1- adrenoceptors since it was blocked by metoprolol, a beta1-antagonist (Ablad et al 1973). However further attempts to reproduce the potentiation failed to demonstrate any effect. This phenomenon is discussed later in conjunction with the effects of tyramine and beta-PEA.

The effects of 5-HT3 antagonists on the '5-HT syndrome' in rats were also examined. This syndrome was induced by 8-OH-DPAT and is believed to be due to activation of postsynaptic 5-HT1a receptors (Trulson et al 1976; Deakin and Green 1978; Hjorth et al 1982; Tricklebank 1985 & Tricklebank et al 1985a&b). Results are preliminary because only single doses of potentially interacting agents were used. ICS 205-930 halved all variables of the 8-OH-DPAT-induced syndrome at the high dose of 1mg/kg used, MDL 72,222 (1mg/kg) only reduced one of the variables (HLA) and GR38032F had no effect on any of the components. The lack of uniformity of these results make it unlikely that the effects of ICS 205-930 were exerted at 5-HT3 receptors. In addition ICS 205-930 had no effect on 5-MeODMT-induced syndrome. An interaction of 5-HT3 and 5-HT1a receptors appears unlikely on the basis of this preliminary evidence.

In addition, the effect of the two 5-HT1a ligands (buspirone and ipsapirone) on 8-OH-DPAT-induced '5-HT syndrome' in rats were examined since at the time this work was done in 1986 there was some debate as to whether 5-HT1a ligands such as ipsapirone and buspirone acted as full agonists, partial agonists or antagonists at postsynaptic 5-HT1a receptors. Ipsapirone inhibited both total '5-HT syndrome' score and FPT and FBP, but not HLA, whereas buspirone did not significantly effect the total '5-HT syndrome' score, FBP or HLA but did inhibit FPT, which of the behaviours which are counted as part of the '5-HT syndrome', is best characterised as being mediated by 5-HT1A receptors

(Tricklebank 1985). The lack of effect of buspirone on total '5-HT syndrome score might be a dosage effect related to the fact that a high dose of 8-OH-DPAT (5mg/kg) was used to produce the syndrome, perhaps the dose of buspirone was not sufficient to inhibit the total '5-HT syndrome' significantly. Ipsapirone, another 5-HT1a partial agonist, more completely inhibited the syndrome than buspirone, however, a larger dose was used than with buspirone (10mg/kg compared to 5mg/kg). Such results suggest that buspirone and ipsapirone act as antagonists at postsynaptic 5-HT1a receptors, a postulate supported by the fact that neither buspirone nor ipsapirone produced any signs of the syndrome in the 20min prior to the injection of 8-OH-DPAT. Other studies have also found that buspirone and ipsapirone when administered alone in doses up to 50mg/kg i.p. did not induce the '5-HT syndrome' (Eison et al 1986; Goodwin et al 1986 & Lucki 1986). However, in contrast, Hjorth and Carlsson (1982) found that buspirone (10mg/kg i.p.) could induce the entire syndrome (FBP, FPT, HLA and Straub tail) while Smith and Peroutka (1986) found that the same dose of buspirone induced a portion of it (HLA, FBP and Straub tail). In the latter study it is interesting to note that the portions of the '5-HT syndrome' which were not produced by buspirone were the ones that buspirone antagonised in the 8-OH-DPAT-induced syndrome (FPT, head-weaving and tremor). In the present study 8-OH-DPAT did not induce head-weaving and tremor, but buspirone did inhibit FPT at a similar dose to that used by Smith and Peroutka (1986). Compared to buspirone, ipsapirone has shown comparatively little ability to induce the '5-HT syndrome' when administered alone, Smith and Peroutka (1986) found a slight flattening of body posture (10mg/kg). Even at doses as high as 80mg/kg, only minimal evidence for the presence of the full 5-HT syndrome was observed (Spencer et al 1984). Such a difference in agonist activity of buspirone and ipsapirone might explain the differences seen in the present study to antagonise the '5-HT syndrome' induced by 8-OH-DPAT. If these results are taken together the best explanation for the effects seen would appear to be that ipsapirone and buspirone are acting as partial agonists at postsynaptic 5-HT1a receptors (with ipsapirone perhaps having more of an antagonist-type profile than buspirone).

Migraine triggers.

Since the work of Burn and Rand (1958), it is generally accepted that indirectly acting sypathomimetic amines, such as the migraine triggers, tyramine and beta-PEA, release NA from noradrenergic nerve endings. Beta-PEA has also been demonstrated to release catecholamines in general (both NA and DA) (Fuxe et al 1967; Backer et al 1976; Braestrup and Randrup 1978 & McQuade and Wood 1983). Tyramine has been demonstrated to release 5-HT from striatal synaptosomes (Raiteri et al 1977).

The hypothesis proposed in the Introduction predicts that tyramine and beta-PEA would initially silence the L.C. by the release of NA onto cell body alpha2-adrenoceptors (see Lundberg et al 1985) and that this could result in a rebound postsynaptic hypersensitivity, especially of beta-adrenergic receptors which would become apparent as neuronal firing began to resume. In view of the evidence for the involvement of the L.C. in modulating head-twitch behaviour (see Introduction section 17.1) it was predicted that tyramine and beta-PEA would thus cause an initial reduction in head-twitches and that this would be followed by a potentiation. The latter should be blockable with a beta-adrenergic antagonist such as metoprolol.

The initial experiments appeared to confirm this hypothesis. Tyramine and beta-PEA (in separate experiments) produced virtually identical time-course pictures, both initially producing inhibition (0.5-5hr after tyramine injection) with potentiation following at 15 hours (15-48hr after tyramine injection) of 5-MeODMT-induced head-twitching. The potentiation produced by both of the migraine triggers was blocked by metoprolol suggesting the involvement of beta1-adrenoreceptors in this response.

As far as the type of beta-receptor involved in these responses, the effect of the beta2-adrenoceptor antagonist, ICI 118,551 (O'Donnell and Wanstall 1980) on the modulation of 5-MeODMT-induced head-twitching by the migraine triggers was also examined. These

-119-

experiments did not show any clear evidence of beta2-adrenoceptor involvement.

Four months later when further investigation of these effects were planned the potentiatory effects of tyramine and beta-PEA were no longer obtainable. Because of the importance of the initial findings to the hypothesis, extensive attempts were made to replicate them :-

1) by repeating the tyramine and beta-PEA (24hr 20mg/kg) experiment to check whether the effect was intermittent, possibly, a seasonal effect,

2) by trying other head-twitch inducers ((±)DOI and 5-HTP) to see whether it was a problem associated with using 5-MeODMT as the twitch inducer,

3) by trying different strains of mice (Olac and Tuk MF1) to see if it was due to some alteration in our home bred MF1 e.g. a genetic change,

4) by looking at different doses/pretreatment times

5) by trying to replicate the original findings using clonidine, instead of the migraine triggers, since it also reduces L.C. firing (Foote et al 1983). Initial pilot studies on the effect of clonidine (0.1mg/kg 15 and 24hr pretreatment) on 5-MeODMT-induced head-twitching indicated no effect was present, therefore it was decided to use sub-chronic dosing. A slight potentiation was seen with the highest dose used but the result was still non-significant. However Handley et al (1986) did find a clonidine withdrawal head-twitch.

Unfortunately none of these measures proved successful.

The crucial nature of the initial findings to the hypothesis put forward for the mechanism of action of migraine triggers (beta-PEA and tyramine) means that it is essential to consider whether they were real effects which then disappeared for unknown reasons or chance findings.

In favour of the effects being real is :-

1) the fact that potentiatory and inhibitory effects of the migraine
triggers were present over the 8 month period it took to complete
these experiments. The potentiation produced by 24hr pretreatment with
tyramine and beta-PEA (20mg/kg) was repeatable (i.e. in the antagonism
experiments using metoprolol) and the metoprolol antagonism was not an
artefact of control variation (Fig. 1.19 & 1.20). In addition, the
metoprolol experiments were balanced across time. Potentiation was
also observed for both tyramine and beta-PEA (24hr pretreatment) in
the ICI 118,551 antagonism experiments, although the ICI 118,551
antagonism was less clear cut. Again these experiments were balanced
across time. The fact that both beta-PEA- and tyramine-induced
potentiation (24hr pretreatment) was repeatable (present with both
agents in 3 separate experiments on three separate occasions) suggests
that it is unlikely to be an artefact of control variation. The
effects produced with other pretreatment times (0.5, 1 & 15hr) are
more in question because they were only found in one experiment each.
The reliability of 24hr pretreatment with beta-PEA and tyramine to
induce potentiation in these initial experiments (3 out of 3
experiments with each migraine trigger) compared with the later
experiments where the effect of beta-PEA and tyramine was consistently
not present is not indicative that the change in effect of the
migraine triggers on 5-MeODMT-induced head-twitching was a product of
random variation.

2) pairing of test and control animals so that day to day variation
would affect test and control groups equally. It is interesting to
note that in experiments where a range of doses was used of drugs
which turned out to be inactive, the changes in control and test
responses closely paralleled each other e.g. Fig. 1.10, 1.11 & 1.12)
so that variation in control responses does not seem to be a major
factor in creating artefactual (i.e. spurious) drug effects. This
validates the pairing method used in these studies.

3) the fact that two different agents (tyramine and beta-PEA) produced similar effects on 5-MeODMT-induced head-twitching.

4) the fact that the potentiation produced by **both** tyramine and beta-PEA was blocked by metoprolol.

5) the disappearance of the potentiatory effect of MDL 72,222 over the same time span. Once these two effects had disappeared I also had problems replicating previous strong and consistent potentiatory effects obtained using 5-MeODMT as the twitch inducing agent, most notably with diazepam (see Table 1.15, Singh et al 1986 & Singh and Handley 1987), FLA-63 (see Chapter 3 results section 1.4 and Singh et al 1986), picrotoxin (see Table 1.15 and Singh et al 1986) and dobutamine (beta1-adrenoceptor agonist) (see Chapter 3 results section 2.1 & Singh and Handley 1987). The problems have been either no effect being produced or effect has diminished. In addition the beta2-adrenoceptor agonists which had in previous work by our laboratory produced potentiation with 5-MeODMT (Singh and Handley 1987) were found to produce either no effect or inhibition (see Chapter 3 results section 2.2). It is perhaps also of significance that, as previously mentioned, another class of beta-adrenergic agents, the beta-adrenoceptor antagonists have also produced differing results on 5-HT-related head-twitching in the hands of different workers. This variability might be related to the variability of beta-agonist effects. Variability of the various metoprolol-blockable potentiations obtained in this study e.g. beta-PEA, tyramine, MDL 72,222 and FLA-63 was only seen where there is some indication at least that beta-receptors could be involved. Possibly some undetected alteration in conditions occurred which altered beta-adrenoceptor effects, although no consistent changes in breeding practice, diet, bedding, water, temperature, light-dark cycle etc were found to have occurred. It may be of importance that in between the work of L.Singh and that of the present study, our laboratory and animal house were moved from one building to another.

In favour of the initial findings being due to chance is :-

1) the variability of the control groups from day to day - indicating the possibility that 'potentiation' and 'inhibition' were merely a product of this variability (see for e.g. figs 1.17 and 1.18). As previously mentioned in the Results section, although the pattern of modulation for the time-courses of both beta-PEA and tyramine were the same, with the exception of the inhibitory effect seen with tyramine (0.5 and 1hr pretreatment), all other effects produced by tyramine and beta-PEA on 5-MeOMT were only revealed by comparison with paired controls i.e. the effects produced were less than the range of control values seen over the time-course experiments.

2) the inability to replicate the initial findings four months later.

It may be commented that the experimental design, examining the animals in pairs, was chosen because of the known day to day variability of the head-twitch rate. Handley & Brown (1982) having found that the within group variability of paired control mice was substantially and significantly less than that of control mice examined on different days.

The fact that the initial findings were not confirmed meant that I could not further study them, thus changing the direction of my experimental work, initially to a study of the effect of noradrenergic ligands on a variety of head -twitch inducers and then a study of the 5-HT2/5-HT1c agonist, (±)DOI.

An examination of the head-twitching distribution of 5-MeODMT and (±)DOI was also done. The purpose of this analysis was to see whether there was a Gaussian distribution of head-twitching frequency or whether there were groups of mice which twitched at different rates, the distribution of which, within the experimental groups, might have affected the outcome of a particular experiment. As reported no such bimodal distribution was found, either in general throughout the

entire period of experimentation, or in a comparison of experiments done before and after the loss of effect of the migraine triggers.

The effect of repeated administration of the head-twitch inducers 5-MeODMT and (±)DOI on twitch frequency was also examined to determine whether each individual mouse had its own inherent twitch frequency which it maintained with repeated administration of the twitch inducer (* - see below).

Looking at the results for 5-MeODMT, they are interesting on two scores :

1) That there was found to be a strong correlation between head-twitch rates in the same mice on four occasions, indicating that the above statement (*) was correct (see Results section 4).

2) A potentiation of the response from the 1st to later occasions was seen, which was replicable (see Results section 4). Such an effect where there is enhanced reponsiveness to a drug resulting from repeated, intermittent administration has been called reverse tolerance or behavioural sensitization (Kuczenski and Leith 1981 & Kuczenski and Segal 1988).

Karler et al (1990) have reported reverse tolerance to 5-HTP-induced head-twitching in mice. Cyproheptadine, but not haloperidol, inhibited this response and they concluded that since cyproheptadine has 5-HT2 receptor blocking activity and head-twitching is thought to be mediated through 5-HT2 receptors then the 'reverse tolerance' is also mediated through 5-HT2 receptors.

It is possible that the reverse tolerance to 5-MeODMT-induced head-twitching seen in the present study is also mediated through 5-HT2 receptor. Possibly via upregulation of 5-HT2 receptors or an increased sensitivity in their transduction mechanism. In line with this theory, ritanserin (a selective 5-HT2/5-HT1c receptor antagonist) has been shown to produce down regulation of 5-HT receptors on chronic

administration (Leysen et al 1986), however, there have also been reports of agonist-induced down regulation of these receptors (see Introduction section 6), and this study is single dose, i.e. a potentiatory effect was seen after a single previous dose of 5-MeODMT, as opposed to a sub-chronic/chronic study.

It also seems possible, however, that the reverse tolerance was not produced via an effect on 5-HT2 receptors but through some other receptors/transmitter system. Such a postulate might gain support from the weaker and unreliable potentiation (and incidentally, intercorrelation) seen with repeated administration of (±)DOI, a more selective 5-HT2 agonist than 5-MeODMT. If 5-MeODMT with its 5-HT1a affinity produced desensitization of 5-HT1a receptors this might be expected to produce potentiation of 5-HT2 effects since the 5-HT1a agonist, 8-OH-DPAT, potently inhibited (±)DOI-induced head-twitching in the present study (see Chapter 2 results section 2.3a). Interestingly, recent work by Beer et al (1990), on the effect of a single dose of 8-OH-DPAT on presynaptic (raphe nuclei) and postsynaptic (frontal cortex and hippocampus) [3H]-8-OH-DPAT-binding has shown a reduction in raphe, but not frontal cortex or hippocampal binding, indicating a decrease in 5-HT1a receptor number only presynaptically. One day after administration of 8-OH-DPAT, an electrically stimulated increase in 5-hydroxyindoleacetic acid in the frontal cortex was significantly potentiated. These results, the authors suggest, are indicative of the ability of 8-OH-DPAT to desensitise presynaptic 5-HT1a receptors and they propose that this may lead to a loss of feedback control, so that, on neuronal stimulation, the increase of 5-HT function is enhanced. Possibly such an effect might underly the increase on repeated administration in the frequency of 5-MeODMT-induced head-twitching.

The relevance of the effects produced by the migraine triggers and treatments on 5-MeODMT-induced head-twitching to the hypothesis of migraine presented in the Introduction is considered in the General Discussion.

EFFECT OF ASPIRIN ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG 1.1

VEHICLE
ASPIRIN
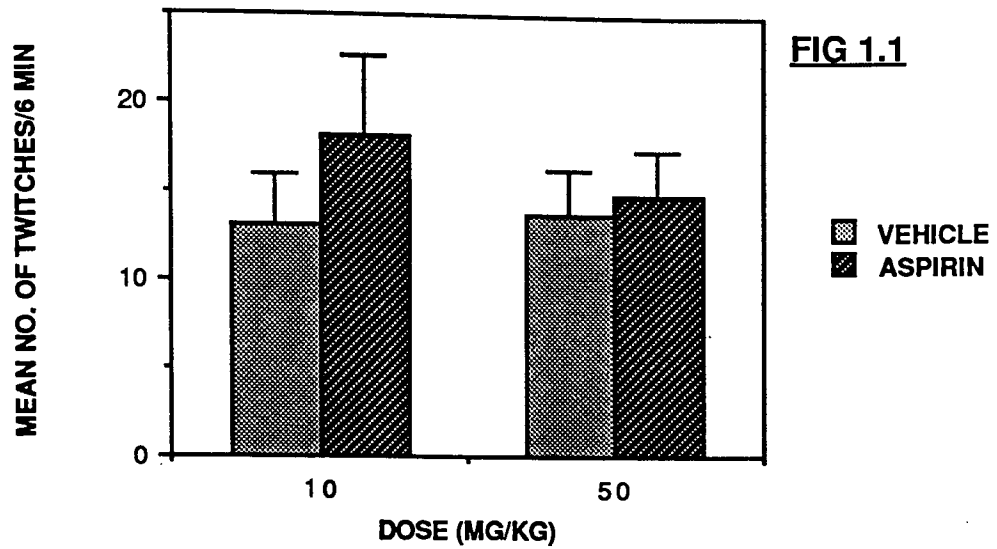
EFFECT OF ERGOTAMINE ON 5-MeODMT-INDUCED HEAD-TWITCHING
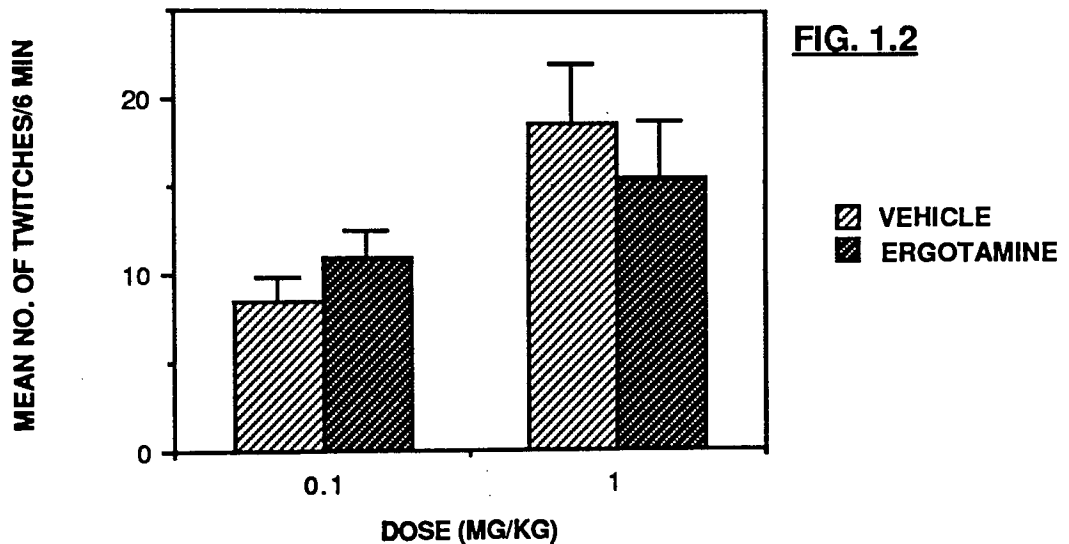
FIG. 1.2

VEHICLE
ERGOTAMINE

All mice recieved 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 1.1 Control mice received vehicle (i.p.) and test mice aspirin (i.p.).

Fig. 1.2 Control mice received vehicle (i.p.) and test mice ergotamine (i.p.).

EFFECT OF METHYSERGIDE ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.3

- ☑ VEHICLE
- ▨ METHYSERGIDE

* P <0.05
** P <0.01
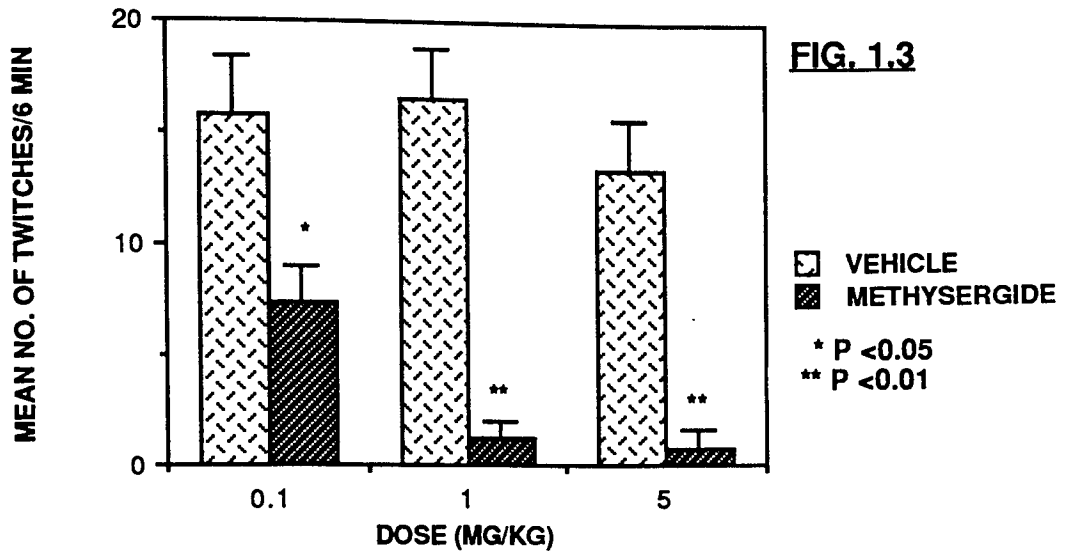
EFFECT OF CYPROHEPTADINE ON 5-MeODMT-INDUCED HEAD-TWITCHING
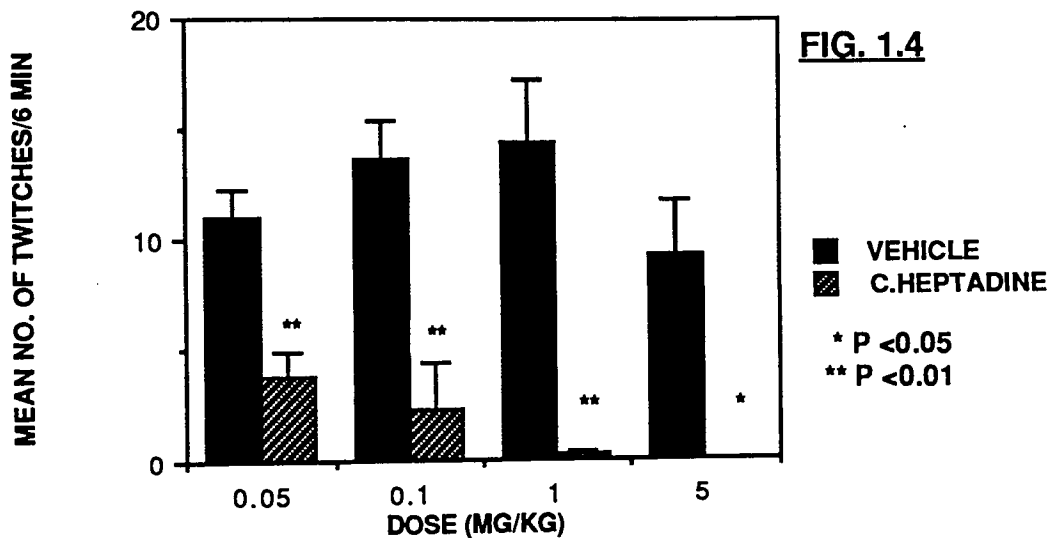
FIG. 1.4

- ■ VEHICLE
- ▨ C.HEPTADINE

* P <0.05
** P <0.01

All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 1.3 Control mice received vehicle (i.p.) and test mice methysergide (i.p.).

Fig. 1.4 Control mice received vehicle (i.p.) and test mice cyproheptadine (i.p.).

EFFECT OF PITZOTIFEN ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.5

■ VEHICLE
▨ PITZOTIFEN

* p <0.05
** p <0.01
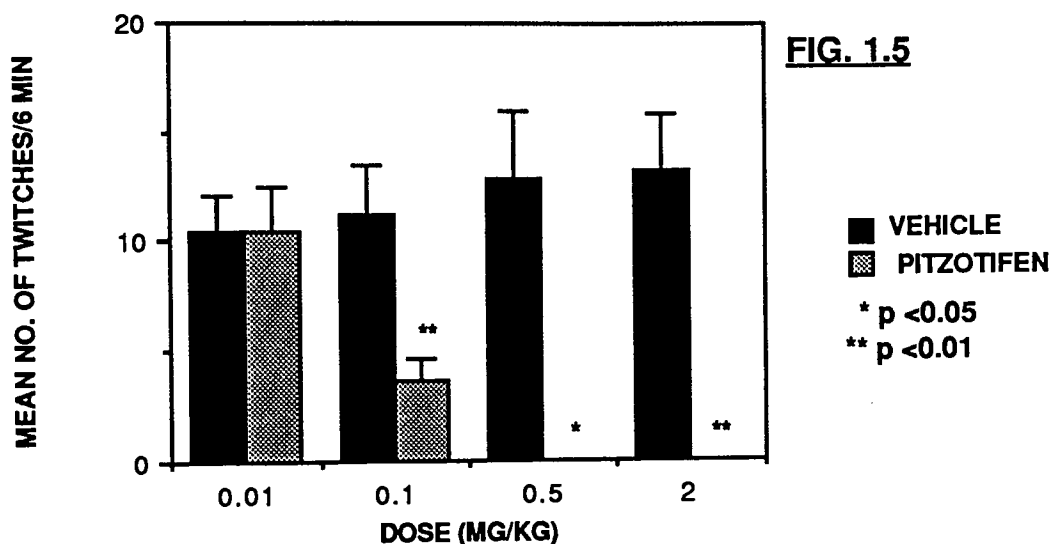
Fig. 1.5 All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice pitzotifen (i.p.). Results are the means of at least 6 determinations and vertical bars represent s.e.m.

EFFECT OF NITRENDIPINE ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.6

MEAN NO. OF TWITCHES/6 MIN

DOSE (MG/KG)

■ VEHICLE
◩ NITRENDIPINE

* p <0.05
** p <0.01
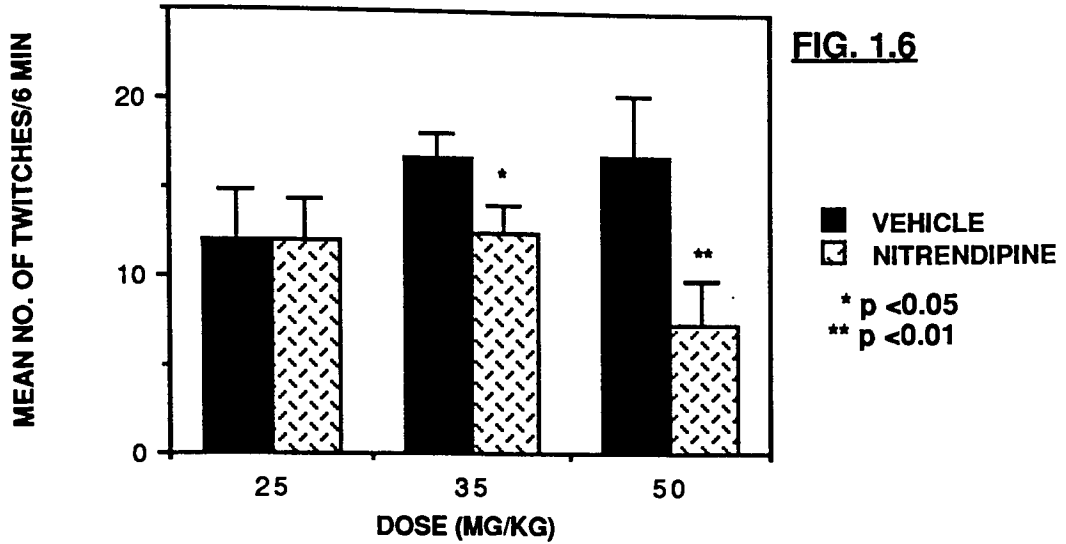
EFFECT OF VERAPAMIL ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.7

MEAN NO. OF TWITCHES/6 MIN

DOSE (MG/KG)

■ VEHICLE
▨ VERAPAMIL

* p <0.05
** p <0.01
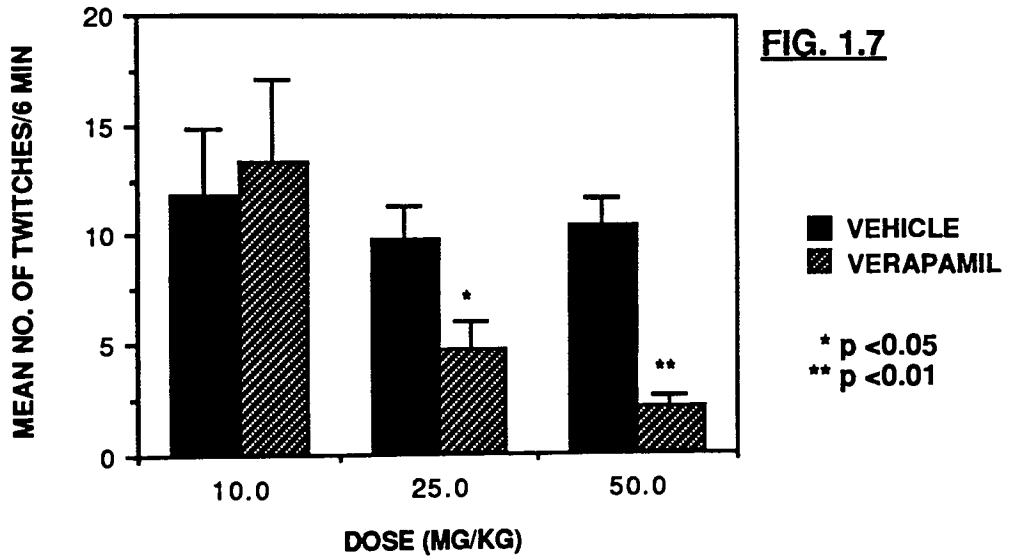
All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control and test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 1.6 Control mice received vehicle (i.p.) and test mice nitrendipine (i.p.).

Fig. 1.7 Control mice received vehicle (i.p.) and the test mice verapamil (i.p.).

EFFECT OF BAY K8644 ON 5-MeODMT-INDUCED HEAD-TWITCHING
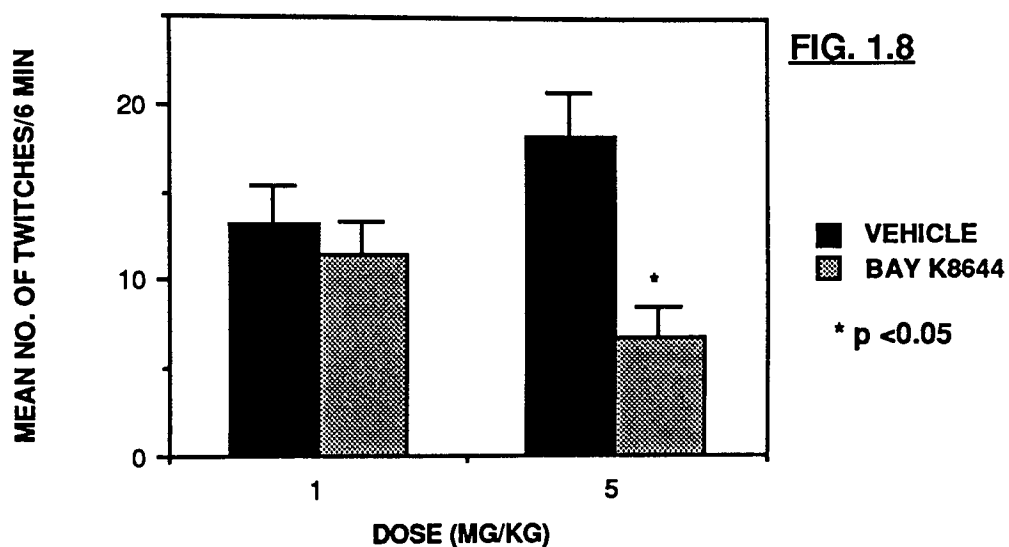
FIG. 1.8

Fig. 1.8 All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice BAY K8644 (i.p.). Results are the means of at least six determinations and vertical bars represent s.e.m.

EFFECT OF NADOLOL ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.9
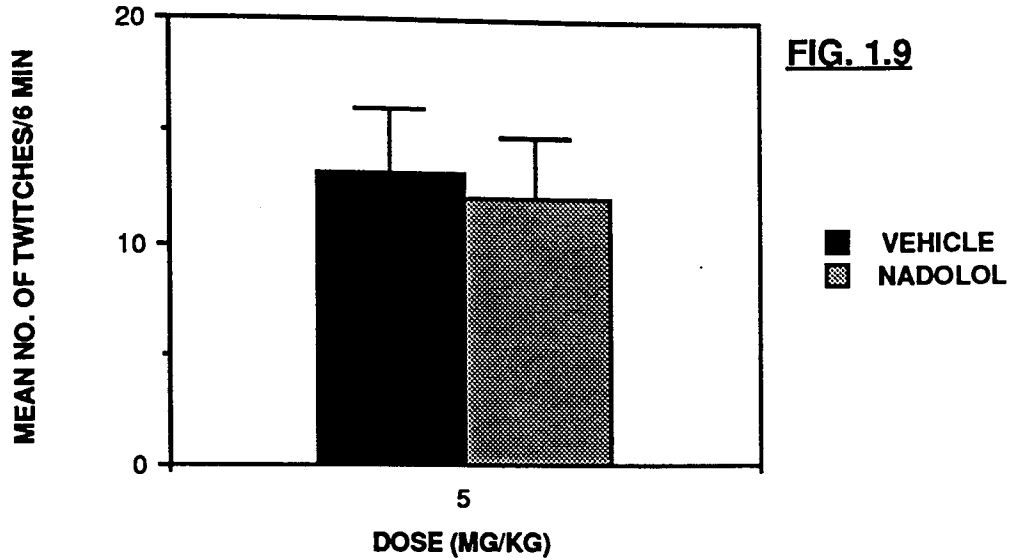
EFFECT OF PINDOLOL ON 5-MeODMT-INDUCED TWITCHING

FIG. 1.10
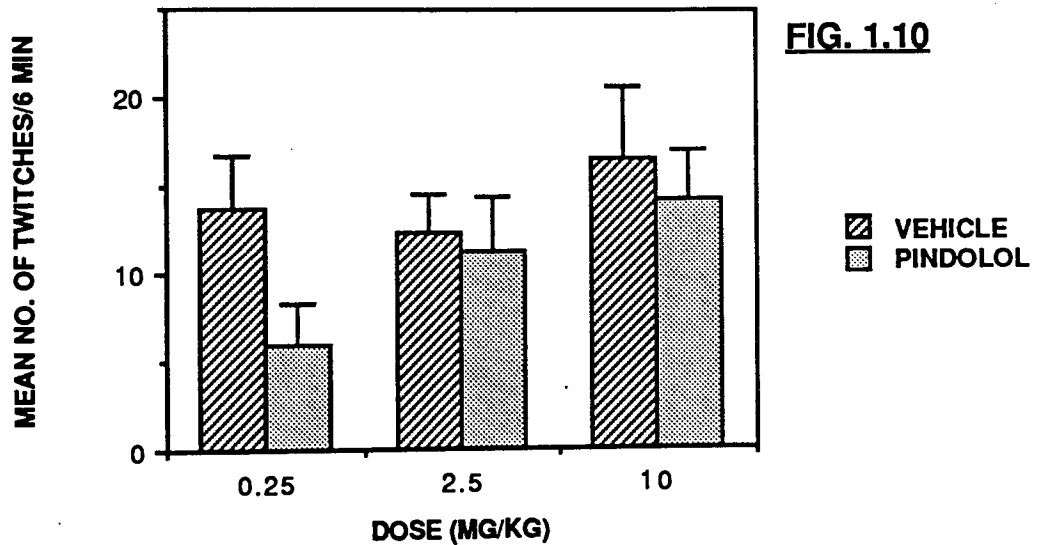
All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were designated at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 1.9 Control mice received vehicle (i.p.) and test mice nadolol (i.p.).

Fig. 1.10 Control mice received vehicle (i.p.) and test mice pindolol (i.p.).

EFFECT OF GR38032F ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.11

Y-axis: MEAN NO. OF TWITCHES/6 MIN
X-axis: DOSE (MG/KG)

Legend:
■ VEHICLE
▤ GR38032F
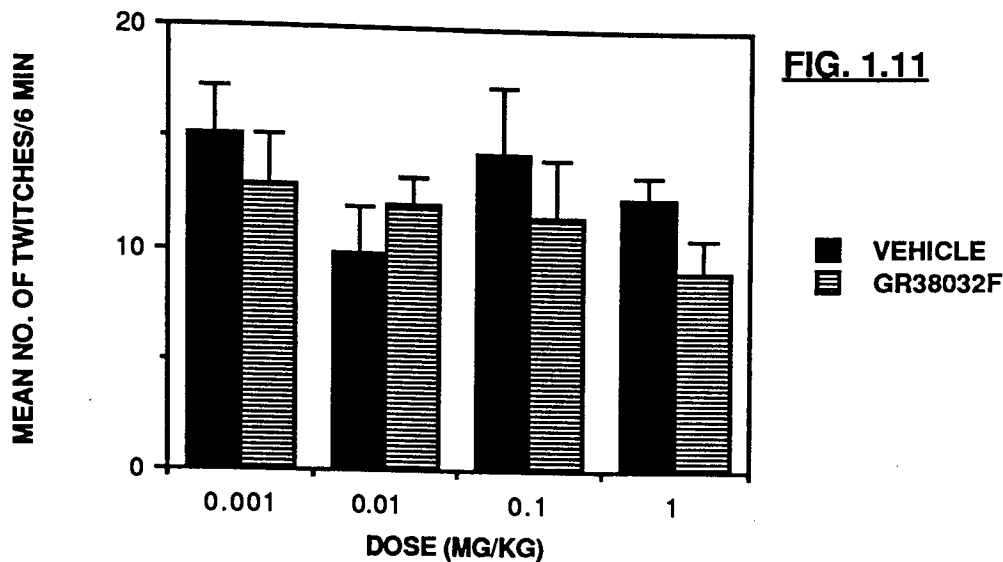
EFFECT OF ICS 205-930 ON 5-MeODMT-INDUCED HEAD-TWITCHING
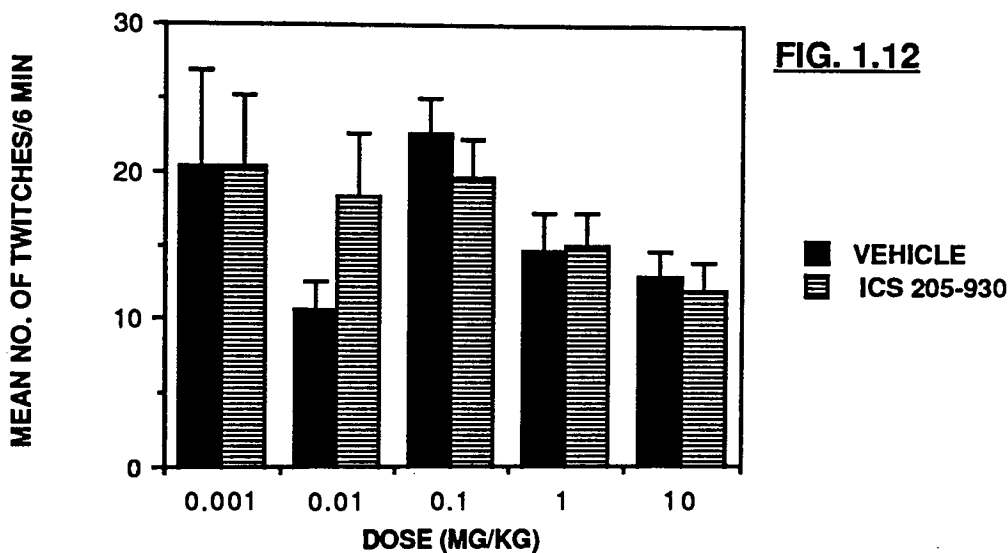
FIG. 1.12

Y-axis: MEAN NO. OF TWITCHES/6 MIN
X-axis: DOSE (MG/KG)

Legend:
■ VEHICLE
▤ ICS 205-930

All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

Fig. 1.11 Control mice received vehicle (i.p.) and test mice GR38032F (i.p.).

Fig. 1.12 Control mice received vehicle (i.p.) and test mice ICS 205,930 (i.p.).

EFFECT OF MDL 72,222 ON 5-MeODMT-INDUCED HEAD-TWITCHING
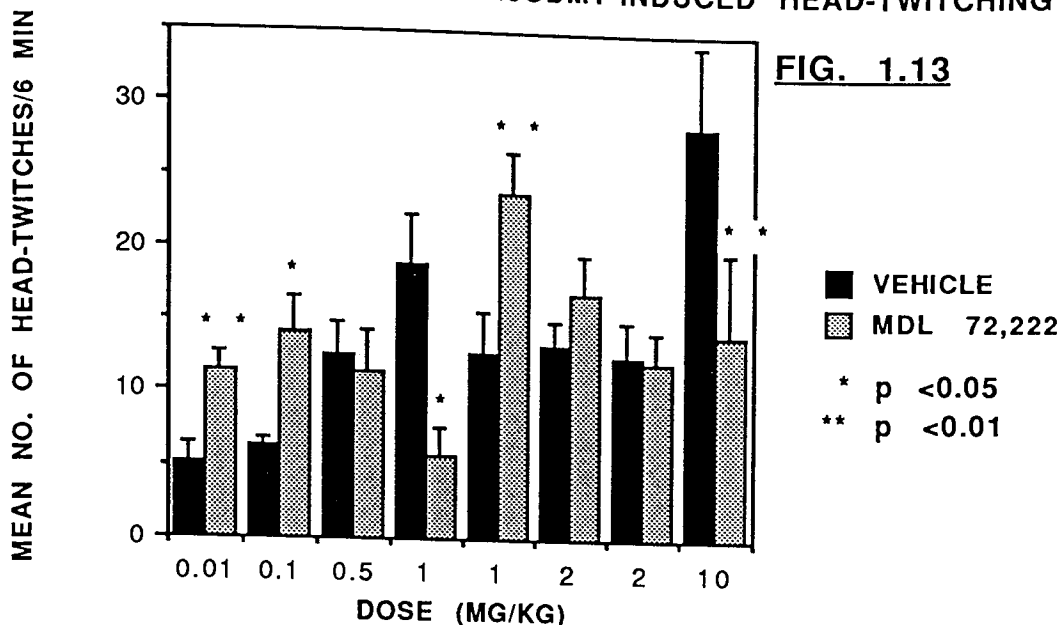
__FIG. 1.13__

TIME-COURSE FOR THE EFFECT OF MDL 72,222 ON 5-MeODMT-INDUCED HEAD-TWITCHING
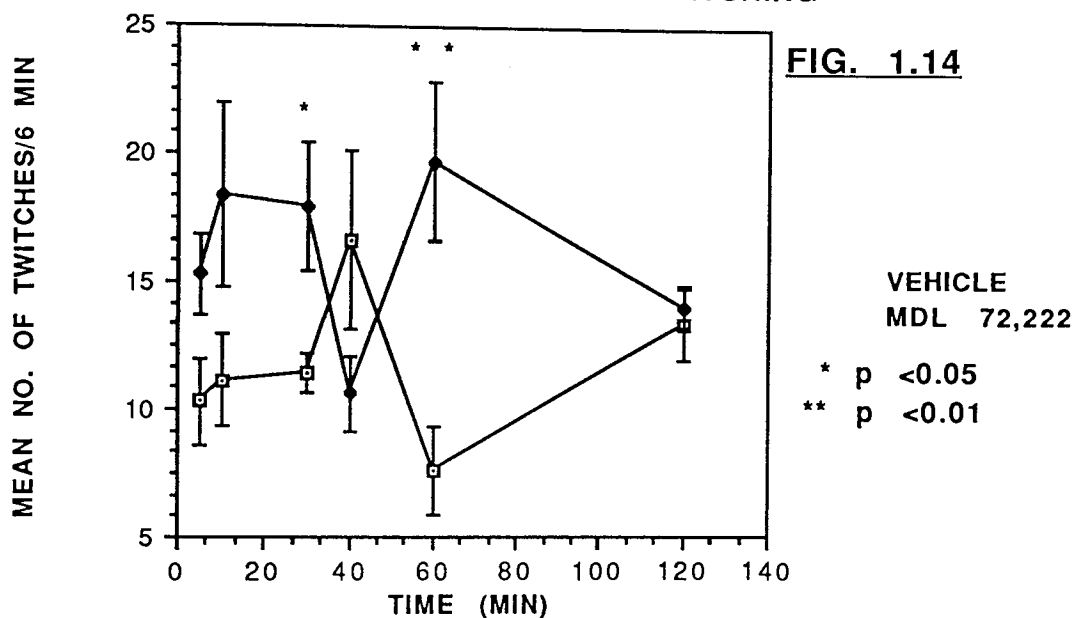
__FIG. 1.14__

All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical lines represent s.e.m.

Fig. 1.13   Control mice received vehicle (i.p.) and the test mice MDL 72,222 (i.p.). With the 1 and 2mg/kg MDL 72,222 results, the experiment was repeated and both results are presented in each case.

Fig. 1.14   Control mice received vehicle (i.p.) and test mice MDL 72, 222 (2mg/kg i.p.) at various times from 5 to 120 min prior to 5-MeODMT.

REPEAT RESULTS :
MDL 72,222 ON 5-MeODMT-INDUCED HEAD-TWITCHING
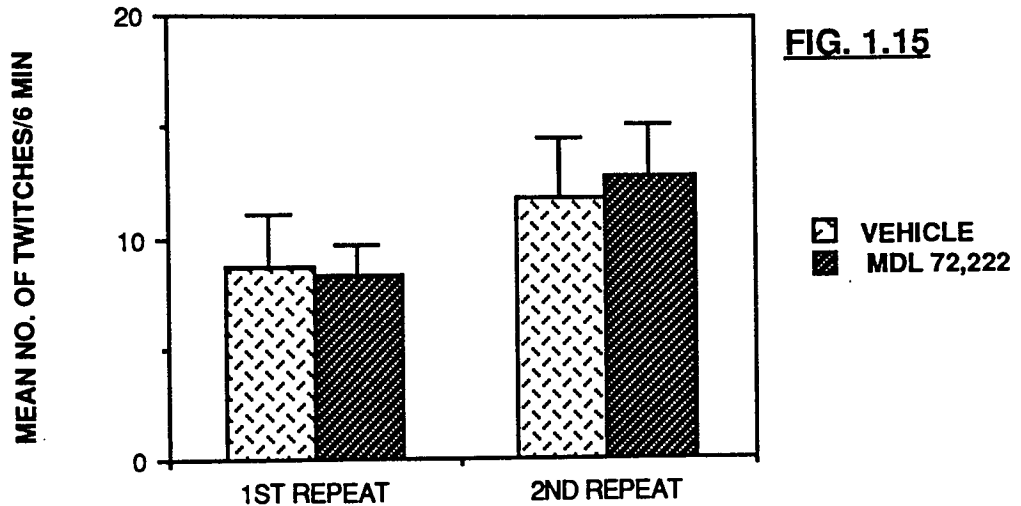
FIG. 1.15

Fig. 1.15 All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice MDL 72,222 (1mg/kg i.p.). The two results illustrated (1st and 2nd repeat) are repetition experiments done 3 and 29 months after the original work on the effect of MDL 72,222 on head-twitching was finished. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

EFFECT OF METOPROLOL ON MDL 72,222-INDUCED POTENTIATION OF 5-MeODMT-INDUCED HEAD-TWITCHING
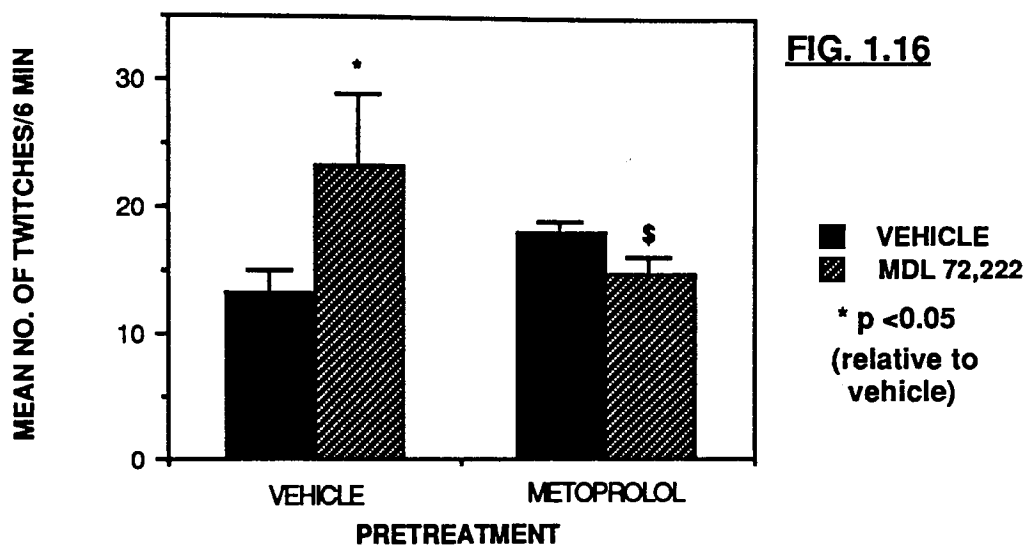
FIG. 1.16

Fig. 1.16 Mice received either MDL 72,222 (1mg/kg i.p.) or vehicle (i.p.) 20min prior, and metoprolol (2.5mg/kg s.c.) or vehicle 15min prior, to 5-MeODMT (5mg/kg i.p.). Results are the means of 6 determinations and vertical bars represent s.e.m. Results were analysed using a two-way between/within subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : F(AB) = 12.08; df 1,10; p <0.01. Interpretation : metoprolol significantly inhibits MDL 72,222-induced potentiation of 5-MeODMT-induced head-twitching.

**TIME-COURSE FOR THE EFFECT OF TYRAMINE ON
5-MeODMT-INDUCED HEAD-TWITCHING**
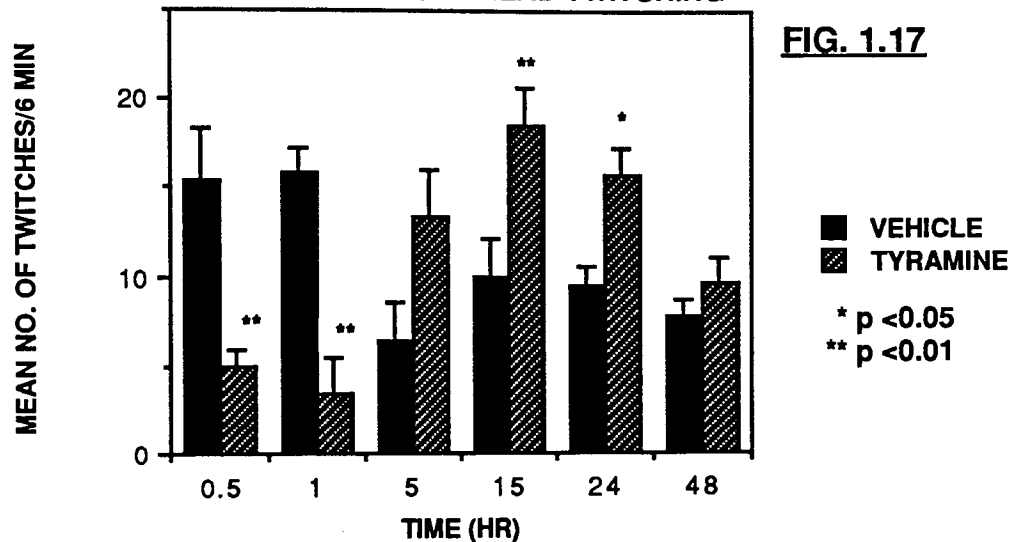
FIG. 1.17

Fig. 1.17 Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (s. c.) or tyramine (20mg/kg s. c.) at various times from 30min to 48hr prior to 5-MeODMT (5mg/kg i. p.). Results are the means of at least 6 determinations and vertical bars represent s. e. m.

TIME-COURSE FOR THE EFFECT OF BETA-PEA ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.18

■ VEHICLE
▨ BETA-PEA

* p <0.05
** p <0.01
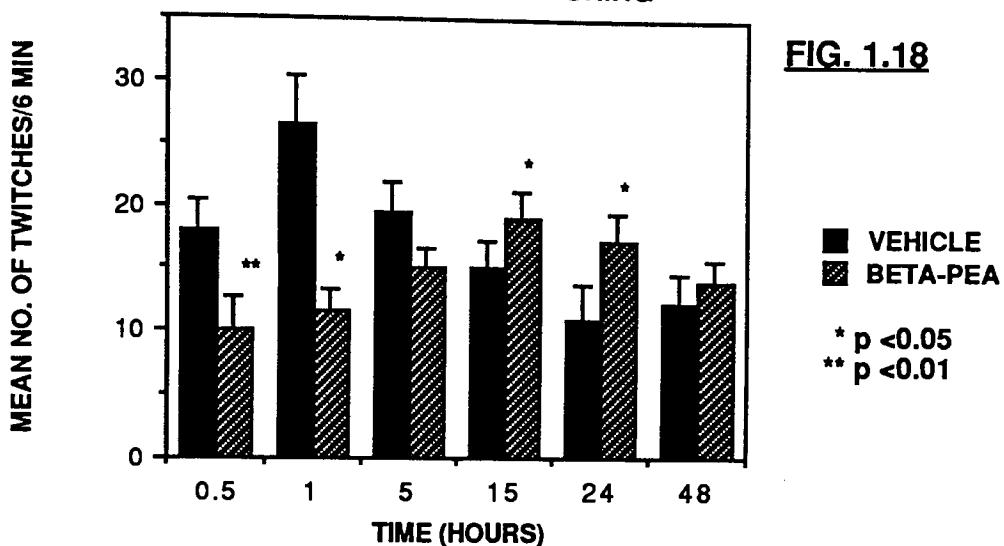
Fig. 1.18 Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (s. c.) or beta-PEA (20mg/kg s. c.) at various times from 30min to 48hr prior to 5-MeODMT (5mg/kg i. p.). Results are the means of at least 6 determinations and vertical bars represent s. e. m.

EFFECT OF METOPROLOL ON BETA-PEA-INDUCED POTENTIATION OF 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.19

VEHICLE
BETA-PEA

* p <0.05
(relative to vehicle)
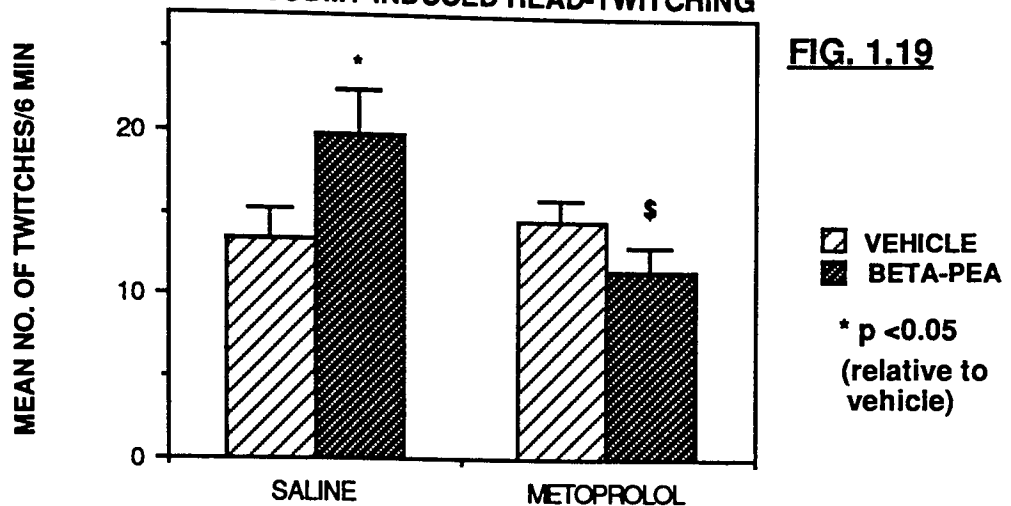
EFFECT OF METOPROLOL ON TYRAMINE-INDUCED POTENTIATION OF 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.20

VEHICLE
TYRAMINE

* p <0.05
(relative to vehicle)
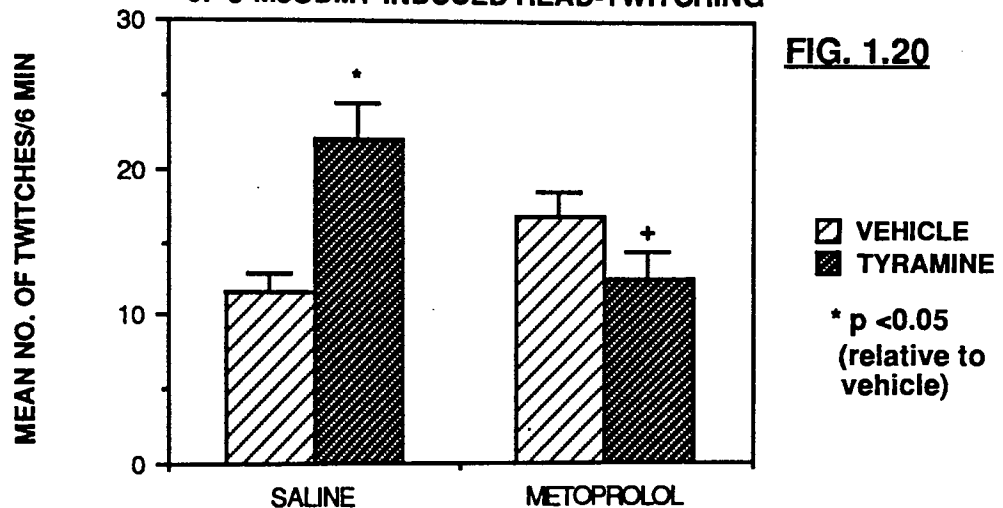
Fig. 1.19   Mice received either beta-PEA (20mg/kg s.c.) or vehicle (s.c.) 24hr prior, and metoprolol (2.5mg/kg s.c.) or vehicle (s.c.) 15min prior, to 5-MeODMT (5mg/kg i.p.).

Fig. 1.20   Mice received either tyramine (20mg/kg s.c.) or vehicle (s.c.) 24hr prior, and metoprolol (2.5mg/kg s.c.) or vehicle (s.c.) 15min prior, to 5-MeODMT (5mg/kg i.p.).

Results are the means of at least 6 determinations and vertical bars represent s.e.m. Results were analysed using a two-way between/within subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : $F(AB) = 10.05$; df 1,14; p <0.01. Interpretation : metoprolol significantly inhibits beta-PEA-induced potentiation of 5-MeODMT-induced head-twitching.

+ ANOVA result : $F(AB) = 14.86$; df 1,10; p <0.01. Interpretation : metoprolol significantly inhibits tyramine-induced potentiation of 5-MeODMT-induced head-twitching.

EFFECT OF ICI 118,551 ON THE EFFECT OF BETA-PEA ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.21

VEHICLE
BETA-PEA

NS (relative to vehicle)
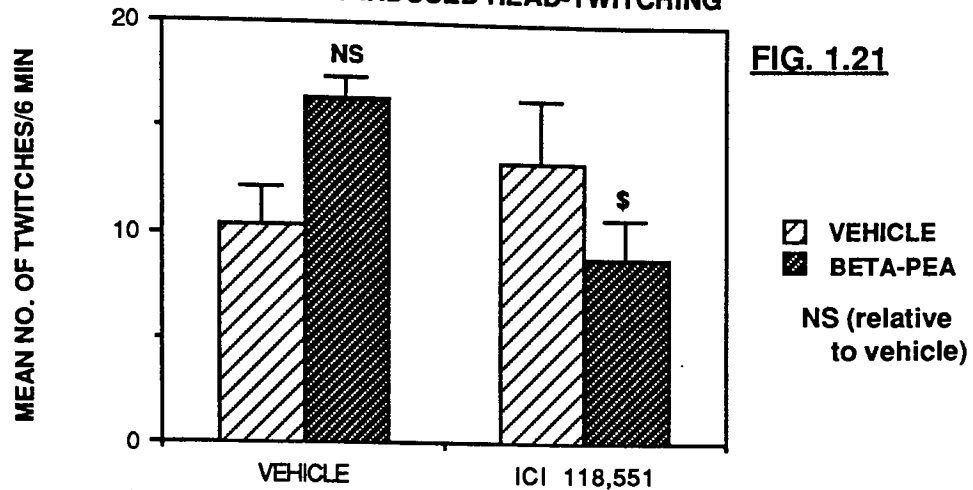
EFFECT OF ICI 118,551 ON TYRAMINE-INDUCED POTENTIATION OF 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.22

VEHICLE
TYRAMINE

* $p < 0.05$ (relative to vehicle)
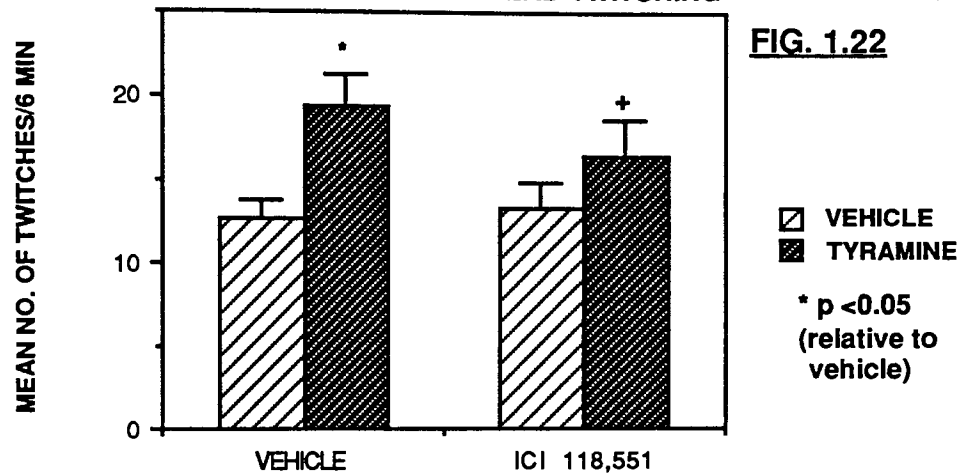
Fig. 1.21 Mice received either beta-PEA (20mg/kg s.c.) or vehicle (s.c.) 24hr prior, and ICI 118,551 (2.5mg/kg s.c.) or vehicle (s.c.) 30min prior, to 5-MeODMT (5mg/kg i.p.).

Fig. 1.22 Mice received either tyramine (20mg/kg s.c.) or vehicle (s.c.) 24hr prior, and ICI 118,551 (2.5mg/kg i.p.) or vehicle (s.c.) 30min prior, to 5-MeODMT (5mg/kg i.p.).

Results are the means of at least 6 determinations and vertical bars represent s.e.m. Results were analysed using a two-way between/within subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : $F(AB) = 7.96$; df 1,12; $p < 0.05$. Interpretation : ICI 118,551 significantly effects the interaction of beta-PEA with 5-MeODMT-induced head-twitching. Analysis using Tukey's U test (for unconfounded means) revealed no effect of beta-PEA on 5-MeODMT-induced head-twitching, however, a paired t-test on this data was significant ($p < 0.05$).

+ ANOVA result : $F(AB) = 0.75$; df 1,30; $p > 0.05$. Interpretation : ICI 118,551 has no effect on tyramine induced potentiation of 5-MeODMT-induced head-twitching.

EFFECT OF TYRAMINE PRETREATMENT ON HEAD-TWITCH-INDUCTION BY 3 DIFFERENT INDUCERS
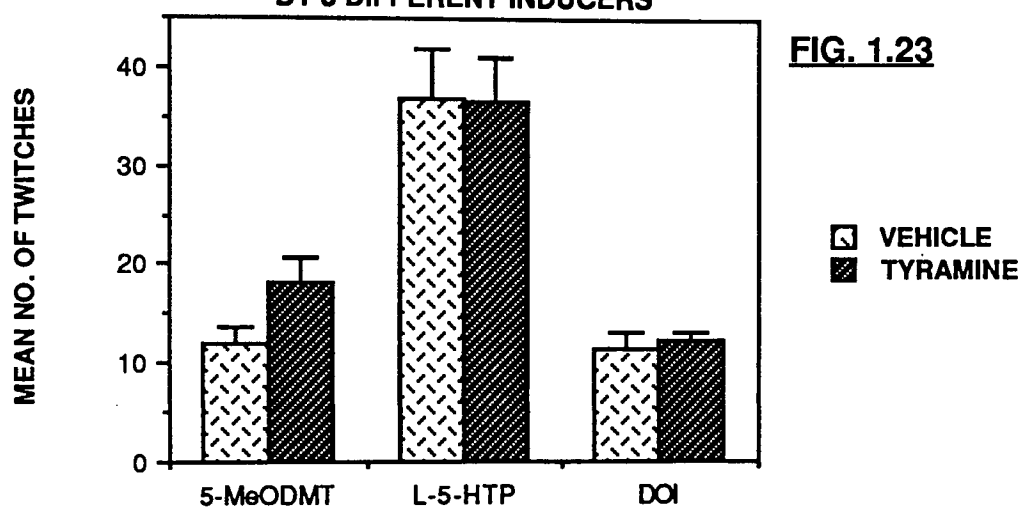
FIG. 1.23

Fig. 1.23 Pairs of mice were assigned at random to control or test conditions and observed in parallel for each experiment. Control mice received vehicle (s. c.) the test mice tyramine (20mg/kg s. c.) 24hr prior to treatment with one of three head-twitch inducers - 5-MeODMT (5mg/kg i. p.), 5-HTP (200mg/kg i. p.) and carbidopa (9mg/kg s. c.) or (±)DOI (0. 5mg/kg i. p.). Results are the means of at least 6 determinations and vertical bars represent s. e. m.

EFFECT OF SUB-CHRONIC TYRAMINE TREATMENT ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 1.24
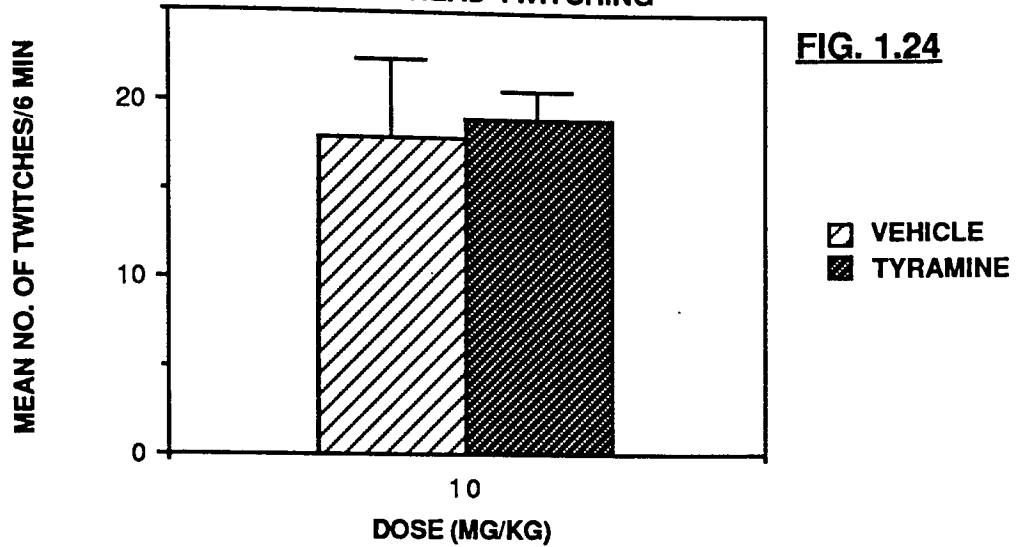
EFFECT OF TYRAMINE (24 HR PRETREATMENT) ON 5-MeODMT-INDUCED HEAD-TWITCHING OF TUK MF1-STRAIN MICE
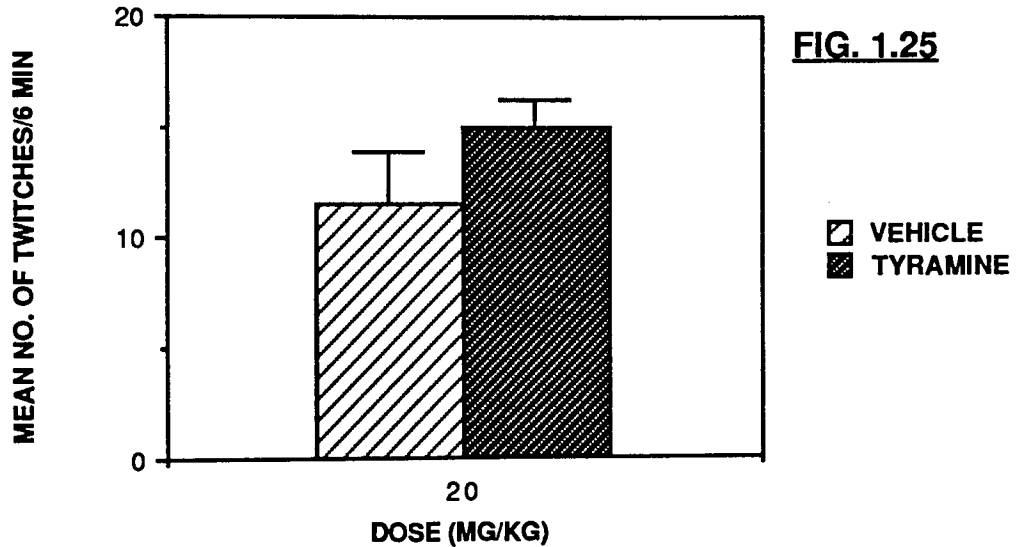
FIG. 1.25

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 1.24 Control mice received vehicle (s.c.) and test mice tyramine (s.c.) daily for 5 days then 5-MeODMT (5mg/kg i.p.) 24hr after the last tyramine injection.

Fig. 1.25 Control mice received vehicle (s.c.) and test mice tyramine (s.c.) 24hr prior to 5-MeODMT (5mg/kg i.p.). Mice used were of the Tuk MF1-strain.

EFFECT OF TYRAMINE (24HR PRETREATMENT) ON 5-MeODMT-INDUCED HEAD-TWITCHING OF OLAC MF1-STRAIN MICE

FIG. 1.26

EFFECT OF SUB-CHRONIC CLONIDINE ON 5-MeODMT-INDUCED HEAD-TWITCHING
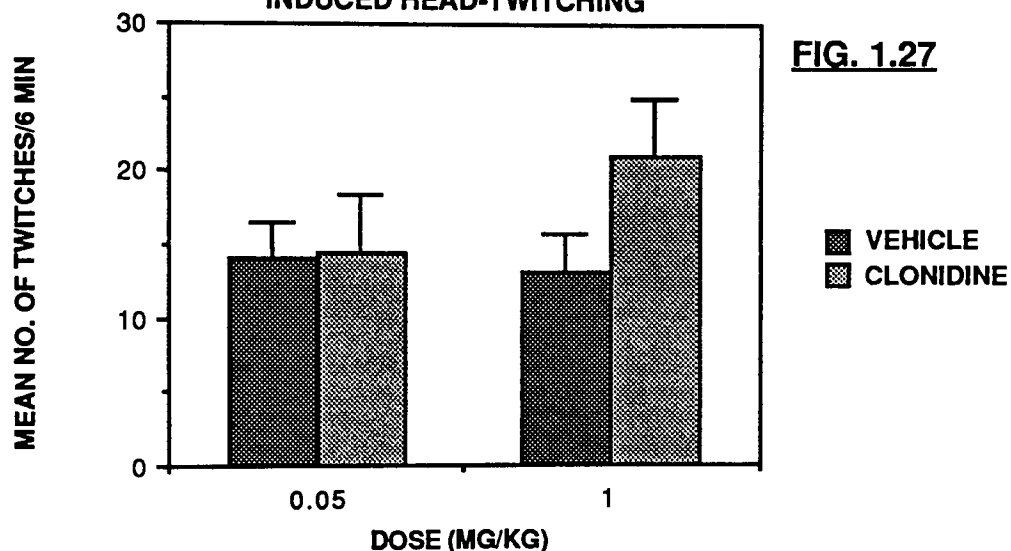
FIG. 1.27

Pairs of mice were observed at random to control or test conditions and observed in parallel. Results are the means of at least 5 determinations and vertical bars represent s.e.m.

Fig. 1.26 Control mice received vehicle (s.c.) and test mice tyramine (s.c.) 24hr prior to 5-MeODMT (5mg/kg i.p.). Mice used were of the Olac MF1-strain.

fig. 1.27 Control mice received vehicle (i.p.) and test mice clonidine (i.p.) daily for 5 days then 5-MeODMT (5mg/kg i.p.) 24 hr after the last clonidine injection.

EFFECT OF REPEATED ADMINISTRATION OF 5-MeODMT
ON HEAD-TWITCHING. EXPERIMENT 1.
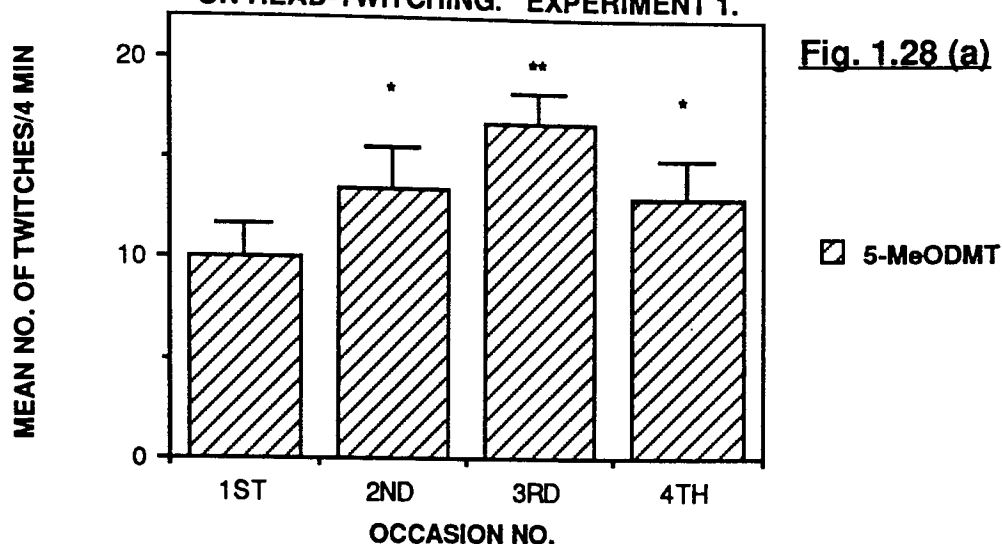
Fig. 1.28 (a)

5-MeODMT

EFFECT OF REPEATED ADMINISTRATION OF 5-MeODMT
ON HEAD-TWITCHING. EXPERIMENT 2.
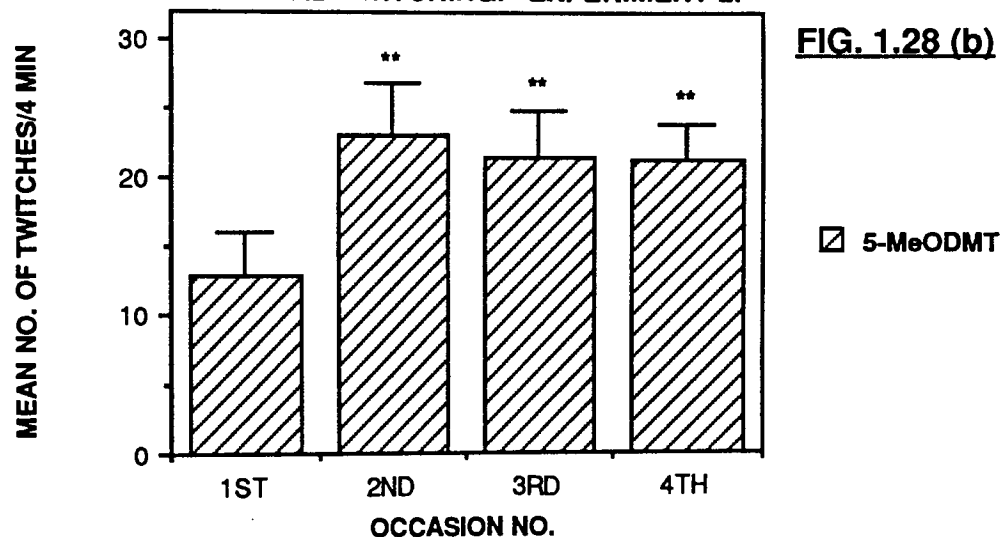
FIG. 1.28 (b)

5-MeODMT

Fig. 1.28 Each occasion was consecutive (1st to 4th) and five days apart. Dose of 5-MeODMT used in each experiment = 5mg/kg i.p.. Results are the means of at least 8 determinations and vertical bars represent s.e.m. Results were analysed statistically using a one-way within subjects ANOVA followed by Duncan's multiple-range test. * p <0.05 ** p <0.01 (relative to vehicle controls).

EFFECT OF REPEATED ADMINISTRATION OF DOI ON HEAD-TWITCHING
EXPERIMENT 1.
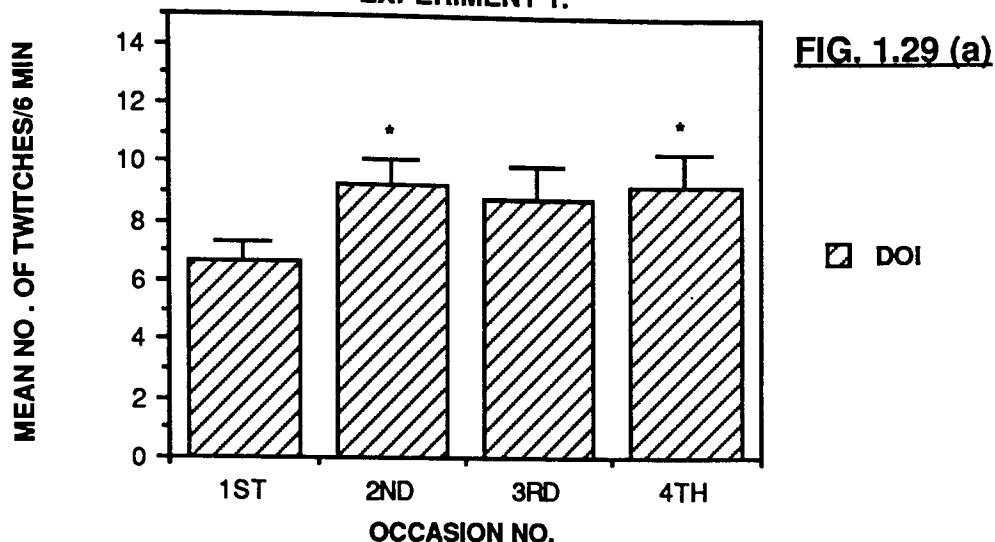
FIG. 1.29 (a)

EFFECT OF REPEATED ADMINISTRATION OF DOI ON HEAD-TWITCHING
EXPERIMENT 2
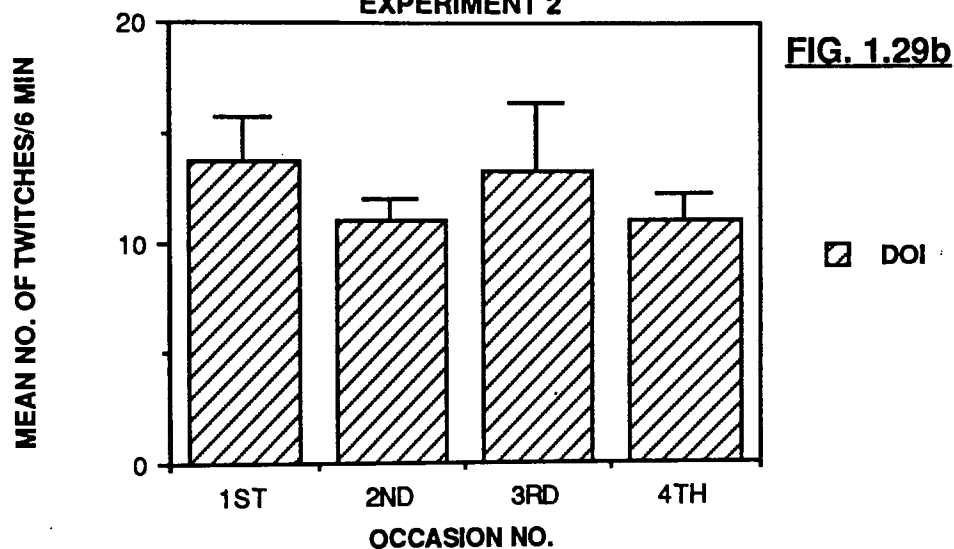
FIG. 1.29b

Fig. 1.29 Each occasion was consecutive (1st to 4th) and five days apart. Dose of (±)DOI used in each experiment = 0.5mg i.p.. Results are the means of at least 6 determinations and vertical bars represent s.e.m. Results were analysed statistically using a one-way within subjects ANOVA followed by Duncan's multiple-range test. * p <0.05 ** p <0.01 (relative to vehicle controls).

**ANALYSIS OF THE DISTRIBUTION OF HEAD-TWITCHING FREQUENCY
(5-MeODMT) IN ALL VEHICLE TREATED EXPT. MICE USED**
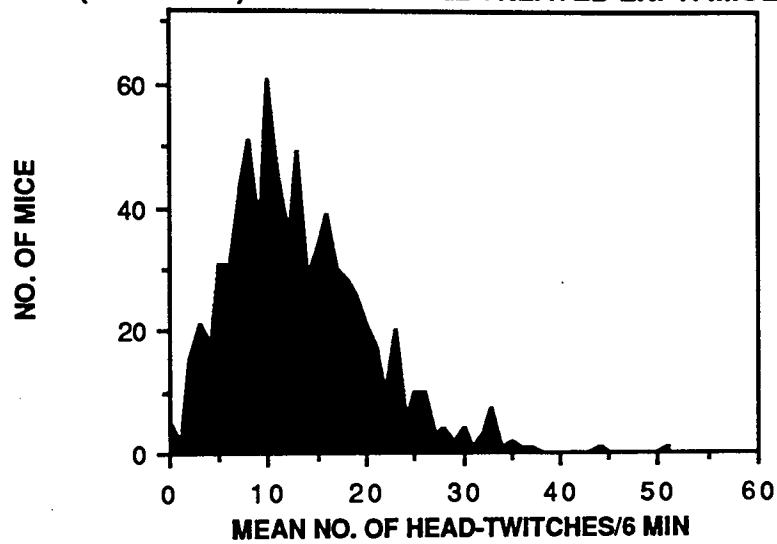
Fig. 1.30 A frequency distribution graph illustrating the skewed
normal distribution of the 5-MeODMT-induced head-twitching
frequencies of all the vehicle-treated control animals used in the
experiments outlined in this thesis.

Table 1.1 The effect of the acute migraine treatments - aspirin and ergotamine - on 5-MeODMT-induced head-twitching.

aspirin (i.p.)	30min prior to 5-MeODMT (5mg/kg i.p.)			
10mg/kg	vehicle (v)	13.2 (2.8)	NS	
	aspirin (a)	18.2 (4.4)		
50mg/kg	v	13.8 (2.4)	NS	
	a	14.8 (2.6)		

ergotamine (i.p.)	30min prior to 5-MeODMT (5mg/kg i.p.)			
0.1mg/kg	v	8.5 (1.4)	NS	
	ergotamine (e)	11.0 (1.5)		
1mg/kg	v	18.6 (3.5)	NS	
	e	15.4 (3.3)		

NS = non significant - p >0.05.
Results are expressed as mean head-twitch counts (from at least 6 determinations), bracketed figures represent s.e.m.

Table 1.2 (a) The effect of some prophylactic migraine treatments on 5-MeODMT-induced head-twitching.

Drug	Dose range tested (mg/kg)	p	r	ID50 (mg/kg)	95% confidence limits (mg/kg)
cyproheptadine	0.05-5	<0.001	-0.66	0.021	5.82×10^{-7} to 0.19
methysergide	0.1-5	<0.002	-0.74	0.070	3.45×10^{-4} to 2.15
pizotifen	0.01-2	<0.001	-0.63	0.12	3.45×10^{-4} to 35.7
verapamil	10-50	<0.031	-0.49	30.75	0.23 to 74400
nitrendipine	25-50	<0.043	-0.42	44.02	15.6 to 855000

p = significance of slope/scatter F ratio of linear regression analysis
r = correlation coefficient
ID50 = dose to decrease head-twitching to 50% of control level.

Cyproheptadine, methysergide, and pizotifen were administered i.p. 30min prior, and verapamil and nitrendipine i.p. 15min prior, to 5-MeODMT (5mg/kg i.p.).

Table 1.2 (b) The effect of two beta-adrenoceptor antagonists (nadolol and pindolol), which have been tested for activity in migraine prophylaxis, on 5-MeODMT-induced head-twitching.

nadolol (i.p.)	20min prior to 5-MeODMT (5mg/kg i.p.)		
5mg/kg	v	13.2 (2.8)	NS
	nadolol (n)	12.0 (2.8)	
pindolol (i.p.)	30min prior to 5-MeODMT (5mg/kg i.p.)		
0.25mg/kg	v	13.8 (2.9)	NS
	pindolol (p)	6.0 (2.3)	
2.5mg/kg	v	12.4 (2.2)	NS
	p	11.2 (3.2)	
10mg/kg	v	16.5 (4.1)	NS
	p	14.2 (2.8)	

Table 1.2 (c) The effect of BAY K8644 on 5-MeODMT-induced head-twitching.

BAY K8644 (i.p.)	15min prior to 5-MeODMT (5mg/kg i.p.)		
1mg/kg	vehicle (v)	13.2 (2.2)	NS
	BAY K8644 (b)	11.5 (1.8)	
5mg/kg	v	18.2 (2.5)	p <0.05
	b	6.7 (1.8)	

NS = non significant - $p > 0.05$.
Results are expressed as mean head-twitch counts (from at least 6 determinations). Bracketed figures represent s.e.m..

Table 1.3 (a) The effect of 5-HT3 antagonists on 5-MeODMT-induced head-twitching.

GR38032F (i.p.)	20min prior to 5-MeODMT (5mg/kg i.p.)			
0.001mg/kg		v	15.2 (2.1)	NS
	GR38032F (g)		12.9 (2.2)	
0.01mg/kg		v	9.8 (2.1)	NS
		g	12.1 (1.2)	
0.1mg/kg		v	14.4 (3.0)	NS
		g	11.6 (2.6)	
1mg/kg		v	12.4 (1.0)	NS
		g	9.1 (1.4)	

ICS 205-930 (i.p.)	20min prior to 5-MeODMT (5mg/kg i.p.)			
0.001mg/kg		v	20.4 (6.4)	NS
	ICS 205-930 (i)		20.4 (4.8)	
0.01mg/kg		v	10.6 (1.8)	NS
		i	18.3 (4.3)	
0.1mg/kg		v	22.5 (2.5)	NS
		i	19.5 (2.6)	
1mg/kg		v	14.6 (2.6)	NS
		i	15.0 (2.2)	
10mg/kg		v	12.9 (1.6)	NS
		i	11.9 (1.8)	

MDL 72,222 (i.p.)	20min prior to 5-MeODMT (5mg/kg i.p.)			
0.01mg/kg		v	5.1 (1.3)	$p < 0.01$
		m	11.4 (1.3)	
0.1mg/kg		v	6.2 (0.7)	$p < 0.05$
		m	14.0 (2.5)	
0.5mg/kg		v	12.5 (2.4)	NS
		m	11.5 (2.8)	
1mg/kg	1st experiment	v	18.8 (3.5)	$p < 0.05$
		m	5.8 (1.8)	
1mg/kg	2nd experiment	v	12.7 (2.9)	$p < 0.01$
		m	23.7 (2.9)	
2mg/kg	1st experiment	v	13.3 (1.8)	NS
		m	16.9 (2.7)	
2mg/kg	2nd experiment	v	12.5 (2.6)	NS
		m	12.2 (2.1)	
10mg/kg		v	28.6 (5.7)	$p < 0.01$
		m	14.1 (5.7)	

Results are expressed as mean head-twitch counts (from at least 6 determinations). Bracketed figures represent s.e.m..
NS = non significant - $p > 0.05$.

Fig 1.3 (b)   Time-course for the effect of MDL 72,222 on 5-MeODMT.

MDL 72,222 (2mg/kg i.p.) prior to 5-MeODMT (5mg/kg i.p.)

time (min)					
5	10	30	40	60	120
v 10.3 (1.7)	11.1 (1.8)	11.4 (0.8)	16.6 (3.5)	7.6 (1.7)	13.4 (1.5)
m 15.3 (1.6)	18.4 (3.6)	17.9 (2.5)	10.6 (1.5)	19.7 (3.1)	14.0 (0.8)
NS	NS	$p < 0.05$	NS	$p < 0.01$	NS

NS = non significant - $p > 0.05$.
Results are expressed as mean head-twitch counts (from at least 5
determinations). Bracketed figures represent s.e.m..

Table 1.4   The effect of metoprolol on MDL 72,222-induced potentiation
of 5-MeODMT-induced head-twitching.

Mice were given either metoprolol (2.5mg/kg s.c.) or vehicle (i.p)
15min prior, and MDL 72,222 (1mg/kg i.p.) or vehicle (i.p.) 20min
prior, to 5-MeODMT (5mg/kg i.p.).

vehicle--------vehicle   13.2 (1.9)   F(A) = 0.09   df 1,10 NS
MDL 72,222----vehicle   23.3 (5.7)   F(B) = 3.73   df 1,10 NS
vehicle--------metoprolol 18.0 (0.9)   F(AB) = 12.08   df 1,10 $p < 0.01$
MDL 72,222----metoprolol 14.7 (1.4)

F(A) = metoprolol.
F(B) = MDL 72,222.
F(AB)= the interaction of MDL 72,222 and metoprolol with respect to 5-
MeODMT-induced head-twitching.

Results are expressed as mean head-twitch scores (from 6
determinations). Bracketed figures represent s.e.m.

The results were analysed using a two-way between/within ANOVA on the
raw data followed by Tukey's U test (for unconfounded means).

Tukey's U test results------v/v compared with v/metoprolol   NS
                           v/v compared with s/MDL 72,222 $p < 0.05$
      v/metoprolol compared with metoprolol/MDL 72,222   NS
      v/MDL 72,222 compared with metoprolol/MDL 72,222   NS

The Tukey's U test results are represented as the treatments that the
mice received whose means were compared using this test.

NS = non significant - $p > 0.05$.

Table 1.5 Repeat experiments performed 3 and 29 months after the original work of the effect of MDL 72,222 (1mg/kg i.p. 20min pretreatment) on 5-MeODMT (5mg/kg i.p.)-induced head-twitching.

Repeat 1 (3 months)
----
	v	8.7 (2.3)	NS
MDL 72,222 (m)		8.3 (1.4)	

Repeat 2 (29 months)
----
v	11.8 (2.7)	NS
m	12.8 (2.3)	

Table 1.6 Time-course for the effect of the migraine triggers – beta-PEA (b) and tyramine (t) – on 5-MeODMT-induced head-twitching.

beta-PEA (20mg/kg s.c.) prior to 5-MeODMT (5mg/kg i.p.)
----
time (hr)

	0.5	1	5	15	24	48
s	17.9 (2.5)	26.4 (4.0)	19.5 (2.3)	15.2 (2.0)	11.0 (2.8)	12.3 (2.3)
b	10.0 (2.6)	11.6 (1.8)	15.2 (1.5)	19.0 (2.2)	17.2 (2.2)	13.9 (1.9)
	p <0.01	p <0.05	NS	p <0.05	p <0.05	NS

tyramine (20mg/kg s.c.) prior to 5-MeODMT (5mg/kg i.p.)
----
time (hr)

	0.5	1	5	15	24	48
s	15.4 (2.9)	15.8 (1.2)	6.6 (1.5)	10.0 (2.1)	9.5 (1.1)	7.7 (1.1)
t	4.9 (1.2)	3.4 (2.1)	8.8 (2.5)	18.5 (2.1)	15.7 (1.5)	9.7 (1.3)
	p <0.01	p <0.01	NS	p <0.01	p <0.05	NS

Results are expressed as mean head-twitch counts (from at least 5 determinations). Bracketed figures represent s.e.m..
NS = non significant – p >0.05.

<u>Table 1.7</u> <u>Effect of metoprolol on the potentiation of 5-MeODMT-</u>
<u>induced head-twitching induced by beta-PEA or tyramine.</u>

Mice were given either tyramine/beta-PEA (20mg/kg s.c.) or vehicle
(s.c.) 24hr prior, and metoprolol (2.5mg/kg s.c.) 15min prior, to 5-
MeODMT (5mg/kg i.p.).

<u>beta-PEA.</u>

vehicle--------vehicle   13.4 (1.8)   F(A) = 2.50   df 1,14 NS
beta-PEA--------vehicle   19.8 (2.7)   F(B) = 1.18   df 1,14 NS
vehicle--------metoprolol 14.6 (1.3)   F(AB) = 10.05   df 1,14 p <0.01
beta-PEA--------metoprolol 11.5 (1.5)

F(A) = metoprolol.
F(B) = beta-PEA.
F(AB)= the interaction between beta-PEA and metoprolol with respect to
5-MeODMT-induced head-twitching.

Tukey's U test results---------v/v compared with v/beta-PEA   p <0.05
                               v/v compared with v/metoprolol   NS
                v/metoprolol compared with metoprolol/beta-PEA NS
                v/beta-PEA compared with metoprolol/beta-PEA   p <0.01

<u>Tyramine.</u>

vehicle--------vehicle   11.5 (1.3)   F(A) = 2.77   df 1,10 NS
tyramine--------vehicle   22.0 (2.5)   F(B) = 1.18   df 1,10 NS
vehicle--------metoprolol 16.8 (1.7)   F(AB) = 14.86   df 1,10 p <0.01
tyramine--------metoprolol 12.5 (1.8)

F(A) = metoprolol.
F(B) = tyramine.
F(AB)= the interaction between tyramine and metoprolol with respect to
5-MeODMT-induced head-twitching.

Tukey's U test results-------v/v compared with v/tyramine   p <0.05
                             v/v compared with v/metoprolol   NS
              v/metoprolol compared with tyramine/metoprolol   NS
               v/tyramine compared with tyramine/metoprolol   p <0.05

Results are expressed as mean head-twitch scores (from 8 (beta-PEA)
and 6 (tyramine) determinations). Bracketed figures represent s.e.m.

The results were analysed using a two-way between/within ANOVA on the
raw data followed by Tukey's U test (for unconfounded means). The
Tukey's U test results are represented as the treatments that the mice
received whose means were compared using this test.

NS = non significant - p >0.05.

Table 1.8   Effect of ICI 118,551 on potentiation/effect of
tyramine/beta-PEA (respectively) on 5-MeODMT-
induced head-twitching.

beta-PEA.

vehicle-------vehicle	10.4 (1.7)	F(A)	= 1.12	df 1,12	NS
beta-PEA-------vehicle	16.4 (1.0)	F(B)	= 0.15	df 1,12	NS
vehicle-------ICI 118,551	13.4 (3.0)	F(AB)	= 7.96	df 1,12	$p < 0.05$
beta-PEA-------ICI 118,551	8.9 (1.8)				

F(A) = ICI 118,551.
F(B) = beta-PEA.
F(AB)= the interaction between beta-PEA and ICI 118,551 with respect
to 5-MeODMT-induced head-twitching.

Tukey's U test results-------v/v compared with v/beta-PEA   NS
                      v/v compared with v/ICI 118,551   NS
       v/ICI 118,551 compared with ICI 118,551/beta-PEA   NS
         v/beta-PEA compared with ICI/118,551/beta-PEA   NS

Tyramine.

vehicle-------vehicle	12.6 (1.2)	F(A)	= 0.49	df 1,30	NS
tyramine------vehicle	19.3 (2.0)	F(B)	= 7.60	df 1,30	$p < 0.01$
vehicle-------ICI 118,551	13.3 (1.6)	F(AB)	= 0.75	df 1,30	NS
tyramine------ICI 118,551	16.4 (2.2)				

F(A) = ICI 118,551.
F(B) = tyramine.
F(AB)= the interaction between tyramine and ICI 118,551 with respect
to 5-MeODMT-induced head-twitching.

Tukey's U test results-------v/v compared with v/tyramine $p < 0.05$
                      v/v compared with v/ICI 118,551   NS
       v/ICI 118,551 compared with ICI 118,551/tyramine   NS
         s/tyramine compared with ICI 118,551/tyramine   NS

Results are expressed as mean head-twitch scores (from 7 (beta-PEA)
and 16 (tyramine) determinations). Bracketed figures represent s.e.m..

The results were analysed using a two-way between/within ANOVA on the
raw data followed by Tukey's U test (for unconfounded means). The
Tukey's U test results are represented as the treatments that the mice
received whose means were compared using this test.

NS = non significant - $p > 0.05$.

Table 1.9 Experiments investigating the loss of effect of the
migraine trigger, tyramine, on 5-MeODMT-induced head-
twitching.

a) Effect of tyramine on (±)DOI-, 5-HTP- and 5-MeODMT-induced head-
twitching.

Mice received either tyramine (20mg/kg s.c.) or vehicle (s.c.) 24hr
prior to (±)DOI (0.5mg/kg i.p.), 5-HTP (200mg/kg i.p.) and carbidopa
(9mg/kg s.c.) or 5-MeODMT (5mg/kg i.p.).

	(±)DOI		5-HTP		5-MeODMT	
vehicle	11.5 (1.5)	NS	37.0 (4.8)	NS	12.0 (1.6)	NS
tyramine	12.2 (0.8)		36.5 (4.6)		18.2 (2.6)	

b) Effect of using two different strains of MF1 mice as the
experimental animals on the effect of tyramine on 5-MeODMT-induced
head-twitching.

Tuk MF1 or Olac MF1 received either tyramine (20mg/kg s.c.) or vehicle
(s.c.) 24hr prior to 5-MeODMT (5mg/kg i.p.).

	Tuk MF1		Olac MF1	
vehicle	11.5 (2.4)	NS	18.8 (4.1)	NS
tyramine	15.0 (1.3)		16.8 (5.8)	

Results are expressed as mean head-twitch counts (from at least 5
determinations). Bracketed figures represent s.e.m..
NS = non-significant - p >0.05.

Table 1.9 continued :

c) Multidose/multipretreatment time study of the effect of tyramine on
   5-MeODMT-induced head-twitching (part I).

Mice recieved either tyramine (t) (10 or 20mg/kg s.c.) or vehicle
(s.c.) 15, 24 or 30hr prior to 5-MeODMT (2.5 or 5mg/kg i.p.). Head-
twitches were counted on alternate minutes from 3rd to 10th min after
injection of 5-MeODMT.

---

tyramine	20mg/kg	15hr pre.					
5-MeODMT	5mg/kg	v 10.5 (1.9)	NS	2.5mg/kg	v 3.3 (1.7)	NS
		t 15.5			t 7.0 (3.1)	

tyramine	10mg/kg	15hr pre.					
5-MeODMT	5mg/kg	v 11.0 (3.5)	NS	2.5mg/kg	v 8.0 (1.8)	NS
		t 11.0 (1.5)			t 8.8 (1.7)	

tyramine	10mg/kg	24hr pre.					
5-MeODMT	5mg/kg	v 11.3 (4.4)	NS	2.5mg/kg	v 9.3 (1.2)	NS
		t 10.0 (1.5)			t 6.3 (1.5)	

tyramine	20mg/kg	30hr pre.					
5-MeODMT	5mg/kg	v 2.5 (1.9)	NS	2.5mg/kg	v 3.5 (1.0)	NS
		t 4.8 (2.0)			t 3.8 (1.4)	

tyramine	10mg/kg	30hr pre.					
5-MeODMT	5mg/kg	v 4.8 (1.7)	NS	2.5mg/kg	v 9.5 (3.4)	NS
		t 3.5 (1.3)			t 6.3 (2.4)	

Results are expressed as mean head-twitch counts (from 4
determinations). Bracketed figures represent s.e.m.. Times "pre" are
the time prior to 5-MeODMT (i.p.) that the mice received tyramine
(s.c.) or vehicle (s.c.).
NS = non significant - $p < 0.05$.

Table 1.9 c) continued PART II :

Effect of tyramine on 5-MeODMT-induced head-twitching.

Mice received either tyramine (20mg/kg s.c.) or vehicle (s.c.) 15 or 24hr prior to 5-MeODMT (5mg/kg i.p.).

	15hr		24hr	
vehicle	10.4 (2.5)	NS	10.8 (2.5)	NS
tyramine	8.0 (1.5)		11.2 (2.0)	

Results are expressed as mean head-twitch scores (from 6 determinations). Bracketed figures represent s.e.m. NS = non significant - p >0.05.

d) Effect of subchronic tyramine on 5-MeODMT-induced head-twitching.

Mice received either tyramine (20mg/kg s.c.) or vehicle (s.c.) daily for 4 days and then 5-MeODMT (5mg/kg i.p.) 24hr after the last injection.

vehicle	11.5 (2.4)	NS
tyramine	15.0 (1.3)	

e) Repeat experiments - Effect of tyramine on 5-MeODMT-induced head-twitching.

Effect of tyramine (20mg/kg s.c.) on 5-MeODMT (5mg/kg i.p.)-induced head-twitching.

Mice received either tyramine or vehicle (s.c.) 30 min or 24hr prior to 5-MeODMT.

24hr.

a)	vehicle	18.8 (1.0)	NS
	tyramine	17.8 (3.4)	

b)	vehicle	13.7 (2.7)	NS
	tyramine	10.2 (2.1)	

30min.

a)	vehicle	9.5 (2.1)	NS
	tyramine	6.5 (1.1)	

Table 1.10 Experiments investigating the loss of effect of the
migraine trigger, beta-PEA, on 5-MeODMT-induced head-
twitching.

a) Effect of beta-PEA (20mg/kg s.c.) on 5-MeODMT (5mg/kg i.p.)-induced
head-twitching

Mice received either beta-PEA or vehicle (s.c.) 30min or 24hr prior
to 5-MeODMT.

30min.

| 1) | vehicle | 8.3 (1.2) | p <0.01 |
| | beta-PEA | 20.2 (3.0) | |

| 2) | vehicle | 8.7 (1.7) | NS |
| | beta-PEA | 10.5 (1.3) | |

| 3) | vehicle | 15.4 (3.2) | NS |
| | beta-PEA | 11.0 (1.6) | |

24hr.

| 1) | vehicle | 13.8 (2.6) | NS |
| | beta-PEA | 15.2 (3.4) | |

| 2) | vehicle | 15.8 (2.0) | NS |
| | beta-PEA | 15.8 (3.7) | |
---

b) Effect of beta-PEA (40mg/kg) on 5-MeODMT (5mg/kg i.p.)-induced
head-twitching.

Mice received either beta-PEA or vehicle (s.c.) 24hr prior to 5-
MeODMT.

| | vehicle | 11.5 (3.5) | NS |
| | beta-PEA | 14.5 (3.5) | |

Table 1.11 Effect of subchronic clonidine on 5-MeODMT-induced head-
twitching.

Mice received either clonidine (0.05 and 1mg/kg i.p.) or vehicle
(i.p.) for 4 days then 5-MeODMT (5mg/kg i.p.) 24hr after the last
injection.

clonidine (mg/kg)	0.05		1	
vehicle	14.0 (2.5)	NS	13.2 (2.5)	NS
clonidine	14.4 (4.0)		21.0 (3.9)	

Results are expressed as mean head-twitch counts (from at least 5
determinations). Bracketed figures represent s.e.m..
NS = non significant - $p > 0.05$.

<u>Table 1.12</u> <u>Effect of repeated, intermittent, administration of (±)DOI</u>
<u>and 5-MeODMT on head-twitch frequency induced by these</u>
<u>agents.</u>

Mice received either (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.)
on four consecutive occasions five days apart and their head twitch
frequency/6min (±)DOI or 4min (5-MeODMT) was determined. The
experiment was repeated twice for each head-twitch inducer.

<u>(±)DOI.</u>

1)	1st occasion	6.7 (0.6)	$F = 2.27$ df 3,33 NS
	2nd occasion	9.3 (0.8) *	
	3rd occasion	8.8 (1.1)	
	4th occasion	9.3 (1.10 *	

2) Repeat :	1st occasion	13.8 (1.9)	$F = 0.61$ df 3,15 NS
	2nd occasion	11.0 (1.0)	
	3rd occasion	13.2 (3.2)	
	4th occasion	11.0 (1.3)	

<u>5-MeODMT.</u>

3)	1st occasion	10.1 (1.6)	$F = 9.77$ df 3,33 $p < 0.01$
	2nd occasion	13.5 (2.1) *	
	3rd occasion	16.8 (1.5) **	
	4th occasion	13.0 (2.0) *	

4) Repeat :	1st occasion	12.9 (3.2)	$F = 6.88$ df 3,21 $p < 0.01$
	2nd occasion	23.0 (3.7) **	
	3rd occasion	21.4 (3.5) **	
	4th occasion	21.3 (2.3) **	

Results are expressed as mean head-twitch counts (from at least 6
determinations). Bracketed figures represent s.e.m.
Results were analysed using a one-way within ANOVA followed by
Duncan's multiple-range test on raw data. The F values quoted indicate
whether there is any significant difference between the four occasions
with regard to the head-twitch frequencies observed for each treatment
group i.e. 1), 2), 3) and 4). The results of Duncan's multiple-range
test are illustrated as * and **, * = $p < 0.05$ and ** = $p < 0.01$
(relative to 1st occasion results).

Table 1.12 continued :

Results were analysed statistically using least square error linear regression analysis to compare the raw data from different occasions :

(±)DOI	r	df	F	
1) 1st and 2nd occasion	0.26	11	0.07	NS
1st and 3rd occasion	0.0036	11	0.0001	NS
1st and 4th occasion	0.23	11	0.59	NS
2nd and 3rd occasion	0.62	11	6.36	$p < 0.05$
2nd and 4th occasion	0.60	11	5.75	$p < 0.05$
3rd and 4th occasion	0.21	11	0.46	NS
2) 1st and 2nd occasion	0.48	5	1.22	NS
1st and 3rd occasion	0.25	5	0.27	NS
1st and 4th occasion	0.032	5	0.006	NS
2nd and 3rd occasion	0.16	5	0.11	NS
2nd and 4th occasion	0	5	0	NS
3rd and 4th occasion	0.76	5	5.61	NS

5-MeODMT	r	df	F	
3) 1st and 2nd occasion	0.53	10	4.01	NS
1st and 3rd occasion	0.45	10	2.53	NS
1st and 4th occasion	0.58	10	5.20	$p < 0.05$
2nd and 3rd occasion	0.69	10	9.09	$p < 0.05$
2nd and 4th occasion	0.83	10	21.46	$p < 0.01$
3rd and 4th occasion	0.71	10	10.41	$p < 0.01$
4) 1st and 2nd occasion	0.76	7	8.30	$p < 0.05$
1st and 3rd occasion	0.70	7	5.67	NS
1st and 4th occasion	0.29	7	0.57	NS
2nd and 3rd occasion	0.91	7	29.54	$p < 0.01$
2nd and 4th occasion	0.77	7	8.79	$p < 0.05$
3rd and 4th occasion	0.82	7	11.92	$p < 0.05$

NS = non significant - $p > 0.05$.

Table 1.13 Effect of 5-HT1a agonist/partial agonists and 5-HT3 antagonists on '5-HT syndrome' induced by 8-OH-DPAT.

a) Total '5-HT syndrome' score.

	dose (mg/kg)	vehicle	drug	
Ipsapirone	10	24.0 (1.2)	8.3 (0.9)	$p < 0.05$
Buspirone	5	24.4 (1.0)	21.4 (1.2)	NS
GR38032F	0.1	24.8 (0.9)	23.0 (1.1)	NS
ICS 205-930	1	24.8 (0.9)	16.5 (1.7)	$p < 0.01$
MDL 72,222	1	24.8 (0.9)	22.0 (1.2)	NS

b) Flat body posture.

	dose (mg/kg)	vehicle	drug	
Ipsapirone	10	10.5 (0.6)	5.5 (0.3)	$p < 0.05$
Buspirone	5	9.8 (0.6)	11.0 (0.3)	NS
GR38032F	0.1	10.1 (0.4)	9.8 (0.7)	NS
ICS 205-930	1	10.1 (0.4)	6.8 (0.4)	$p < 0.01$
MDL 72,222	1	10.1 (0.4)	10.4 (0.6)	NS

c) Forepaw treading.

	dose (mg/kg)	vehicle	drug	
Ipsapirone	10	7.5 (0.9)	0 (0)	$p < 0.05$
Buspirone	5	8.0 (1.0)	2.0 (0.7)	$p < 0.05$
GR38032F	0.1	8.4 (0.7)	10.6 (0.5)	NS
ICS 205-930	1	8.4 (0.7)	5.7 (1.0)	$p < 0.05$
MDL 72,222	1	8.4 (0.7)	7.8 (0.9)	NS

Table 1.13 continued :

d) Hindlimb abduction.

	dose (mg/kg)	vehicle	drug	
Ipsapirone	10	5.3 (0.3)	2.8 (1.0)	NS
Buspirone	5	6.6 (1.1)	8.4 (0.9)	NS
GR 38032F	0.1	6.1 (0.8)	3.6 (0.8)	NS
ICS 205-930	1	6.1 (0.8)	3.2 (0.3)	p <0.01
MDL 72,222	1	6.1 (0.8)	3.8 (0.4)	p <0.05

Rats received either vehicle (i.p.) or drug (i.p.) 20min prior to
8-OH-DPAT (5mg/kg i.p.). Animals were then individually observed for
20min, being scored (0-3 for increasing severity) for 5 components of
the 5-HT syndrome - head-weaving, tremor, flat body posture (FBP),
forepaw treading (FPT) and hindlimb abduction (HLA) - every 5min.
These scores were combined to give a component score, the component
scores were added to give a total '5-HT syndrome' score. No head-
weaving was seen in any of the animals and tremor was only seen in a
small number of animals, its score never exceeding 1, so while it was
included in the total syndrome score, the effect of individual
treatments on tremor was not evaluated.

Results were analysed statistically using Mann-Whitney U test.

Table 1.14   Effect of ICS 205-930 on '5-HT syndrome' induced by
5-MeODMT.

	FBP	FPT	HLA	Total
vehicle	9.0 (0.6)	4.3 (1.8)	4.5 (0.3)	17.0 (1.2)
ICS 205-930	7.0 (1.5)	2.8 (1.4)	3.2 (0.8)	13.0 (3.0)
	NS	NS	NS	NS

This experiment was performed, scored and statistically analysed in
the same manner as those presented in fig. 1.12.. Rats received either
ICS 205-930 (1mg/kg i.p.) or vehicle (i.p.) 20min prior to 5-MeODMT
(5mg/kg i.p.).

Table 1.15 Effect of diazepam and picrotoxin on 5-MeODMT-induced
head-twitching.

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diazepam i.p.	15min prior to 5-MeODMT (5mg/kg i.p.)			
0.5mg/kg	vehicle (v)	12.3 (1.9)	NS
	diazepam (d)	18.8 (2.8)	

diazepam i.p.	30min prior to 5-MeODMT (5mg/kg i.p.)			
1mg/kg	v	6.2 (1.7)	NS
	d	14.8 (6.8)	

picrotoxin i.p.	30min prior to 5-MeODMT (5mg/kg i.p.)			
0.5mg/kg	v	13.0 (3.4)	NS
	p	8.2 (3.6)	

Bracketed figures represent s.e.m. Results are the means of at
least 5 determinations (in the case of the diazepam 0.5mg/kg,
10 determinations were done, but diazepam still did not
significantly effect 5-MeODMT-induced head-twitching).

NS = non-significant - $p > 0.05$.

CHAPTER 2

EFFECTS OF (±)DOI ON ON-GOING BEHAVIOUR IN MICE AND RATS.
MODULATION OF (±)DOI-INDUCED HEAD-TWITCHING, SCRATCHING AND
INCREASED LOCOMOTOR ACTIVITY BY NORADRENERGIC AND SEROTONERGIC
AGENTS.

CHAPTER 2.

Introduction.

Until recently there has been no agonist with a high selectivity for
5-HT2 as opposed to 5-HT1 receptors. Glennon et al (1986) reported
that (±)DOI was such an agent, the most selective at present available
(see Table 2.1). Since the 5-HT-related head-twitch has been
demonstrated to be a function of 5-HT2 receptor activation, it was
decided to investigate the effect of (±)DOI on the on-going behaviour
of mice and rats.

Table 2.1 Binding characteristics of selective serotonergic agents.

| site   Ki (nm) | 5-HT1 [3H]-5-HT | 5-HT1 [3H]-LSD | 5-HT1a [3H]-8-OH-DPAT | 5-HT2 [3H]-KET |
ligand				
amphetamine	7600			43000
8-OH-DPAT	160		2	5500
DOF		3470		1100
RU24969	2		5	780
5-HT	2		3	560
5-MeO-DMT			20	390
quipazine	230		3300	230
TFMPP	20		1950	160
DOB(+)		5000		120
DOM		2890		100
DOB		3340		63
DOM(-)		3550		60
DOB(-)		4200		60
DOI(+)		1670		36
DOI		2240		19
DOI(-)		2290		10

Binding data from Glennon (1987) and Glennon et al (1986).

During the course of the work on the behavioural effects of (±)DOI, the high 5-HT1c receptor affinity of this and other 5-HT2-related agents has been demonstrated (see Hoyer 1988b). This obviously has to be taken into account when interpreting the effects of (±)DOI on on-going behaviour as documented below. As has the binding of (±)DOI to the 'DOB binding site' (Pierce and Peroutka 1989b).

Results.

1. (±)DOI-induced effects on behaviour.

1.1 The effect of (±)DOI on on-going behaviour in mice.

Three doses were tested (0.2, 1 & 10mg/kg i.p.) using a modified version of Irwin's behavioural analysis (see Methods section 3.9), the response of the mice being observed 15 min after i.p. injection. As can be seen from Fig. 2.1 and Table 2.2a, the main effects of (±)DOI on mouse on-going behaviour found were :

1) induction of head-twitching.
2) increased grooming and scratching.
3) increased fearfulness.
4) increases in startle and touch response.
5) increase in the pinna reflex.

These effects were found to be dose-related (0.2-10mg/kg). A non dose-related increase in sniffing was also seen, and at the highest dose investigated (10mg/kg), restlessness, tremor, ptosis, body sag and corneal reflex were all increased ((±)DOI produced no effect on these behaviours at the lower doses tested). A dose-related increase in cyanosis was observed. No signs of the '5-HT syndrome' were present (0.2-10mg/kg).

The nature of the excessive scratching response was bouts of reciprocal hindlimb scratching (hindlimb scratching alternating from one hindlimb to the other) directed towards caudal parts of the body,

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principally the flanks. Little scratching was directed towards the ears or face, as is observed in the 'normal' behavioural repertoire of mice.

A decrease in spontaneous activity was seen with 1 and 10mg/kg. However, on observing the animals while the head-twitching dose-response experiments were being done, an increase in spontaneous activity in (±)DOI-treated animals seemed more prevalent, especially with the lower doses of (±)DOI used (0.1, 0.25, 0.5 and 1mg/kg). It was thus decided that the effect of this agent on spontaneous locomotor activity should also be investigated.

1.2 The effect of (±)DOI on on-going behaviour in rats.

The effect of (±)DOI (5mg/kg i.p.) on on-going behaviour was assessed 5, 15 and 30min after injection using the scoring method of Deakin and Green (1978) (see Methods section 3.9).

The dose was chosen to reveal any 5-HT1 receptor-mediated effects of (±)DOI, as observation of the response of rats while the twitching dose-response experiments were being done, indicated that at the lower doses tested (0.5 & 1mg/kg i.p.) twitching was the only behaviour induced by (±)DOI, at 5mg/kg some evidence of 5-HT1-related behaviour was observed (but not scored) and thus it was decided to investigate the response of rats to 5mg/kg (±)DOI in more detail.

The main behaviours seen in rats with the 5mg/kg dose tested was head-twitching and wet dog shaking (Fig. 2.2 and Table 2.2b). As previously mentioned in the Introduction, in mice the 5-HT-related twitching response consists of a rapid intermittent side to side movement of the head. In mice only the head is involved but in the rat the upper trunk may move as well resulting in the so called 'wet dog shakes' (WDS) (Bedard and Pycock 1977). As outlined in methods both head-twitches (Matthews and Smith 1980) and WDS were counted and are collectively referred to as the 'twitch response'.

There was some chewing and gnawing (score 1), slight fore limb tremor (of maximal intensity 1), fore paw treading (of maximal intensity 1), flat body posture (of maximal intensity 2), all of these were intermittent in nature i.e. not present the whole of the observation time (see Fig. 2.2 and Table 2.2b).

2. (±)DOI-induced twitch production.

2.1 Dose-response in : 1) mice.

Head-twitch frequency was found to be dose-dependent between 0.1 and 1mg/kg. Higher doses up to 10mg/kg produced slightly smaller responses than 1mg/kg (see Fig. 2.4a and Table 2.3). Slope/scatter F ratio from linear regression analysis of this dose-response data was found to be 4.93 with a significant probability value of $p < 0.032$ (df 1,32).

2) rats.

Twitch frequency was found to be dose-dependent between 0.5 and 5mg/kg (Fig. 2.4b and Table 2.3). Slope/scatter F ratio from linear regression analysis of this dose-response data was found to be 5.22 with a significant probability value of $p < 0.036$ (df 1,15).

2.2 Time-course in :   1) mice.

Using 1mg/kg, onset of head-twitching was found to be 2-4 min after (±)DOI injection, peak head-twitch frequency occurring 10min after injection, head-twitching had returned to the spontaneous rate 30min after injection (Fig. 2.3a and Table 2.3).

2) rats.

Using 1mg/kg, peak twitch frequency was observed 7min after injection, the twitch frequency then plateaued at this peak frequency for the final 54min of observation (Fig. 2.3b and Table 2.3).

For the interaction experiments (in mice), the results of which are presented below (sections 2.3-2.6), a dose of (±)DOI in the centre of the dose-response curve was chosen, 0.5mg/kg (0.25mg/kg initially) to allow both inhibition and potentiation of head-twitch production to be observed. The dose of (±)DOI used in the interaction experiments was changed from 0.25mg/kg to 0.5mg/kg as, after the initial experiments using 0.25mg/kg, this dose was found to produce unreliable frequencies of head-twitching. Head-twitch production was counted between the 3rd and 15th minutes (alternate minutes) as this period contained the peak period of head-twitching as indicated by the time-course experiment.

Effect of a variety of agents on (±)DOI induced head-twitch production in mice:

A variety of agents were examined for their effect on (±)DOI-induced head-twitching, the results of many of these experiments will be covered in chapter 3 as they involve the modulation of (±)DOI-induced head-twitching by noradrenergic ligands. The results summarised below mainly involve the effect of 5-HT agonists, partial agonists and antagonists on this behaviour.

The pretreatment times mentioned below refer to the number of minutes prior to the injection of (±)DOI (0.5mg/kg i.p.) that the test agents were injected. Figures in [brackets] following ID50 values are the 95% confidence limits.

2.3 a) 8-OH-DPAT

8-OH-DPAT (0.2-2mg/kg 10min pretreatment) was found to produce dose-dependent inhibition of (±)DOI-induced head-twitching (Fig. 2.6 and Table 2.10 (3)). ID50 = 0.33 [0.035 - 2.09] mg/kg.

b) <u>buspirone</u>

Buspirone (1-10mg/kg 30min pretreatment) was found to produce dose-dependent inhibition of (±)DOI-induced head-twitching (Fig. 2.7 and Table 2.10 (4)). ID50 = 4.21 [0.14 - 158.49] mg/kg.

c) <u>ipsapirone.</u>

Ipsapirone (5mg/kg 30min pretreatment) was found to produce no effect on (±)DOI-induced head-twitching, but potentiation of 5-MeODMT-induced head-twitching (5mg/kg i.p.) (see Fig. 2.8 and Table 2.10 (5)).

2.4   <u>xylamidine.</u>

Xylamidine (10mg/kg 15min pretreatment) was found to produce no effect on (±)DOI-induced head-twitching (Fig. 2.10 and Table 2.10 (2)).

2.5   <u>ritanserin.</u>

Ritanserin (0.5-5mg/kg 30min pretreatment) was found to produce dose-dependent inhibition of (±)DOI-induced head-twitching (Fig. 2.12 and Table 2.10 (1)). ID50 = 0.24 [0.05 - 0.86] mg/kg.

2.6   <u>Effect of idazoxan (0.5mg/kg s/c 30min pretreatment) on
        inhibition of (±)DOI-induced head-twitching by 8-OH-DPAT
        (1mg/kg 10min pretreatment).</u>

Idazoxan (0.5mg/kg) was found to produce no significant effect on the inhibition of (±)DOI-induced head-twitching by 8-OH-DPAT (1mg/kg) (the interaction term, $F_{(AB)}$, from the two-way between/within subjects analysis of variance (ANOVA) = 0.38; df 1,20; p >0.05). Analysis of data using Tukey's test for unconfounded means (after the ANOVA) indicated that 8-OH-DPAT produced significant inhibition of (±)DOI-induced head-twitching (p <0.01) and idazoxan (0.5mg/kg) produced no effect on (±)DOI-induced head-twitching itself (p >0.05) (see Fig. 2.13 and Table 2.11) .

3.   (±)DOI-induced scratching in mice.

3. 1 Dose-response.

Two sets of doses were examined for the dose-response relationship of
(±)DOI -induced scratching. Initially a small number of doses was
examined to determine a suitable dose of (±)DOI for use in interaction
experiments. At a later date a more comprehensive dose-response was
assembled, this was partly for the sake of completeness, and partly as
a downward trend in the scratch rate achieved with the dose of (±)DOI
chosen in the original experiment (2.5mg/kg) had been noticed i.e.
initial dose-response result (dose-response 1), mean no. of scratching
episodes in the 10min period after (±)DOI administration (± s.e.m.) =
36.6 (16.0), dose-response 2, mean no. of scratching episodes/10min
observation period (± s.e.m.) = 9 (6.6). However, on comparison of
these two means using unpaired t-test no significant difference was
found between them, p = 1.70 (df = 9). This is probably due to the
large variability of this data indicated by the large s.e.m. of the
two means.

Dose-response 1. (dose-range tested = 1 - 5mg/kg).

(±)DOI-induced scratching was found to be dose-dependent between 1 and
5mg/kg (observation period from 0 to 30 minutes after injection) (Fig.
2.15 and Table 2.4 (1)). Slope/scatter F ratio from linear regression
analysis of this dose-response data was found to be 34.58 with a
significant probability value of $p < 0.001$ (df 1, 15).

Dose-response 2. (dose-range tested = 0.1 - 10mg/kg).

(±)DOI-induced scratching was found to be dose-dependent between 0.1
and 10mg/kg (observation period from 0 to 10 minutes after injection)
(Fig. 2.16 and Table 2.4 (2)). Slope/scatter F ratio from linear
regression analysis of this dose-reponse data was found to be 28.14
with a significant probability value of $p < 0.001$ (df 1, 46).

3.2 Time-course.

Using 2.5mg/kg (observation period of 30min), the onset of scratching occurred between the 3rd and 5th minute after (±)DOI injection, peak scratching frequency being observed 8-9min after injection, scratching returning to the spontaneous rate 24-30min after injection (Fig. 2.17 and Table 2.4).

For the interaction experiments whose results are presented below (see sections 3.3-3.12), a dose of (±)DOI which produced scratching frequency in the centre of dose-response curve 1. (Fig. 2.15 and Table 2.4 (1)), 2.5mg/kg, was chosen to allow both inhibition and potentiation of scratch production. No. of episodes of scratching was counted for the first ten minutes after (±)DOI injection as this period contained the peak period of scratch production.

Effect of a variety of agents on (±)DOI-induced scratching :

The pretreatment times mentioned below refer to the number of minutes prior to the injection of (±)DOI 2.5mg/kg i.p. (unless otherwise stated) that the test agents were injected.

3.3 ritanserin and ICI 169,369.

Ritanserin (0.02-2mg/kg 30min pretreatment) was found to produce dose-dependent inhibition of (±)DOI-induced scratching (Fig. 2.11 and Table 2.12 (1)). ID50 = 0.03 [0.0001 - 2.93]. ICI 169,369 (2mg/kg 30min pretreatment) also inhibited (±)DOI-induced scratching (Fig. 2.14 and Table 2.12 (2)).

3.4 8-OH-DPAT.

8-OH-DPAT (0.01-1mg/kg 10min pretreatment) was found to produce potentiation of (±)DOI-induced scratching which was not dose-dependent (Fig. 2.5 and Table 2.12 (4)).

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3.5 xylamidine.

Xylamidine (2.5 & 10mg/kg 15min pretreatment) was examined against two doses of (±)DOI (2.5 & 5mg/kg). Only the 10mg/kg dose produced a significant effect, inhibition, on scratching induced by the 5mg/kg dose of (±)DOI, no effect being seen on the lower dose (2.5mg/kg) of (±)DOI (see Fig. 2.9 and Table 2.12 (3)).

3.6 naloxone.

Naloxone (2mg/kg 15min pretreatment) produced no effect on (±)DOI-induced scratching (Fig. 2.18 and Table 2.12 (10)).

3.7 terfenadine.

Terfenadine (5mg/kg 30min pretreatment) produced no effect on (±)DOI-induced scratching (Fig. 2.19 and Table 2.12 (11)).

3.8 FLA-63.

FLA-63 (40mg/kg 4hr pretreatment) produced inhibition of (±)DOI-induced scratching (Fig. 2.20 and Table 2.12 (5))..

3.9 clonidine.

Clonidine (0.05 & 1mg/kg 30min pretreatment) produced inhibition of (±)DOI-induced scratching (Fig. 2.21 and Table 2.12 (6)).

3.10 idazoxan.

Idazoxan (0.5 & 1mg/kg 30min pretreatment) produced no effect on (±)DOI-induced scratching (Fig. 2.22 and Table 2.12 (7)).

3.11 prenalterol.

Prenalterol (10mg/kg 15 min pretreatment) produced no effect on
(±)DOI-induced scratching (Fig. 2.23 and Table 2.12 (8)).

3.12 procaterol.

Procaterol (5mg/kg 15min pretreatment) produced no effect on (±)DOI-
induced scratching (Fig. 2.24 and Table 2.12 (9)).

4. (±)DOI-induced increase in locomotion in mice.

4.1 Dose-response.

Two dose ranges were examined for the dose-response relationship of
(±)DOI-induced increase in locomotion. The initial three dose study
was a pilot study to see if (±)DOI did in fact have any effect on
mouse locomotor activity. The second set used the information gained
from the first to choose more appropriate doses to look at in terms of
(±)DOI-induced increase in locomotion.

Dose-response 1 (dose-range tested = 0.1-5mg/kg).

Slope/scatter F ratio from linear regression analysis of this dose-
response data was found to be 0.54 with a non-significant probability
value of p <0.48 (F = 3.12; df 1,19). However on analysis of the data
using one-way between subjects analysis of variance (F = 3.12; df
3,17; p >0.05) followed by Duncan's multiple range test, it was found
that 1mg/kg (±)DOI did significantly increase locomotion in comparison
with vehicle control (p <0.05) (see Fig 2.25 and Table 2.5 (1)).

Dose-response 2 (dose-range tested = 0.25-1mg/kg).

(±)DOI-induced increase in locomotion was found to be dose-dependent
between 0.25 and 1mg/kg. Slope/scatter F ratio for this dose-response

data was found to be 11.36 with a significant probability value of p <0.003 (F = 11.36; df 1,33) (see Fig. 2.26 and Table 2.5 (1)).

Analysis of the data using a one-way between subjects analysis of variance (F = 3.83; df 4,30; p <0.05) followed by Duncan's multiple range test, indicated that of the doses tested 0.25 (p <0.05), 0.75 (p <0.01) and 1mg/kg (p<0.01) all significantly increased locomotion in comparison with vehicle controls (0.5mg/kg did not).

For the interaction experiment involving ritanserin whose results are presented below (see section 4.2), a dose of (±)DOI showing a strong consistent increase in locomotor activity over several experiments was chosen - 1mg/kg.

4.2   Effect of ritanserin on (±)DOI-induced increase in locomotor activity.

Ritanserin (2mg/kg 30min pretreatment) was found to significantly inhibit the potentiation of locomotor activity induced by (±)DOI (1mg/kg) (the interaction term, F(AB), from the two-way between subjects analysis of variance (ANOVA) = 6.19; df 1,40; p <0.05). Analysis of the data using Tukey's test for unconfounded means (after the ANOVA) indicated that (±)DOI produced a significant effect (p <0.05) on locomotor activity in comparison with the vehicle controls, whereas ritanserin produced no effect (p >0.05) on locomotor activity in comparison with the vehicle controls. There was a significant difference (p <0.01) between animals treated with saline and (±)DOI and those treated with ritanserin and (±)DOI, as expected (see Fig. 2.27 and Table 2.13).

5.   (±)DOI-induced effects on grooming in mice.

5.1 Dose-response.

The effect of (±)DOI on grooming was analysed in terms of :

a) total grooming : 1) the mean no. of bouts in 15min.

                    2) the mean time spent grooming in 15min.

   face/paw grooming, lick elsewhere and penile grooming :

                    1) the mean no. of bouts in 15min.

                    2) the mean time spent grooming in 15min.

b) total grooming, face/paw grooming, lick elsewhere and penile

   grooming:

                    1) mean no. of bouts in the 0-5, 5-10 and 10-15min

                       time periods.

                    2) mean time spent grooming in 0-5, 5-10 and

                       10-15min time periods.

Total grooming scores were comprised of the scores achieved for the

individual forms of grooming (face/paw, penile and lick elsewhere)

summed for each experimental animal.

The effects of (±)DOI on grooming were slight and not dose-dependent :

a) Total grooming.

(±)DOI produced no significant effects on either mean no. of grooming

bouts or mean time spent grooming/15min, with the one exception of

5mg/kg (±)DOI where inhibition of mean time spent grooming/15min was

seen in comparison with vehicle treated controls (see Fig. 2.28 and

Table 2.6a).

0-5min period.

In the first five minute period, (±)DOI produced no significant effect on either mean no. of grooming bouts or time spent grooming, with the one exception of 5mg/kg (±)DOI. where a significant increase in no. of grooming bouts over vehicle treated controls was seen (see Fig. 2.29, 2.30 and Table 2.6b).

5-10min period.

In the second five minute period, (±)DOI produced no significant effects on either mean no. of grooming bouts (Fig. 2.29 and Table 2.6b) or mean time spent grooming (see Fig. 2.30 and Table 2.6b).

10-15min period.

In the third five minute period, no significant effect was produced by (±)DOI on mean no. of grooming bouts (see Fig. 2.29 and Table 2.6b). However, in the case of total time spent grooming, three doses of (±)DOI showed significantly lower scores than vehicle treated controls (2.5, 0.1 and 5mg/kg, in order of increasing effect) (see Fig. 2.30 and Table 2.6b).

b) Face/paw (F/P) grooming.

(±)DOI produced no significant effects on mean time spent in F/P grooming/15min with the exception of 1mg/kg (±)DOI which produced an increase in this form of grooming over vehicle controls (see Fig. 2.31 and Table 2.7a).

(±)DOI produced no significant effects on mean no. of bouts of F/P grooming/15 min, with the exception of 2.5mg/kg (±)DOI which produced an increase in this form of grooming, over vehicle controls (Fig. 2.32 and Table 2.7a).

<u>0-5min period.</u>

In the first five minute period, (±)DOI produced no significant
effects on both mean no. of grooming bouts and mean time spent
grooming, with the one exception of 5mg/kg (±)DOI where an increase in
the mean no. of grooming bouts, over vehicle controls, was seen (see
Fig. 2.33, 2.34 (respectively) and Table 2.7b).

<u>5-10min period.</u>

In the second five minute period, again (±)DOI produced a significant
increase in mean no. of grooming bouts at only one dose (2.5mg/kg
(±)DOI). (±)DOI produced no effects on mean time spent grooming (see
Fig. 2.33, 2.34 (respectively) and Table 2.7b).

<u>10-15min period.</u>

In the third five minute period, again (±)DOI produced a significant
effect on mean no. of F/P grooming bouts and mean time spent in F/P
grooming at only one dose. However, in this instance, it was a
decrease in mean time spent in this form of grooming (at 5mg/kg) in
comparison with vehicle controls (see Fig. 2.33, 2.34 (respectively)
and Table 2.7b).

<u>c) Penile grooming.</u>

(±)DOI produced no significant effects on mean no. of bouts of penile
grooming/15min or mean time spent in penile grooming/15 min compared
with vehicle treated controls (Fig. 2.32, 2.31 (respectively) and
Table 2.8a).

<u>0-5min period.</u>

In the first five minute period, (±)DOI produced no significant
effects on either mean no. of grooming bouts or mean time spent
grooming, with the exception of 5mg/kg dose of (±)DOI which produced

an increase in mean no. of grooming bouts over vehicle controls (see Fig. 2.35, 2.36 (respectively) and Table 2.8b).

5-10min period.

In the second five minute period, (±)DOI produced no significant effects on either mean no. of grooming bouts or mean time spent in grooming (see Fig. 2.35, 2.36 (respectively) and Table 2.8b).

10-15min period.

In the third five minute period, (±)DOI produced a significant increase in mean no. of grooming bouts with one dose of (±)DOI (0.5mg/kg) and a significant decrease in mean time spent grooming with another (5mg/kg). No other doses of (±)DOI produced significant effects on either mean no. of grooming bouts or mean time spent grooming in this time interval compared with vehicle controls (see Fig. 2.35, 2.36 (respectively) and Table 2.8b).

d) Lick elsewhere grooming.

(±)DOI produced dose-related decreases in both mean total time spent in lick elsewhere grooming/15 min (statistically significant at 0.5, 1, 2.5 and 5mg/kg) and mean total no. of grooming bouts (statistically significant at 2.5 and 5mg/kg) of lick elsewhere grooming/15min in comparison with vehicle treated controls (see Fig. 2.31, 2.32 (respectively) and Table 2.9a).

0-5min period.

In the first five minute period, only two doses of (±)DOI (0.1 & 0.5mg/kg) produced any lick elsewhere grooming, and the scores were low (in comparison with those seen for F/P and penile grooming) for both mean no. of grooming bouts and mean time spent grooming. Only the increase in mean no. of grooming bouts induced by 0.1mg/kg (±)DOI (in comparison with vehicle controls) was statistically significant. There

was no lick elsewhere grooming observed in the vehicle treated animals
(see Fig. 2.37, 2.38 and Table 2.9b).

5-10min period.

In the second five minute period, lick elsewhere grooming was seen
with all doses of (±)DOI and in the vehicle treated animals, however,
again both mean no. of grooming bouts and mean time spent grooming
scores were low. None of the doses of (±)DOI produced significant
effects on mean no. of lick elsewhere grooming bouts or mean time
spent in lick elsewhere grooming in the 5-10min time period (see Fig.
2.37, 2.38 and Table 2.9b).

10-15min period.

In the third five minute period, all doses of (±) DOI (apart from
0.2mg/kg in the case of mean no. of grooming bouts) showed
significantly lower scores for both mean no. of grooming bouts and
mean time spent grooming compared with vehicle treated controls (see
Fig. 2.37, 2.38 (respectively) and Table 2.9b).

6. Effect of quipazine and mescaline on on-going behaviour in mice.

Initial experiments with quipazine and mescaline indicated that in
addition to inducing head-twitching in mice, at high dose these agents
also induced scratching behaviour identical to that produced by (±)DOI
i.e. reciprocal hindlimb scratching. In the case of quipazine the
group sizes used were too small for statistical evaluation, however,
excessive scratching behaviour was seen with doses of quipazine
greater than 5mg/kg i.p. In the case of mescaline, two doses of
mescaline were tested for scratch production, 20mg/kg and 50mg/kg
(both doses i.p.), the animals were observed and scratching episodes
counted (in the same manner as those produced by (±)DOI) for 60min.
With 20mg/kg mescaline, no significant difference between vehicle and
mescaline treated animals with respect to scratching frequency was
observed, although there was a trend towards greater scratch frequency

in those animals treated with mescaline (mean no. of scratching episodes/60min = 10.4 ± 2.6 for vehicle treated animals, and 66.5 ± 45.4 for mescaline treated animals. t = 1.44, p >0.05 for an unpaired t-test). The large standard error of the mean produced in the latter result is indicative of the variability of the scratching response to mescaline. With 50mg/kg, a significant difference was observed between vehicle and mescaline treated animals with respect to scratching behaviour (mean no. of scratching episodes/60min = 9.7 ± 3.3 for vehicle treated animals, and 204.8 ± 64.2 for mescaline treated animals, p < 0.01) (see Fig. 2.39).

Discussion.

The behavioural profile of (±)DOI was examined because at the time
these experiments were performed it had only just become available and
was considered a major improvement on other 5-HT2 agonists such as 5-
MeODMT due to the selectivity it was thought to have between 5-HT2 and
5-HT1 receptors. (±)DOI has since been found to bind with nanomolar
affinity not only to the 5-HT2 site but also to the 5-HT1c site (Hoyer
1988b and Pierce and Peroutka 1989b). There has been speculation that
the 5-HT1c receptor is actually a sub-type of the 5-HT2 receptor due
to the high degree of homology between these two sites (e.g Hoyer
1988b), indeed, many of the agents with activity at the latter show
affinity for the former e.g. ritanserin, which is considered to be a
selective 5-HT2 antagonist (Leysen and Gommeren, 1986) has a high 5-
HT1c affinity (Hoyer and Karpf 1988). In addition, (±)DOI also binds
with high affinity to the recently identified 'DOB binding site'
labelled by 3H-DOB (Titeler et al 1985 & Lyon et al 1987) or
77BR-R(-)DOB (Wang et al 1988). Whether this site represents an actual
subtype of the 5-HT2 receptor (tentatively designated 5-HT2a) or just
the high affinity component of the 5-HT2 binding site has prompted
debate (Pierce and Peroutka 1989a & Frazer et al 1990). It would seem
necessary to take into account all three of these high affinity
binding sites (5-HT2, 5-HT1c and 'DOB binding site') when interpreting
the behavioural effects of (±)DOI, although at present there have been
no functional correlates for the 'DOB binding site' reported.

Despite these recent discoveries (±)DOI is still one of the most
selective 5-HT2 agonists available, having 100-fold higher affinity
for 5-HT2 than 5-HT1 sites (5-HT2 sites labelled with 3H-ketanserin;
5-HT1 with 3H-LSD in presence of ketanserin)(Shannon et al 1984), 600-
fold higher affinity for the 5-HT2 than 5-HT1a sites (5-HT2 sites
labelled with 3H-spiperone; 5-HT1a with 3H-8-OH-DPAT) (Pierce and
Peroutka 1989b) and 1800-fold higher affinity for 5-HT2 than 5-HT1b
sites, although in this latter case it should be noted that 3H-DOB was
used to label 5-HT2 sites (5-HT1b were labelled with 3H-5-HT in
presence of 8-OH-DPAT and mesulergine)(Titeler et al 1988). The

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selectivity of (±)DOI between 5-HT2 and 5-HT1a sites is important when a proposed interaction between these two receptors is considered later.

Effect of (±)DOI on on-going behaviour of mice and rats.

The main effects observed in mice were head-twitch production and increased scratching. Head-twitch production by (±)DOI is consistent with its high affinity for 5-HT2 receptors since head-twitching has been demonstrated to be related to 5-HT2 receptor activation (see Introduction plus Peroutka et al 1981; Lucki et al 1984 & Green and Heal 1985). Other effects of (±)DOI observed were increases in fearfulness and the startle and touch responses which might possibly be related to the hallucinogenic activity of (±)DOI (Titeler et al 1988). Sniffing, cyanosis and at the highest dose tested (10mg/kg), ptosis and tremor were also produced by (±)DOI and the pinna and corneal reflexes were increased.

In rats the main effect observed on on-going behaviour was twitch production. Doses of (±)DOI of 0.5 and 1mg/kg produced no effects other than the twitch response, however, using a dose of 5mg/kg (±)DOI some signs of 5-HT1a/b receptor activation were also seen i.e. two components of the '5-HT syndrome' - flat body posture and forepaw treading which were intermittent in nature. Increased scratching was not observed in rats at doses up to 5mg/kg.

The fact that a higher dose (threshold - 5mg/kg) of (±)DOI was required to produce weak and partial signs of the '5-HT syndrome' in the rat than that required to produce twitching (threshold below 0.5mg/kg), is in line with the high selectivity for the 5-HT2-receptor in comparison with the 5-HT1-receptor subtypes found in binding studies. In view of the 100-fold selectivity of (±)DOI for 5-HT2 cf 5-HT1 receptors (Shannon et al 1984), it is surprising that the ratio of (±)DOI doses required to induce the '5-HT syndrome' v twitching was only ten, highlighting the difference between in vitro binding studies and effects produced in behavioural studies.

It would be difficult at present to demonstrate absolutely that (±)DOI produces its behavioural effects via either the 5-HT2 or the 5-HT1c receptor as, although there are selective antagonists available for the 5-HT2 receptor, they mostly have affinity for the 5-HT1c receptor (see Hoyer et al 1985b and Introduction), and there are at present no selective 5-HT1c antagonists available. Since this thesis was originally submitted Kennett and Curzon (1991) have published a very significant paper in which the ID50 values for ten antagonists against 5-HTP-induced head-shakes was determined and a significant correlation with their affinities for 5-HT2 but not 5-HT1a, 5-HT1b, 5-HT1c or 5-HT1d receptors found, supporting the hypothesis that 5-HT-related shaking behaviour is mediated by 5-HT2 and not 5-HT1c receptors.

It might have been interesting to examine the effect of ketanserin on (±)DOI-induced behavioural effects as this agent has high selectivity for 5-HT2 over 5-HT1c receptors (at least 50-fold - Hoyer et al 1985b; Hoyer 1988a&b & Hoyer and Karpf 1988), however, this agent has, in addition to its 5-HT2-antagonist activity, high affinity for alpha1-adrenoceptors (Leysen et al 1981). It was considered that since such receptors have a strong modulatory effect on head-twitching in mice and twitching in rats (see Introduction section 17.1), it would have been difficult to reach a firm conclusion in terms of effect of (±)DOI on on-going behaviour being mediated via 5-HT2 receptors only. Since these experiments were performed other authors have examined the effect of ketanserin e.g. Darmani et al (1990) and found that it dose-dependently inhibited (±)DOI-induced head-twitching in mice, but a role for alpha1-adrenoceptor binding in this effect has not yet been excluded.

There is evidence in favour of at least some of the behavioural reponses engendered by (±)DOI being 5-HT2-related, for example Arnt and Hyttel (1989) found that forepaw treading induced by (±)DOI plus 8-OH-DPAT was potently inhibited by ketanserin. They also found that TFMPP, which has high 5-HT1c affinity (Hoyer 1988a&b) and is active in in vivo models suggested for 5-HT1c activity (e.g. Kennett and Curzon,

1988a&b), had little effect on 8-OH-DPAT-induced forepaw treading. In addition, (±)DOI has been found to exert an anorexic action in rats which was completely reversed by ketanserin (Schechter and Simansky 1988). However the role of alpha1-adrenoceptors in these behaviours has not yet been excluded.

Effect of a variety of 5-HT-related agents on (±)DOI-induced head-twitching.

1) ritanserin.

At the time this study was done, (±)DOI was claimed to be a selective 5-HT2 agonist (although now known to be 5-HT2/5-HT1c agonist). It was therefore important to check whether head-twitching induced by (±)DOI was blocked by ritanserin (at that time claimed to be a selective 5-HT2 antagonist, now known to be 5-HT2/5-HT1c antagonist). The expected antagonism was found (Fig. 2.12 and Table 2.10).

2) xylamidine.

Head-twitching is thought to be of central origin (see Handley and Singh 1986b), however, it is not known whether any component of (±)DOI-induced head-twitching is peripheral in origin, therefore it was decided to examine the effect of the 5-HT2 antagonist, xylamidine on this behaviour. This agent, which is thought to block peripheral 5-HT2 receptors (Fuller et al 1986) and not to penetrate the blood brain barrier (Copp et al 1967 & Mawson and Whittington 1970)), produced no effect on (±)DOI-induced head-twitching, indicating that the head-twitching induced by (±)DOI probably has no peripheral component.

The dose of xylamidine used in the above experiment was quite high, 10mg/kg i.p. Lower doses have been shown to inhibit peripheral 5-HT2-mediated effects, for example doses of 0.1 and 0.3mg/kg i.p. have been shown to inhibit the contractile response to 5-HT in the rat jugular vein and doses of 1 and 3mg/kg had no effect on the central 5-HT2-mediated elevation of serum corticosterone concentration by quipazine

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in rats (Fuller et al 1986). A high starting dose was used to reveal any weak modulatory effect of peripheral 5-HT2 receptors. If an inhibitory effect had been observed, lower doses of xylamidine would have been examined.

3) 5-HT1a agonists.

8-OH-DPAT, buspirone and ipsapirone are three compounds that bind to the 5-HT1a receptor selectively. In contrast to 8-OH-DPAT which appears to be a full 5-HT1a agonist, buspirone and ipsapirone show both agonist, partial agonist and antagonist activity in behavioural, biochemical and electrophysiological studies depending on whether the 5-HT1a receptors are located pre- or postsynaptically (see Introduction section 16; Sprouse and Aghajanian 1986; 1988; Van der Maelen et al 1986; Andrade and Nicoll 1987; Martin and Mason 1987 & Traber and Glaser 1987).

The initial work on the effect of 8-OH-DPAT on (±)DOI-induced head-twitching was done prior to the present interest in 5-HT2/5-HT1a interactions in an attempt to replicate the behavioural effects of 5-MeODMT by combining a selective 5-HT1a agonist (8-OH-DPAT) with a selective 5-HT2 (5-HT1c) agonist ((±)DOI), since 5-MeODMT is a non-selective 5-HT agonist with affinity at 5-HT1a, 5-HT1b and 5-HT2 receptors. The purpose of this study was then to look at the effect of FLA-63 on the (±)DOI/8-OH-DPAT combination, as we had found that FLA-63 produced inhibition of (±)DOI-induced head-twitching but potentiation of 5-MeODMT-induced head-twitching (see Chapter 3). Such an experiment it was thought might reveal the reason for this difference.

In mice, 5-MeODMT produced both head-twitching and a behavioural syndrome similar to the '5-HT syndrome' observed in rats. This consisted of hindlimb abduction, forepaw treading, flat body posture, head weaving and tremor. In rats this syndrome is mainly under the control of 5-HT1a and 5-HT1b receptors (see Lucki et al 1984 and Tricklebank 1985). In mice, however, the situation is more complex

since neither the 5-HT1a agonist 8-OH-DPAT nor the 5-HT1b agonist RU 24969 have been found to induce the '5-HT syndrome' when administered alone (Green et al 1984 and Goodwin & Green 1985). It was thought that a combination of receptor activation e.g. 5-HT2 and 5-HT1a, might be required to produce the syndrome in mice, and thus that the combined administration of (±)DOI and 8-OH-DPAT might simulate the behavioural effects of 5-MeODMT. In fact, the combination produced none of the behaviours observed with 5-MeODMT, but instead, 8-OH-DPAT dose-dependently inhibited (±)DOI-induced head-twitching (Fig. 2.6 and Table 2.10). The effect of two other 5-HT1a-selective agents were then examined, buspirone (Fig. 2.7 and Table 2.10) also produced inhibition while ipsapirone produced no effect with the single high dose tested (Fig. 2.8 and Table 2.10).

These results are very interesting as they suggest a functional link between 5-HT1a and 5-HT2 receptors. Whether this link is direct or via another transmitter system is open to further experimentation.

Table 2.14. The modulation of shaking behaviour by 5-HT1a agents in mice and rats.

Shake inducer   Modulatory effect of 5-HT1a agent on shake inducer.

5-HT1a ligand	8-OH-DPAT	buspirone	gepirone	ipsapirone
5-MeODMT	no effect (1,2) M	potentiation (2) M		potentiation (*,2,3) M
5-HTP	inhibition (1) M	no effect (2) M		no effect (2,3) M
(±)DOI	inhibition (*,4,5) M R	inhibition (*) M		no effect (*) M
quipazine	inhibition (6) R	inhibition (6,7) R	inhibition (6) R	inhibition (6,7) R

* = In the present study.   M = mouse   R = rats.

1. Goodwin and Green (1985). 2. Moser (1990). 3. Goodwin et al (1986).

4. Arnt and Hyttel (1989). 5. Darmani et al (1990). 6. Yocca et al (1990). 7. Lucki (1986).

Goodwin and Green (1985) first provided evidence of the modulation of 5-HT-head-twitching in mice by a 5-HT1a ligand, 8-OH-DPAT. Since the present experiments were performed, Arnt and Hyttel (1989) reported an inhibitory effect of 8-OH-DPAT on shaking behaviour induced by (±)DOI **in rats.** In the same year our group published my finding that head-twitching induced by (±)DOI was also inhibited by 8-OH-DPAT **in mice** (Heaton and Handley 1989). Darmani et al (1990) repeated this finding. Similar results were also reported using quipazine to induce head-shaking in rats (Lucki 1986 & Yocca et al 1990).

Mice were the the main experimental animal used in the studies in Table 2.14, with the exception of those using quipazine as a twitch inducer. It is perhaps wise therefore to use caution when evaluating these results in comparison with the mice based studies. Although in the one set of studies in Table 2.14 where both mice and rats were used i.e the effect of 8-OH-DPAT on (±)DOI-induced twitching, the same result was found for both species.

From table 2.14 it can be seen that there is not a simple pattern of modulation of 5-HT-related shaking behaviour by 5-HT1a agonists/partial agonists. If 5-MeODMT is used as a shake inducer, the 5-HT1a ligands either produce no effect or potentiation. If 5-HTP, (±)DOI or quipazine are used as shake inducers, either no effect or inhibition is produced. The main difference between 5-MeODMT and (±)DOI/quipazine as a 5-HT agonist is its much greater affinity for 5-HT1a receptors (see Tricklebank 1985 and Table 2.1). Possibly it is this which is responsible for the potentiation produced by some of the 5-HT1a ligands on 5-MeODMT-induced head-twitching. A mechanism for this effect could be that 5-MeODMT while inducing head-twitching is also activating inhibitory 5-HT1a receptors (the existence of these receptors being indicated by the inhibitory effect of 8-OH-DPAT on the other twitch inducers) reducing the peak head-twitch frequency obtainable. In this situation partial agonists such as ipsapirone and

buspirone at the appropriate dose might be able to remove the
inhibitory effect of 5-MeODMT on its own twitch production causing
potentiation. 8-OH-DPAT on the other hand being virtually a full
agonist at 5-HT1a receptors would not be able to produce such an
effect and it might be expected that further stimulation by 8-OH-DPAT
of the inhibitory 5-HT1a receptors (already stimulated by 5-MeODMT
itself) would not produce a further decrease in head-twitching
frequency.

Whether the 5-HT1a ligands act as full or partial agonists at the 5-
HT1a receptor does not appear to affect their inhibitory activity on
quipazine- induced head-twitching. However, on further analysis of the
data such a distinction is still present, thus for quipazine-induced
head-shaking, although all of the 5-HT1a ligands tested produced
inhibition, their ED50 values show a marked difference between 8-OH-
DPAT and the rest (8-OH-DPAT - 0.0013mg/kg; buspirone - 0.27mg/kg;
gepirone - 0.24mg/kg and ipsapirone - 0.47mg/kg)(Yocca et al 1990). In
the present study 8-OH-DPAT (ED50 - 0.33mg/kg) was also found to be
much more potent than buspirone (4.21mg/kg) in inhibiting (±)DOI-
induced head-twitching while ipsapirone did not inhibit it at all.

There does not appear to be a direct correlation between agonist
potency of the 5-HT1a ligands and their potency in inhibiting 5-HT-
related head-twitching as the order of decreasing potency as measured
by the inhibition of forskolin-stimulated adenylate cyclase activity
(IC50) in mice is 8-OH-DPAT (7.1nM) >> ipsapirone (82nM) > buspirone
(128nM)(Bockaert et al 1987)(similar figures were also observed for 8-
OH-DPAT and buspirone in rats (De Vivo and Maayani 1986)). Thus
although 8-OH-DPAT was considerably more potent than both buspirone
and ipsapirone, as seen in the inhibitory activity of the 5-HT1a
ligands on (±)DOI and quipazine-induced shaking behaviour, ipsapirone
was less potent than buspirone in inhibiting quipazine-induced shaking
behaviour but more potent than buspirone in inhibiting forskolin-
stimulated adenylate cyclase activity. The same pattern was repeated
with (±)DOI-induced head-twitching.

It is uncertain why only 8-OH-DPAT of the 5-HT1a ligands in Table 2.14 was capable of inhibiting 5-HTP-induced head-twitching, although 8-OH-DPAT does possess the highest agonist efficacy of these 5-HT1a ligands at both pre- and postsynaptic 5-HT1a receptors (De Vivo and Maayani 1986; Bockaert et al 1987; Sprouse and Aghajanian 1988 & Schoeffter and Hoyer 1988). Possibly the effect is related to the lack of selective action of 5-HTP in inducing head-twitching via the release of 5-HT onto postsynaptic 5-HT receptors (Grahame-Smith 1971). Of the head-twitch inducing agents in table 2.14, only quipazine also has a presynaptic 5-HT releasing component to its twitch-inducing activity, but, in contrast to 5-HTP, quipazine is also thought to act directly on 5-HT receptors to induce twitch production (Malick et al 1977). Goodwin and Green (1985) suggested that 8-OH-DPAT inhibits 5-HTP-induced head-twitching by inhibiting 5-HT release since they found that 8-OH-DPAT greatly decreased the rate of 5-HT synthesis. Such a mechanism would seem unlikely as 5-HT synthesis/turnover is also decreased by buspirone, gepirone and ipsapirone (see Traber and Glaser 1987) and these agents did not reduce 5-HTP-induced head-twitching.

Yocca et al (1990) propose a variation on the mechanism of Goodwin and Green (1985) to explain the inhibitory effect of 5-HT1a partial agonists/agonists on quipazine-induced head-shaking. They suggest that the 5-HT1a receptors involved in inhibitory effect of 5-HT1a agonists/partial agonists on quipazine-induced head-shaking are presynaptic since the doses of 8-OH-DPAT and the buspirone analogues used were similar to those required to inhibit central 5-HT synthesis (Galloway et al 1985 & Torrente et al 1988). The authors argue that stimulation of presynaptic 5-HT1a receptors would reduce 5-HT impulse flow and synthesis which might alter the degree of postsynaptic receptor stimulation by 5-HT in the presence of quipazine. Such a mechanism might also explain the inhibitory effect of the 5-HT1a ligands on (±)DOI-induced shaking.

It is also possible that the effects of the 5-HT1a agents on 5-HT2 receptor-mediated shaking behaviour are being produced via interaction with a transmitter system other than 5-HT, for example, noradrenergic

-189-

or dopaminergic systems. Thus 8-OH-DPAT has the ability to release NA onto alpha2-adrenoceptors (Heal et al 1989b), has antagonist activity at alpha1-adrenoceptors (Trezise et al 1990) and partial agonist activity at dopamine D2 receptors (Bull et al 1990). All three of these activities might be expected to produce inhibition of (±)DOI-induced head-twitching (see Chapter 3 results sections 3.2; 3.3; Corne et al 1963 & Bedard and Pycock 1977). In the case of buspirone, however, a dopaminergic mechanism for its inhibitory effect on (±)DOI-induced head-twitching would seem unlikely as buspirone has antagonist, rather than agonist, activity at dopaminergic receptors (Hjorth and Carlsson 1982 & Pich and Samanin 1986). Ipsapirone was found to produce no effect on (±)DOI-induced head-twitching and has been reported not to possess dopaminergic activity (Yevich et al 1983). A more recent report by Hamon et al (1988) found that ipsapirone, gepirone and buspirone all accelerated dopamine turnover by a 5-HT1a-insensitive mechanism. 8-OH-DPAT was not found to produce this effect.

The possibility of an alpha2-adrenoceptor mechanism was investigated by looking at the effect of idazoxan on the inhibition of (±)DOI-induced head-twitching by 8-OH-DPAT. It was expected that idazoxan might have fully or partially blocked the inhibitory effect of 8-OH-DPAT. No such effect was found, however, since only one dose of idazoxan was used, further experimentation would be necessary to rule out this explanation for 8-OH-DPAT's activity totally.

Another possible cause of the inhibition of (±)DOI-induced head-twitching in the present study by 8-OH-DPAT and buspirone, is a sedative effect of these drugs, especially as ipsapirone, a 5-HT1a agonist with which sedative effects are not associated did not produce inhibition of (±)DOI-induced head-twitching in the present study at a dose which has been demonstrated to be non-sedative (Goodwin et al 1986). This would seem unlikely, however, as sedation was not observed in the experiments in the present study and Corne et al (1963) showed that a wide range of sedative drugs were inactive in inhibiting the 5-HT-induced head-twitch.

In recent years, a large amount of evidence from other behavioural studies has accumulated which indicates an interaction between central 5-HT1a and 5-HT2 receptors. This includes the work of Pericic and Manev (1988) who showed that imipramine which dose-dependently inhibited 5-HT2-mediated behaviours in mice, stimulated the '5-HT syndrome' in rats, which has been proposed to be mediated in part by 5-HT1a receptors (see above). Also the recent work on the potentiatory effect of 5-HT2-related agents, (±)DOI, ritanserin and ketanserin on the behavioural syndrome induced by 8-OH-DPAT (Arnt and Hyttel 1989; Backus et al 1989; 1990); gepirone (Backus et al 1989) and 5-MeODMT (Backus et al 1989; 1990). In the study of Arnt and Hyttel (1989), the potentiatory effect of (±)DOI on 8-OH-DPAT-induced behavioural syndrome was inhibited by ketanserin and ritanserin. Foster and Fletcher (1990) could not repeat this finding nor the ritanserin-induced potentiation of 8-OH-DPAT-induced syndrome. They suggest that the facilitatory effect of (±)DOI on 5-HT1a agonist-induced behaviour is either a non-5-HT2-mediated behaviour or mediated by a ritanserin-insensitive sub-type of the 5-HT2 receptor.

In addition to the behavioural data, there is evidence from physiological and biochemical studies for a 5-HT1a/5-HT2 interaction. For example, Araneda and Andrade (1988) demonstrated that when 5-HT1a and 5-HT2 receptors coexisted on the same cell in rat prefrontal cortex the activation of 5-HT2 receptors reduced the ability of 5-HT1a receptors to hyperpolarize these cells. Similarly, treatment in humans with ritanserin potentiated the prolactin response to L-tryptophan (Charig et al 1986), an effect that is thought to be mediated by 5-HT1 receptors.

Scratching.

(±)DOI-induced scratching was investigated to see if its pharmacology was the same as that of (±)DOI-induced head-twitching. The production of scratching by (±)DOI was unexpected, as it had not been seen with other 5-HT-related twitch inducing agents I had examined e.g. 5-HTP, quipazine and 5-MeODMT.

Scratching has, however, been found on i.t. administration of 5-HT. Fasmer et al (1983) reported that the behavioural response to i.t. 5-HT, which included biting/licking, as well as scratching, was similar to the response induced by i.t. substance P. From the description of the scratching produced by i.t. substance P and 5-HT, it appears identical in nature (reciprocal hindlimb scratching) to that seen in the present study with (±)DOI. With low doses of (±)DOI less intense scratching involving only one side of the body in each episode was seen (a situation found to be paralleled by intraventricular injection of substance P (Dobry et al 1981)). This is perhaps not surprising, since it has been demonstrated that 5-HT coexists with substance P in some raphe-spinal neurones projecting predominately to the ventral horn (Hökfelt et al 1975; 1978), and there is evidence to suggest that they interact functionally (Mitchell and Fleetwood-Walker 1981; Hylden and Wilcox 1983; Tremblay et al 1986 & Murphy and Zemlan 1987). There is biochemical evidence to suggest that tachykinins may exert a tonic excitatory input on serotonergic neurones (Reubi et al 1978 & Reisine et al (1982)).

Effect of a variety of agents on the (±)DOI-induced scratching response.

1) ritanserin.

Ritanserin was found to potently inhibit scratching suggesting that, as with (±)DOI-induced head-twitching, the behaviour is mediated through 5-HT2/5-HT1c receptors. Another 5-HT2/5-HT1c antagonist, ICI 169,369 (Blackburn et al 1988) also inhibited (±)DOI-induced scratching.

It would seem likely that (±)DOI-induced scratching in mice is 5-HT2- rather than 5-HT1c-receptor mediated as neither mCPP nor TFMPP, compounds with 5-HT1c affinity and active in in vivo models suggested for 5-HT1c activity (Hoyer 1988a&b and Kennett and Curzon 1988a&b) have been reported to induce scratching (Simansky and Schecter 1988, Darmani et al 1990 and personal communication by K. Njunge).

Why has scratching not been observed after other 5-HT2 agonists e.g. quipazine, 5-MeODMT & d-LSD ? Since (±)DOI is one of the most specific agonists for 5-HT2 receptors (Glennon et al 1986), it may be that other 5-HT receptors are inhibitory and block the production of this behaviour by less selective agonists. Mescaline, another 5-HT2 agonist (Leysen et al 1982; Niemegeers et al (1983); Davis 1987 & Appel and Callahan 1989) produced scratching on central (i.c.v.) administration (Meisenberg 1982) and, in the present study on peripheral administration, however, the dose found necessary to produce scratching was high (50mg/kg i.p.). This might be due to the low affinity of mescaline for 5-HT2 receptors (Ki = 5,900nm – Leysen et al 1982) compared with (±)DOI (Ki = 19nm – Glennon et al 1986). An initial small study examining higher doses of quipazine than had been previously examined in twitching studies indicated that scratching behaviour could be produced but required doses > 5mg/kg i.p. (quipazine's Ki at 5-HT2 receptors = 230nm – Glennon 1987).

The ED50 for the inhibition of (±)DOI-induced scratching by ritanserin was lower than that for the inhibition of head-twitching induced by (±)DOI despite using a larger dose of (±)DOI to induce the scratching. Possibly this suggests that a larger number of 5-HT2 receptors require to be occupied to induce scratching than head-twitching, thus it might consequently be easier to inhibit. If the dose-response curve for the two behaviours is examined, it becomes apparent that larger doses of (±)DOI seem to be required to induce scratching behaviour in comparison with twitching behaviour (peak head-twitch frequency was observed using 1mg/kg, higher doses producing a reduction in this behaviour, whereas with scratching, the highest frequency of this behaviour was observed with the highest dose tested 10mg/kg, so the top of the dose-response curve was probably not reached).

2) 8-OH-DPAT.

8-OH-DPAT produced potent potentiation of (±)DOI-induced scratching. This result suggests that it is not activity at 5-HT1a receptors which

prevents less selective agonists such as 5-MeODMT from inducing scratching.

In contrast to the potentiation of (±)DOI-induced scratching induced by 8-OH-DPAT, (±)DOI-induced head-twitching was inhibited by 8-OH-DPAT. The fact that (±)DOI-induced scratching and head-twitching were differently affected by 8-OH-DPAT implies that the two behaviours may be controlled by different 5-HT pathways.

3 xylamidine.

A dose of xylamidine (2.5mg/kg) which has been shown to inhibit responses mediated by peripheral but not central 5-HT2 receptors (Fuller et al 1986) had no effect on scratching induced by (±)DOI 5mg/kg. In a further experiment, 10mg/kg xylamidine appeared to inhibit scratching after 5mg/kg but not 2.5mg/kg (±)DOI. However, the 5mg/kg dose of (±)DOI produced an unusually high rate of scratching. These experiments do not eliminate a peripheral component to (±)DOI-induced scratching especially when compared with the lack of effect of 10mg/kg of xylamidine on (±)DOI-induced head-twitching.

4) terfenadine.

The effect of terfenadine (a histamine1-receptor blocker which does not pass the blood brain barrier (Wiech and Martin 1982)) on (±)DOI-induced scratching was examined to see if this behaviour had a peripheral component possibly involving stimulation or release of histamine onto H1-receptors. In Man, intradermal injection of histamine consistently produces itching and the associated scratching, and H1-antagonists block this effect (Rothman 1960).

No effect was seen with the chosen dose of terfenadine (which has been shown to block a peripheral histamine-induced response (in the guinea pig - see Carr and Meyer (1982)) but not to produce measurable levels of terfenadine in CNS (Wiech and Martin 1982)) indicating that

peripheral histamine is unlikely to be involved in the scratching response to (±)DOI.

5) Noradrenergic ligands.

The NA depleting agent FLA-63, and clonidine, an alpha2 agonist produced inhibition, the same effect as was observed for (±)DOI-induced head-twitching. However, prenalterol, a beta1-agonist, procaterol, a beta2-agonist and idazoxan, an alpha2-antagonist, all produced no effect on (±)DOI-induced scratching, whereas (±)DOI-induced head-twitching was inhibited by procaterol, and potentiated by another beta1-agonist, dobutamine, and by idazoxan at the same doses (see Chapter 3 results sections 2.3, 2.1 and 3.3).

The fact that the noradrenergic depleting agent FLA-63 produced inhibition (almost total blockade) of scratching induced by (±)DOI is suggestive of a role for NA in the modulation of the expression of this behaviour. The marked differences in the effects seen with the NA agonists between (±)DOI-induced head-twitching and scratching is indicative that the two behaviours are likely to be controlled by different pathways.

6) naloxone.

It was decided to examine the effect of naloxone on (±)DOI-induced scratching on the basis of work done on what appears to be a virtually identical scratching response seen with substance P (i.t. or i.c.v.) to mice (Dobry et al 1981 & Share and Rackham 1981) which was found to be modulated by opioid agonists and antagonists. Opioid agonists e.g. morphine, dihydromorphine, etonitazine, etorphine, levorphanol, phenazocine and methadone, produced inhibition whereas the opioid antagonist naloxone produced potentiation of this behaviour (Share and Rackham 1981). Naloxone however had no effect on (±)DOI-induced scratching at a dose which had been shown, in the Share and Rackham 1981 study, to potentiate substance P (i.c.v.)-induced scratching in mice.

Grooming.

The effects of (±)DOI on on-going grooming behaviour were investigated as, in addition to scratching and head-twitching, increases in other forms of grooming behaviour i.e. face/paw grooming, penile grooming and lick-elsewhere grooming were noted in the initial behavioural analysis of (±)DOI. On further investigation, the effect of (±)DOI on such grooming behaviour was found to be slight and not dose-dependent. Significant increases and decreases in grooming activity were seen but only at certain doses.

Of the doses tested 5mg/kg produced significant effects most consistently, although it produced both inhibitory and potentiatory effects on on-going grooming behaviour. It decreased mean total time spent in grooming/15 min, decreased mean no. of grooming bouts and time spent in lick elsewhere grooming/15 min. In terms of the three 5min periods of grooming examined (0-5, 5-10 and 10-15min after administration), this dose of (±)DOI increased mean no. of bouts of total grooming, face/paw grooming and penile grooming in the 0-5min period but decreased mean time spent in total grooming, face/paw grooming, penile grooming and lick elsewhere grooming in the 10-15min period. One possible reason for the reduction in grooming produced by 5mg/kg in the 10-15min period is behavioural competition from other forms of behaviour potentiated by (±)DOI e.g. scratching behaviour, which, for a 2.5mg/kg dose, peaks between 7 and 12min after injection.

The major difficulty in interpreting the results of the effect of (±)DOI on on-going grooming behaviour in mice was a paper, found after this work was completed, by Rodriguez-Echandia et al (1983). They demonstrated that injection of saline (the vehicle used in the present experiments) induced excessive grooming in its own right. Their study used rats and two i.p. injections rather than the mice and one i.p. injection used in the present study, thus it cannot be certain that this effect would be produced in the present study. If it was, then it would complicate the assessment of the effect of (±)DOI on on-going grooming behaviour as it would imply that grooming was probably

already being potentiated by the injection process before any effect of (±)DOI was superimposed on it.

Rodriguez-Echandia et al (1983) also provided evidence for the involvement of serotonergic pathways in saline-induced excessive grooming, as two 5-HT antagonists, methysergide and pitzotifen prevented the excessive grooming response. The 5-HT antagonists used, methysergide and pitzotifen, bind with highest affinity to 5-HT2/5-HT1c receptors (Hoyer 1988a), which might suggest the involvement of such receptors in the saline-induced grooming response.

Locomotion.

The effect of (±)DOI on locomotor activity on Animex using unhabituated mice was examined since, in contrast to the decrease in spontaneous activity seen in the behavioural analysis of (±)DOI (Fig. 2.1), during head-twitching experiments (±)DOI appeared to produce a marked increase in locomotion.

The increase in locomotor activity seen with (±)DOI, in experiments specifically designed to measure it, occurred over a small dose range 0.25-1mg/kg, doses above and below this range produced no effect. The increase in locomotor activity between 0.25-1mg/kg, while not marked, was found to be dose-dependent through linear regression analysis, although the log-dose-response slope was found to be shallow.

Yamamoto and Ueki (1975) found similar results with DOM, a compound structurally related (both are phenalkylamines) to (±)DOI which also has high affinity for 5-HT2 receptors (Glennon et al 1986). They studied ambulation in an open-field situation. In rats, DOM showed no change at a dose of 0.1mg/kg, but caused a significant increase in ambulation at 0.5-1mg/kg. They found the maximum increase in ambulation was of approximately the same magnitude with all doses from 0.5 to 30mg/kg. The effects of DOM on ambulation in mice were found to be similar to those in rats. Ambulation was increased by DOM at a dose

of 0.5mg/kg but showed no change at 1mg/kg and was decreased at doses larger than 5mg/kg.

Ritanserin inhibited the increase in locomotor activity induced by (±)DOI. This suggests that the increased locomotor activity was mediated via 5-HT2/5-HT1c receptors. However, Kennett and Curzon (1988a) found that mCPP and TFMPP, two agonists with affinity for the 5-HT1c receptor (Hoyer 1988a), reduced locomotion of rats placed in a novel observation cage (as used in the present study) - an effect which they concluded was produced by 5-HT1c receptor activation. This observation suggests that activation of 5-HT1c receptors is unlikely to be the mechanism by which (±)DOI produces increase in locomotor activity, although, the work in this study has not eliminated an involvement of 5-HT1c receptors in (±)DOI-induced hyperlocomotion.

Several other agents with potency for the 5-HT2 receptor have also been shown to produce increases in locomotor activity i.e. mescaline (Yamamoto and Ueki 1975), quipazine (Simansky and Schecter (1988) and LSD (Dandiya et al 1969; 1970) a non-selective 5-HT agonist with high affinity (in order of decreasing affinity) for 5-HT2 = 5-HT1a > 5-HT1c > 5-HT1b receptors (Engel et al 1986). Also 5-HTP, the 5-HT precursor has been reported to increase locomotor activity (Schlosberg and Harvey 1979).

Schlosberg and Harvey (1979) found that when they administered quantities of 5-HTP greater than 220mg/kg (plus a decarboxylase inhibitor) the '5-HT syndrome' was produced, which was associated with a reduction in locomotor activity, whereas, in amounts 27.5-220mg/kg, an increase in locomotor activity was seen without the symptoms of the serotonin syndrome. They did not test if this increase in locomotor activity was 5-HT2-related but the fact that the '5-HT syndrome', a 5-HT1a/b-related behaviour in rats, was associated with reduction in locomotor activity might suggest that the locomotor potentiation seen with lower doses of 5-HTP is a 5-HT2 receptor mediated effect. Although RU24969, a selective 5-HT1b agonist (Sills et al 1984 & Middlemiss 1985b) has been shown to produce hyperlocomotion (Green et

al 1984) as has 8-OH-DPAT (Tricklebank et al 1985), the selective 5-
HT1a agonist (Hoyer 1988a).

In line with a stimulant role for the 5-HT2 receptor in locomotion,
Lassen (1989) found that nialamide (an MAOI) produced an abnormal
hyperlocomotion (in familiar conditions) which was inhibited by the 5-
HT2/5-HT1c antagonist, ritanserin, but not by the 5-HT1a&b antagonist,
L-propranolol.

However, Wing et al (1990) found that the 5-HT2 agonists, mescaline,
quipazine, DOI, DOM and 2,5-dimethoxy-4-ethylamphetamine (DOET)
produced an inhibition of locomotor and investigatory behaviour in
unfamiliar conditions - such as those used in the present study -
which was blocked by the ketanserin (mescaline, DOM & quipazine) or
ritanserin (quipazine). The suppression of exploratory behaviour was
attenuated when familar conditions were used. The fundamental
difference between their work and the present study was the use of
rats as the experimental animals as opposed to mice and the presence
in the experimental chamber of holes in the floor and walls, possibly
these differences are responsible for the opposite effects on
locomotor activity in the two tests.

The observation that there was a decrease in spontaneous activity in
the behavioural analysis of (±)DOI in contrast to the increase in
locomotor activity on Animex is possibly related to the fact that in
the former test the animals were habituated whereas in the latter the
conditions under which hyperlocomotion was produced were unfamiliar to
the experimental animals.

FIG. 2.1 Effect of (±) DOI on mouse behaviour intensity 15 min after after IP injection
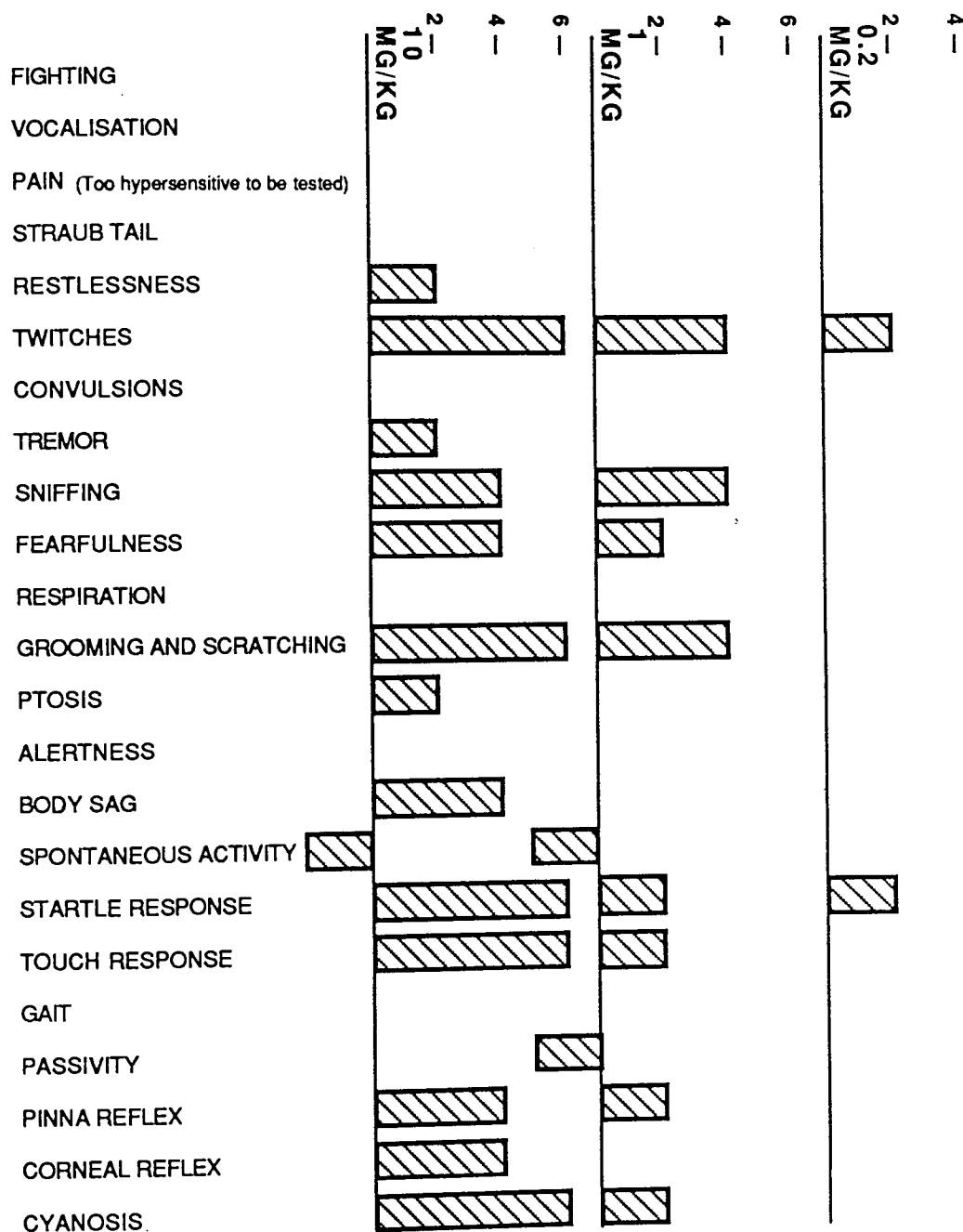
Fig. 2.1 Behaviour intensity was scored on the scale +6 to -6, using the criteria of Irwin (1968), 15min after the injection of 0.2, 1.0 and 10mg/kg of (±)DOI i.p. in mice.

Fig. 2.2 Effect of (±) DOI 5 mg/kg on rat gross behaviour
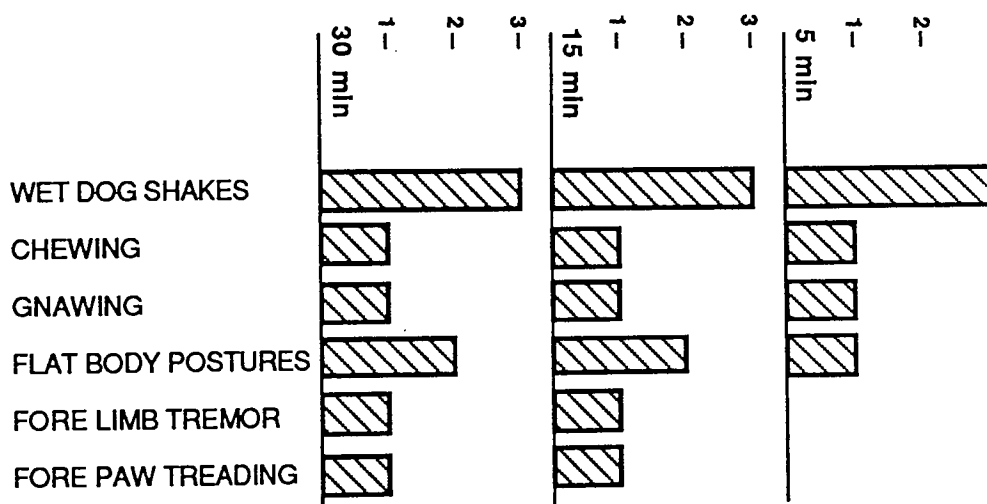
Fig. 2.2 Intensity of 5-HT-mediated behaviour was scored on a scale of 0 to 3, at 5, 15 and 30min after i.p. injection of 5mg/kg (±)DOI i.p.

MOUSE - TIME-COURSE FOR DOI-INDUCED HEAD-TWITCHING

FIG. 2.3 (a)

□ VEHICLE
◆ DOI mn 3

RAT - TIME-COURSE FOR DOI-INDUCED TWITCHING
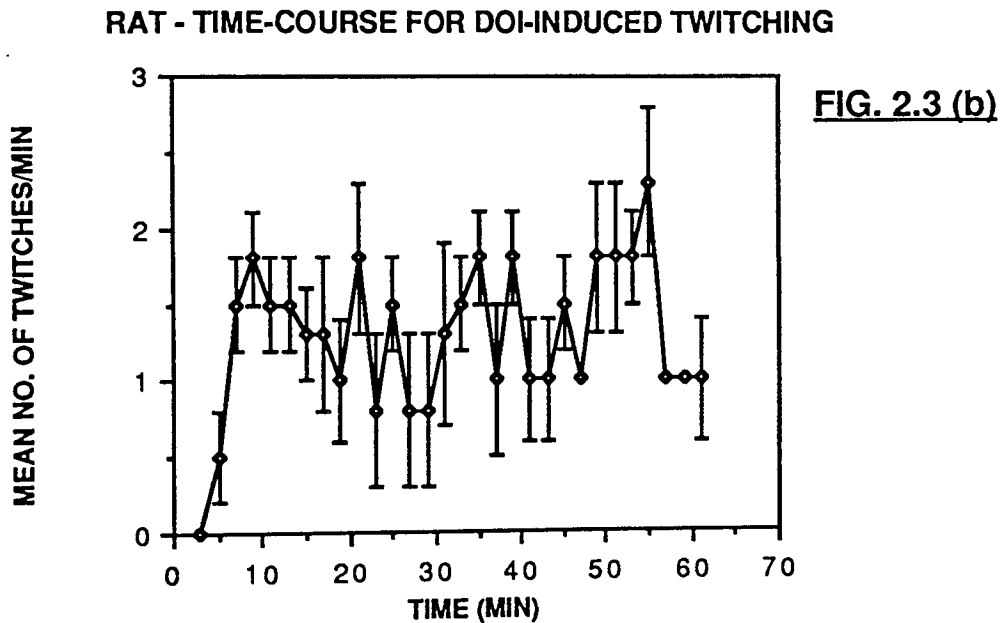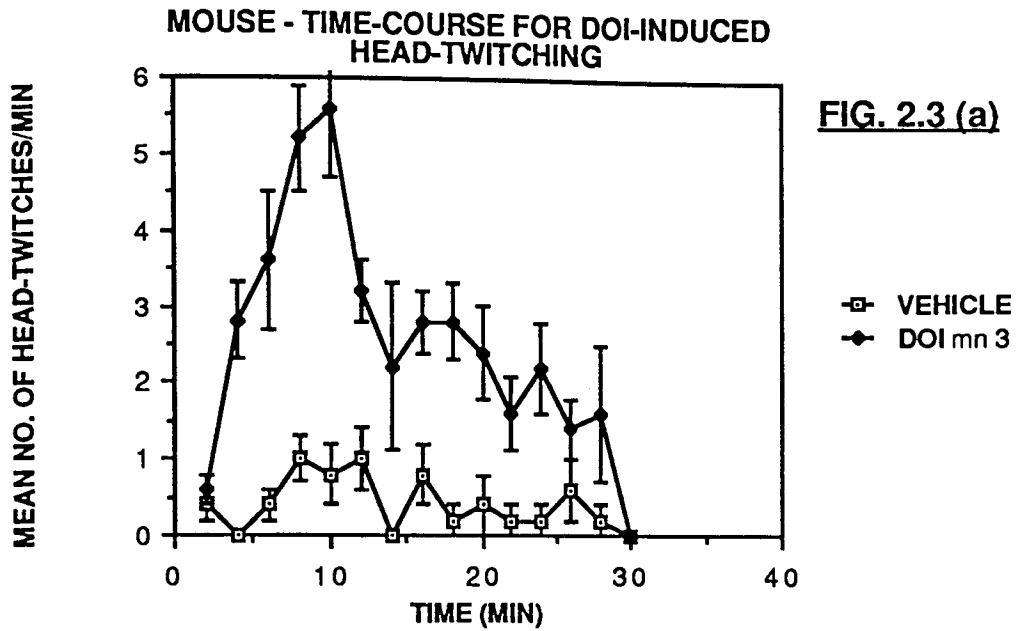
FIG. 2.3 (b)

Fig. 2.3 (a)   One mouse from each pair was injected with vehicle (i.p.), the other received (±)DOI (1mg/kg i.p.) at time 0.

Fig. 2.3 (b)   Each experimental rat was injected with (±)DOI (1mg/kg i.p.) at time 0.

MOUSE - DOSE-RESPONSE EFFECT FOR DOI-INDUCED HEAD-TWITCHING
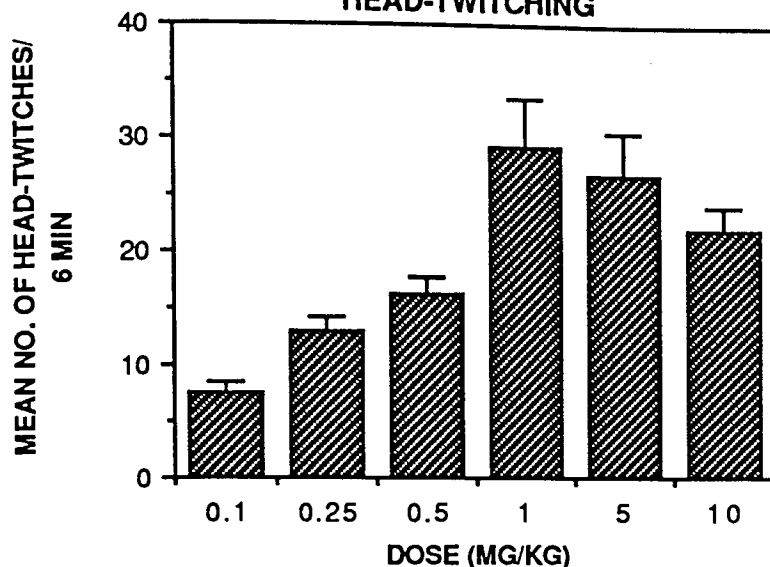
FIG. 2.4 (a)

RAT - DOSE-RESPONSE EFFECT FOR DOI-INDUCED TWITCHING
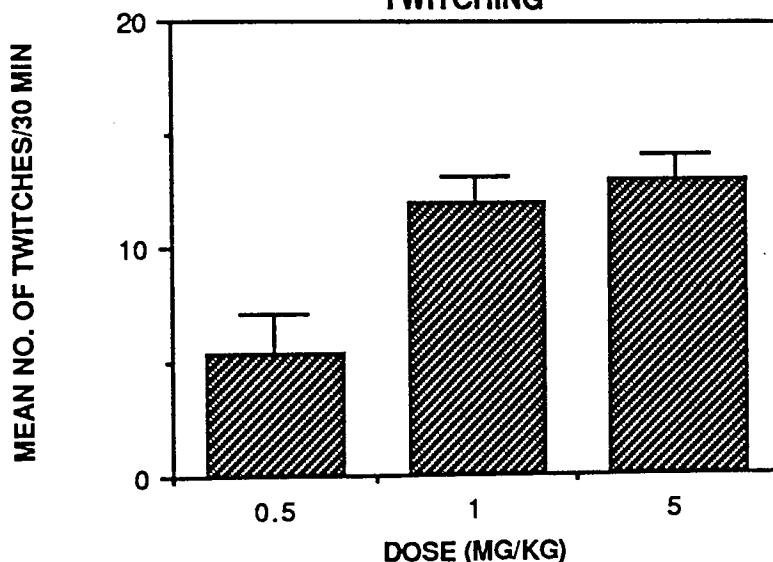
FIG. 2.4 (b)

Results were statistically evaluated using linear regression analysis on the raw data. Results were the means of at least 5 determinations and vertical bars represent s.e.m..

Fig. 2.4 (a) Induction of (±)DOI head-twitching in mice (0.1-10mg/kg i.p.) was found to be dose-dependent (F = 4.93; df 1,32; p <0.032).

Fig. 2.4 (b) Induction of (±)DOI twitching in rats (0.5-5mg/kg i.p.) was found to be dose-dependent (F = 5.22; df 1,15; p <0.036).

EFFECT OF 8-OH-DPAT ON DOI-INDUCED SCRATCHING
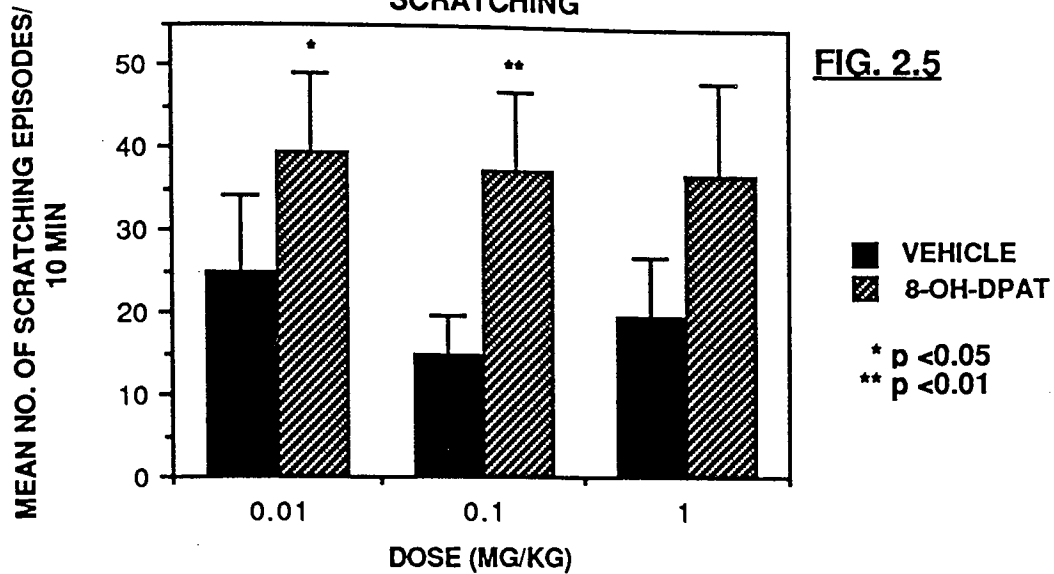
FIG. 2.5

EFFECT OF 8-OH-DPAT ON DOI-INDUCED HEAD-TWITCHING

FIG. 2.6

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s. e. m.

Fig. 2.5 All mice received ($\pm$)DOI (2.5mg/kg i.p.). Control mice received vehicle (i.p.) and test mice 8-OH-DPAT (i.p.).

Fig. 2.6 All mice received ($\pm$)DOI (0.5mg/kg i.p.). Control mice received vehicle (i.p.) and test mice 8-OH-DPAT (i.p.).

EFFECT OF BUSPIRONE ON DOI-INDUCED HEAD-TWITCHING

FIG. 2.7

(Bar chart: MEAN NO. OF TWITCHES/6 MIN vs DOSE (MG/KG))

Legend:
- VEHICLE
- BUSPIRONE
- * p <0.05
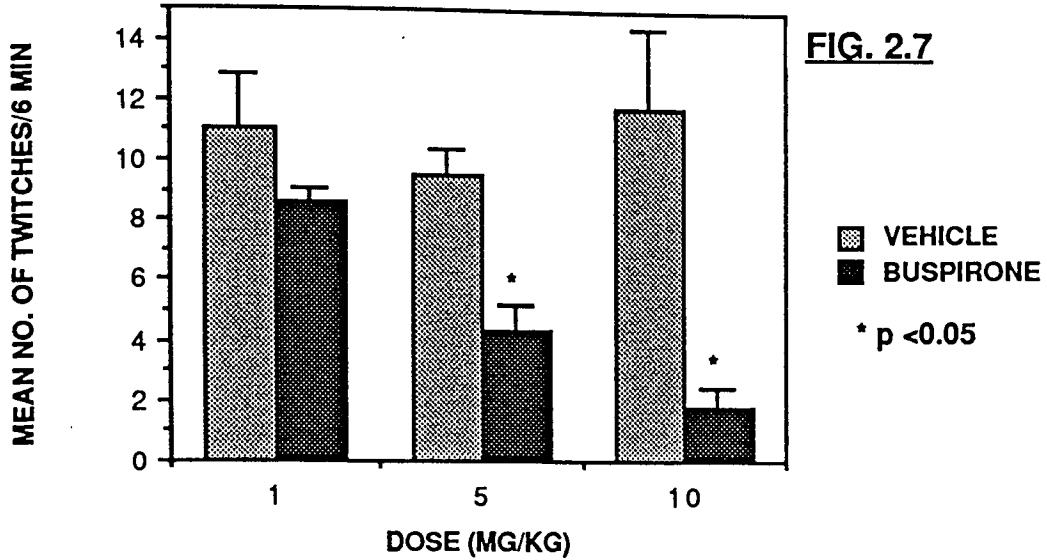
EFFECT OF IPSAPIRONE ON HEAD-TWITCHING INDUCED BY TWO DIFFERENT INDUCERS

FIG. 2.8

(Bar chart: MEAN NO. OF TWITCHES/6 MIN for DOI and 5-MeODMT)

Legend:
- VEHICLE
- IPSAPIRONE
- * p <0.05

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s. e. m.

Fig. 2.7 All mice received (±)DOI (0.5mg/kg i. p.). Control mice received vehicle (i. p.) and test mice buspirone (i. p.).

Fig. 2.8 Mice received either (±)DOI (0.5mg/kg i. p.) or 5-MeODMT (5mg/kg i. p.). Control mice received vehicle (i. p.) and test mice ipsapirone (i. p.).

EFFECT OF XYLAMIDINE ON DOI-INDUCED SCRATCHING

FIG. 2.9

- ■ VEHICLE
- ▨ XYLAMIDINE

** p <0.01

EFFECT OF XYLAMIDINE ON DOI-INDUCED HEAD-TWITCHING

FIG. 2.10

- ■ VEHICLE
- ▨ XYLAMIDINE
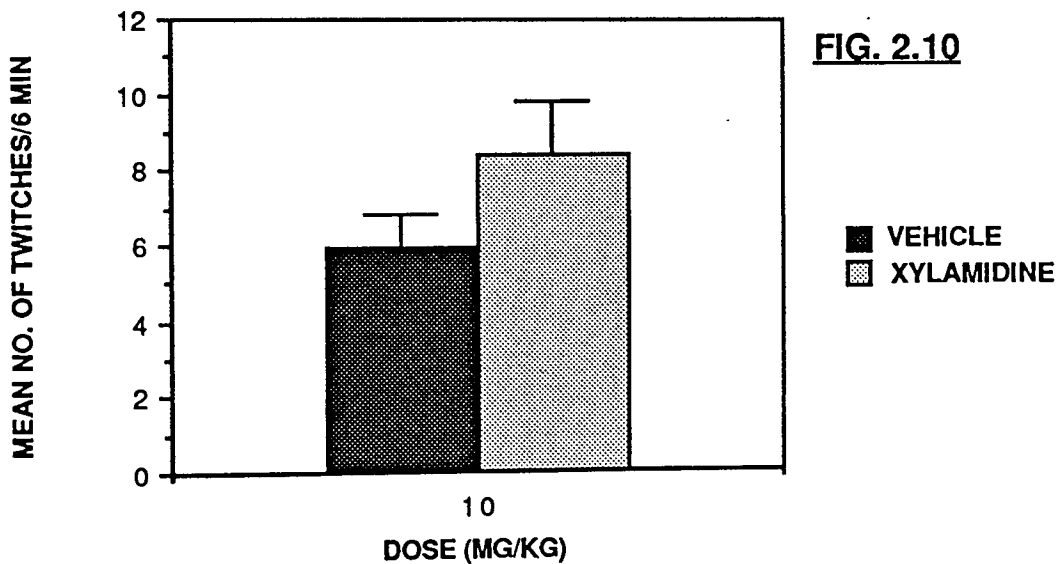
Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s. e. m.

Fig 2.9 Mice received either 2.5 or 5mg/kg (±)DOI (i. p.). Control mice received vehicle (i. p.) and test mice xylamidine (i. p.).

Fig 2.10 All mice received (±)DOI (0. 5mg/kg i. p.). Control mice received vehicle (i. p.) and test mice xylamidine (i. p.).

EFFECT OF RITANSERIN ON DOI-INDUCED SCRATCHING

FIG. 2.11

VEHICLE
RITANSERIN

* p <0.05
** p <0.01

EFFECT OF RITANSERIN ON DOI-INDUCED HEAD-TWITCHING

FIG. 2.12

VEHICLE
RITANSERIN

* p <0.05
** p <0.01
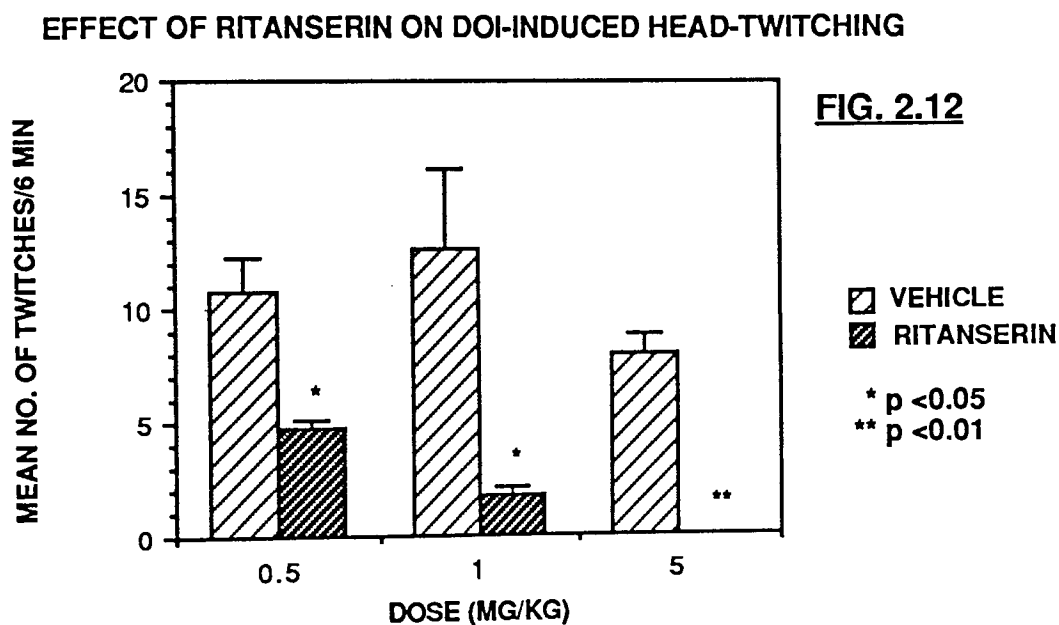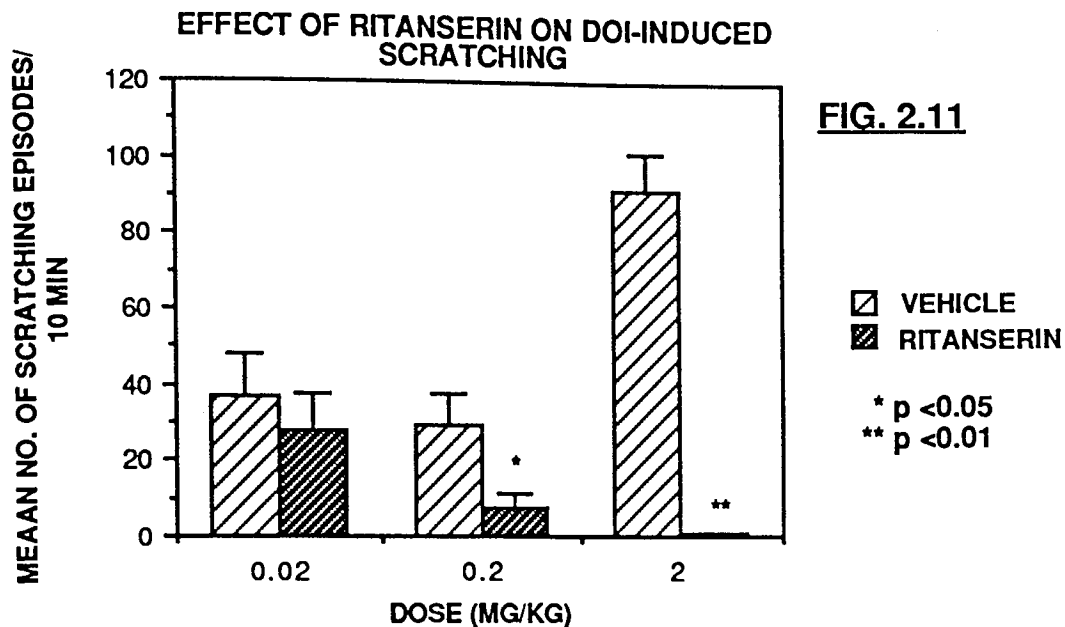
Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 2.11 All mice received (±)DOI (2.5mg/kg i.p.). Control mice received vehicle (i.p.) and test mice ritanserin (i.p.)

Fig. 2.12 All mice received (±)DOI (0.5mg/kg i.p.). Control mice received vehicle (i.p.) and test mice ritanserin (i.p.).

EFFECT OF IDAZOXAN ON INHIBITION OF DOI-INDUCED HEAD-TWITCHING BY 8-OH-DPAT

FIG. 2.13

■ VEHICLE

▨ 8-OH-DPAT

** p <0.01
(relative to
vehicle)
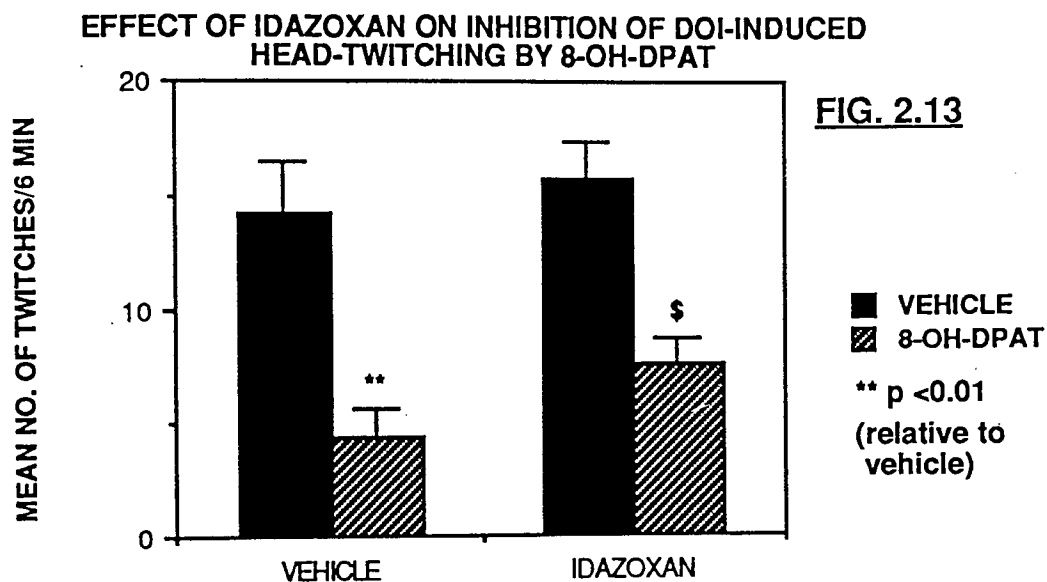
Fig. 2.13 Mice received idazoxan (0.5mg/kg s.c.) or vehicle (s.c.) 30min prior, and 8-OH-DPAT (1mg/kg i.p.) or vehicle (i.p.) 10min prior, to (±)DOI (0.5mg/kg i.p.). Results were analysed using a two-way between/within subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : F(AB) = 0.38; df 1,22; p >0.05. Interpretation : Idazoxan had no effect on 8-OH-DPAT-induced inhibition of (±)DOI-induced head-twitching.

Results are the means of at least 6 determinations and vertical bars represent s.e.m.

EFFECT OF ICI 169,369 ON DOI-INDUCED SCRATCHING
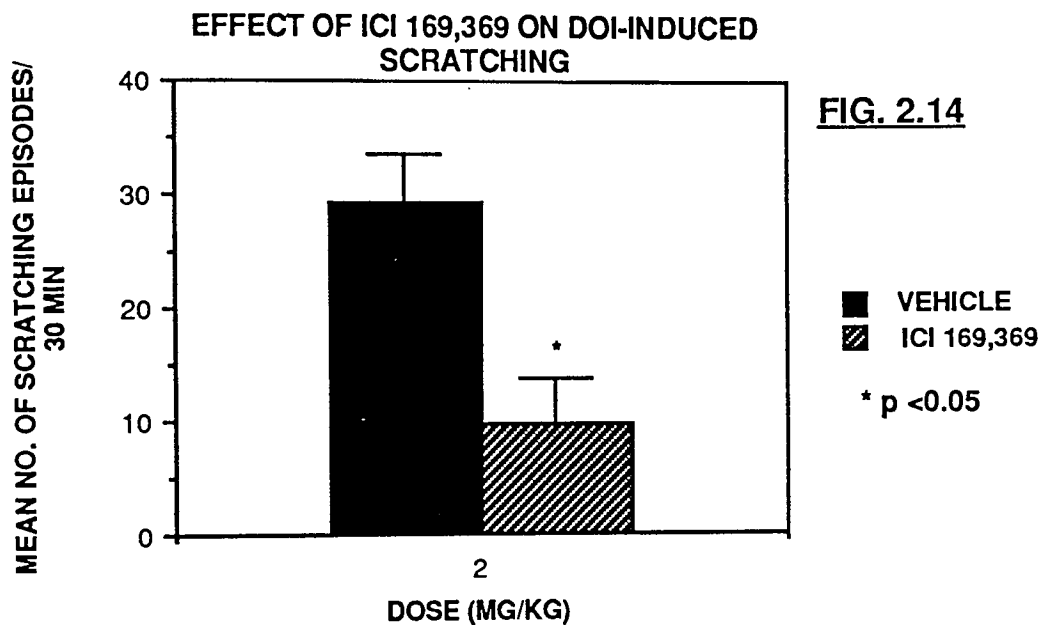
FIG. 2.14

Fig 2.14 Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice ICI 169,369 (i.p.). All mice received (±)DOI (2.5mg/kg i.p.). Results are the means of at least 6 determinations and vertical bars represent s.e.m.

DOSE-RESPONSE EFFECT FOR DOI-INDUCED SCRATCHING IN MICE
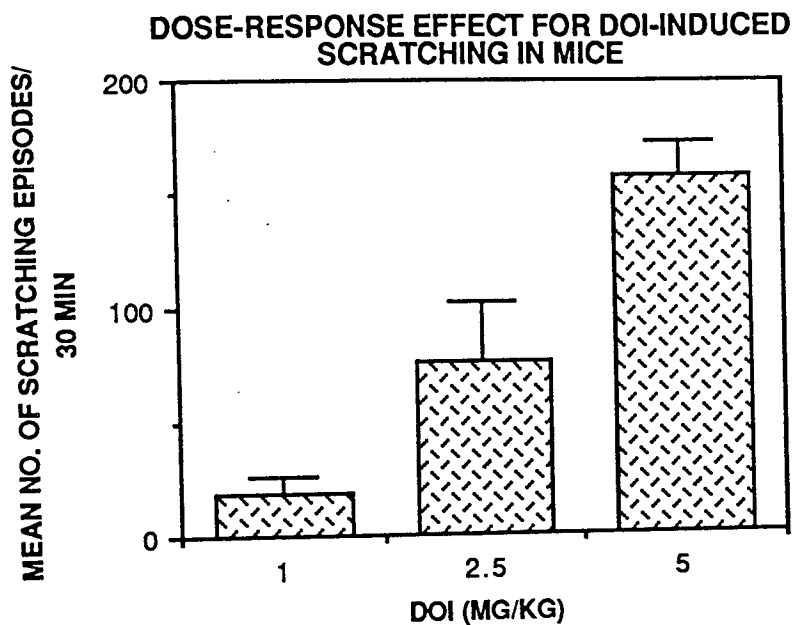
FIG. 2.15

Fig. 2.15 Results were statistically evaluated using linear regression analysis on the raw data. Induction of (±)DOI scratching (1-5mg/kg i.p.) was found to be dose-dependent (F = 34.58; df 1,15; p <0.001. Results are the means of at least 5 means determinations and vertical bars represent s.e.m.

FIG. 2.16 EFFECT OF DOI ON SCRATCHING IN MICE
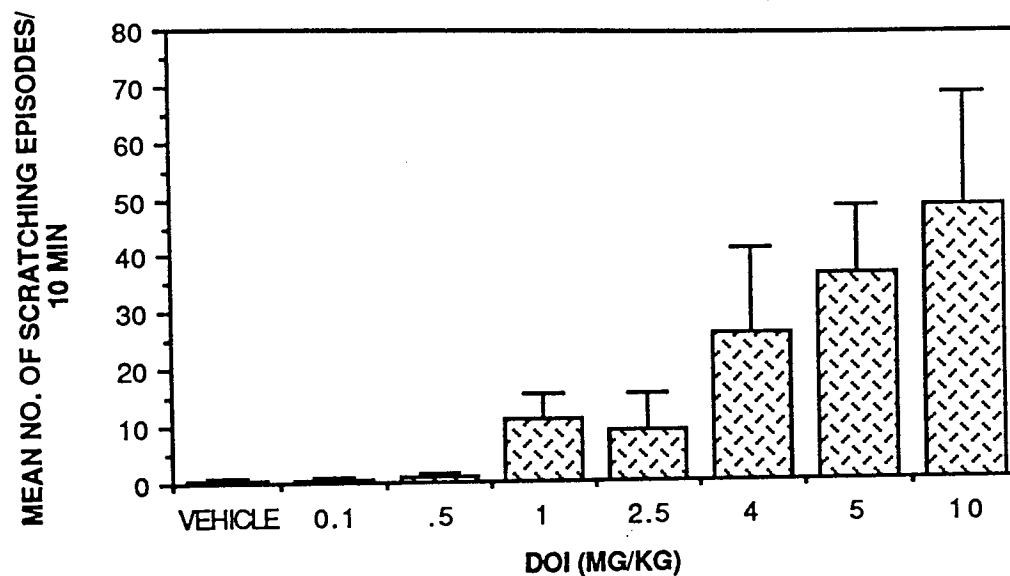
Fig. 2.16 Results were statistically evaluated using linear regression analysis on the raw data. Induction of ($\pm$)DOI scratching (0.1-10mg/kg i.p.) was found to be dose-dependent (F = 28.14; df 1,46; p <0.001). Results are the means of at least 5 determinations and vertical bars represent s.e.m.

TIME-COURSE FOR DOI-INDUCED SCRATCHING
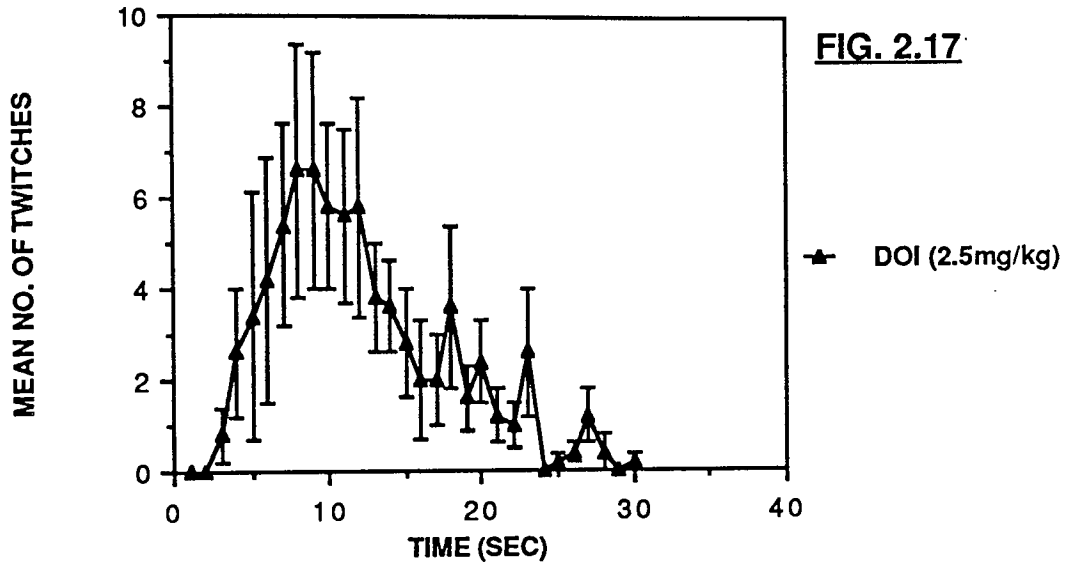
FIG. 2.17

Fig. 2.17 All mice received (±)DOI (2.5mg/kg i.p.). Scratching episodes were counted each minute from the 1st to 30th after (±)DOI administration. Results are the means of 6 determinations and vertical bars represent s.e.m.

EFFECT OF NALOXONE ON DOI-INDUCED SCRATCHING

FIG. 2.18

☑ VEHICLE
■ NALOXONE
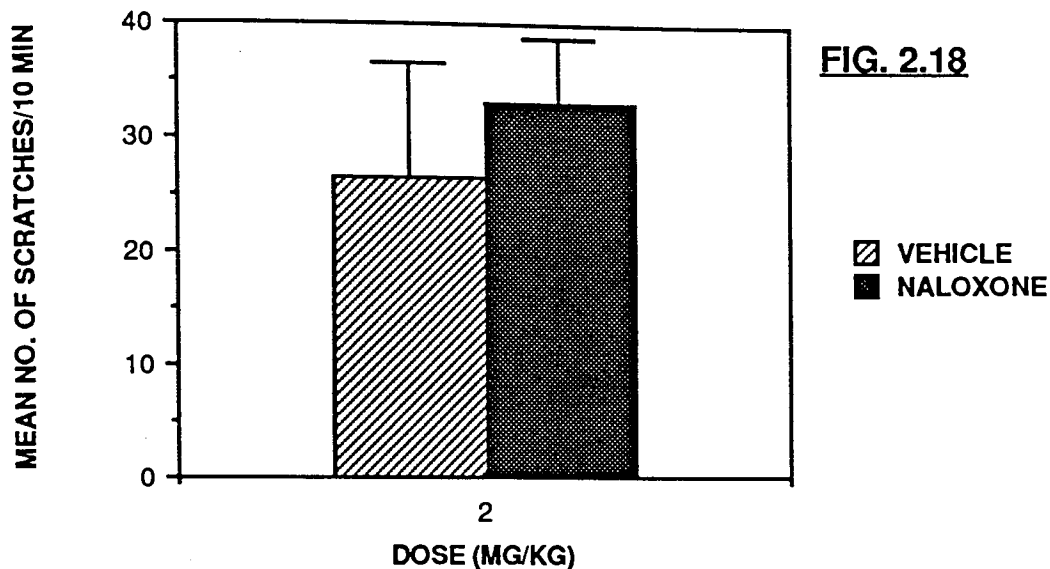
EFFECT ON TERFENADINE ON DOI-INDUCED SCRATCHING
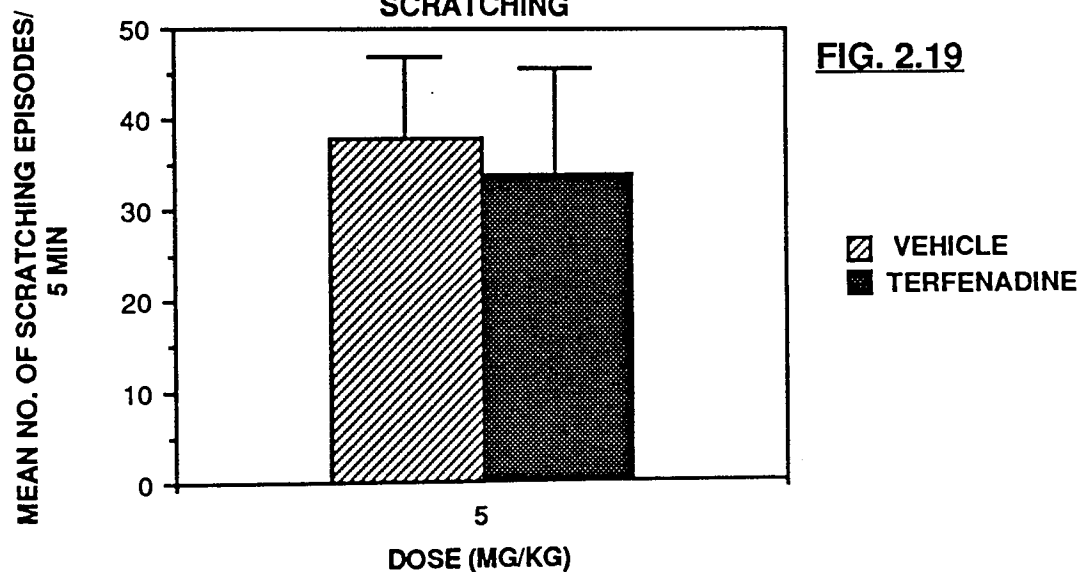
FIG. 2.19

☑ VEHICLE
■ TERFENADINE

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least six determinations and vertical bars represent s.e.m.

Fig. 2.18 All mice received (±)DOI (2.5mg/kg i.p.). Control mice received vehicle and the test mice, naloxone (i.p.).

Fig. 2.19 All mice received (±)DOI (0.5mg/kg i.p.). Control mice received vehicle (i.p.) and the test mice, terfenadine (i.p.).

EFFECT OF FLA-63 ON DOI-INDUCED SCRATCHING

FIG. 2.20

⬚ VEHICLE
▨ FLA-63 ·

** p <0.01

Y-axis: MEAN NO. OF SCRATCHING EPISODES/ 10 MIN
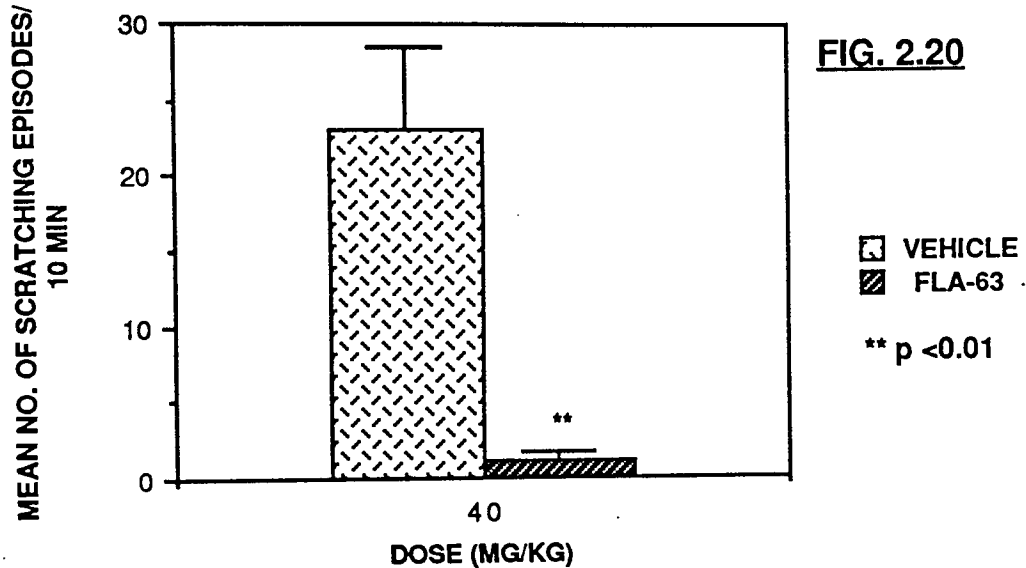
X-axis: DOSE (MG/KG) — 40

Fig. 2.20 All mice received (±)DOI (2.5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice FLA-63 (i.p.). Results are the means of at least 6 determinations and vertical bars represent s.e.m.

EFFECT OF CLONIDINE ON DOI-INDUCED SCRATCHING

FIG. 2.21

VEHICLE
CLONIDINE

* p <0.05
** p <0.01
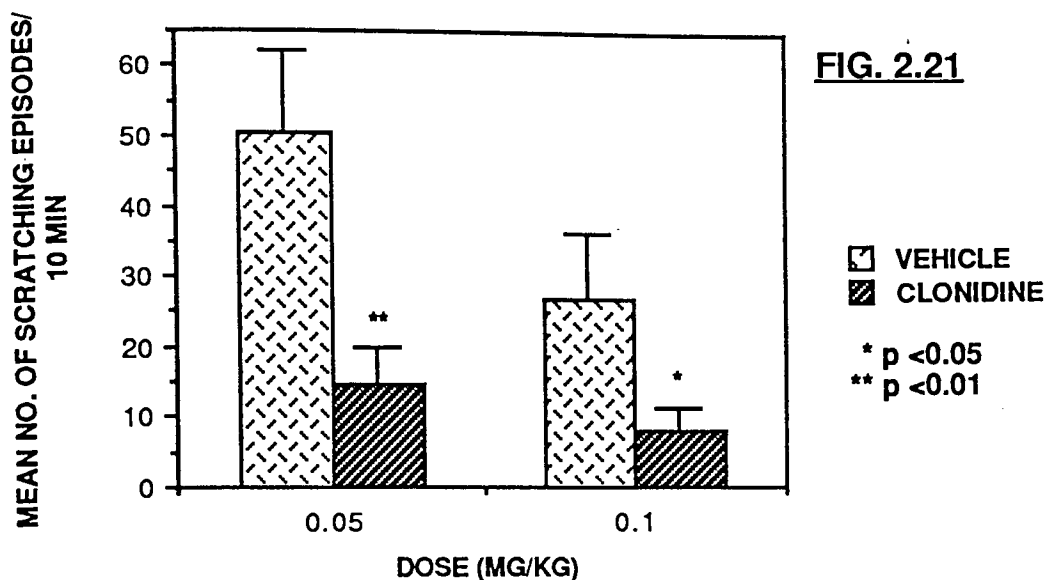
EFFECT OF IDAZOXAN ON DOI-INDUCED SCRATCHING
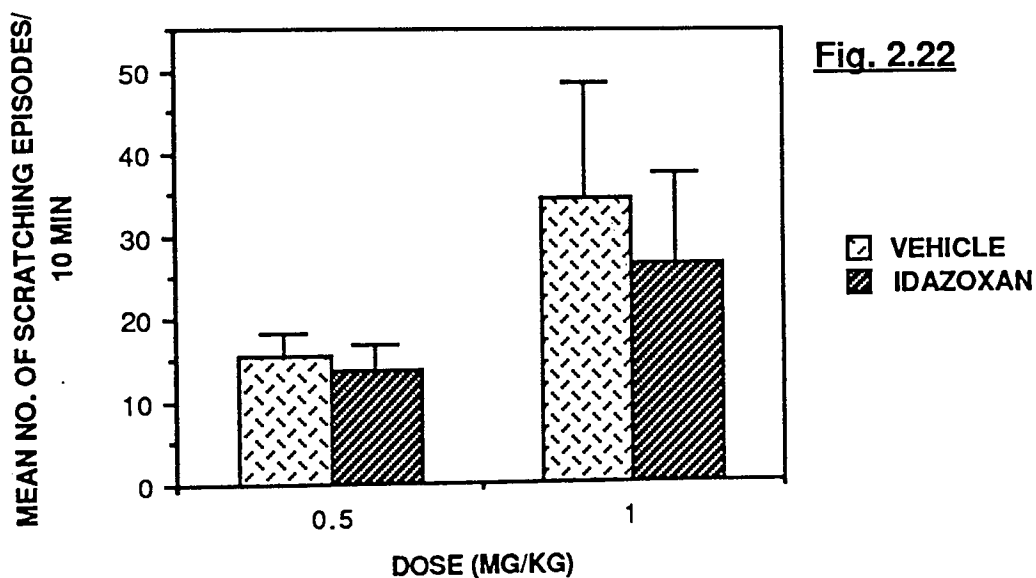
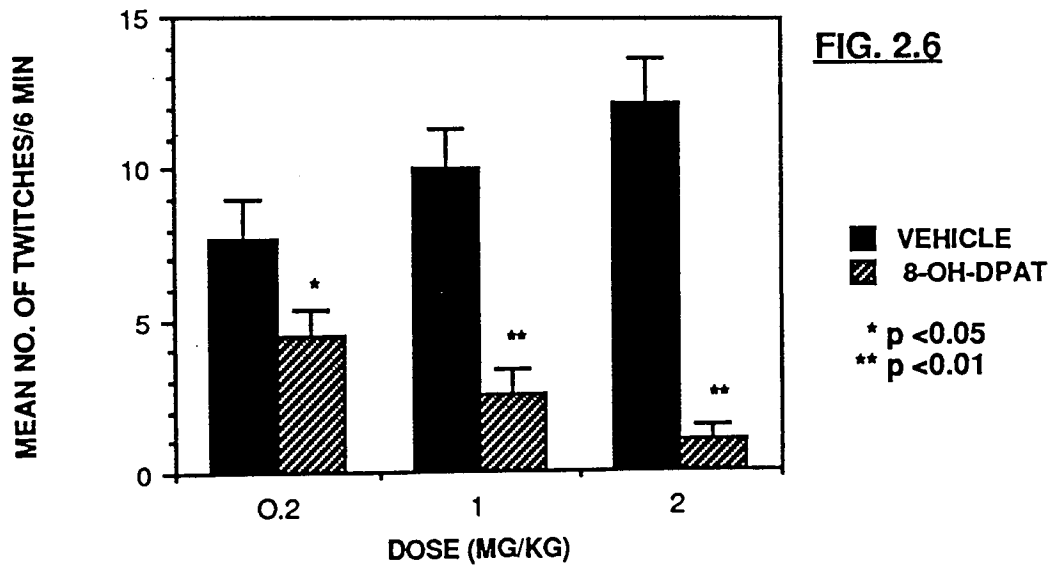Fig. 2.22

VEHICLE
IDAZOXAN

All mice received (±)DOI (2.5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 2.21   Control mice received vehicle (i.p.) and test mice clonidine (i.p.).

Fig. 2.22   Control mice received vehicle (i.p.) and test mice idazoxan (i.p.).

EFFECT OF PRENALTEROL ON DOI-INDUCED SCRATCHING
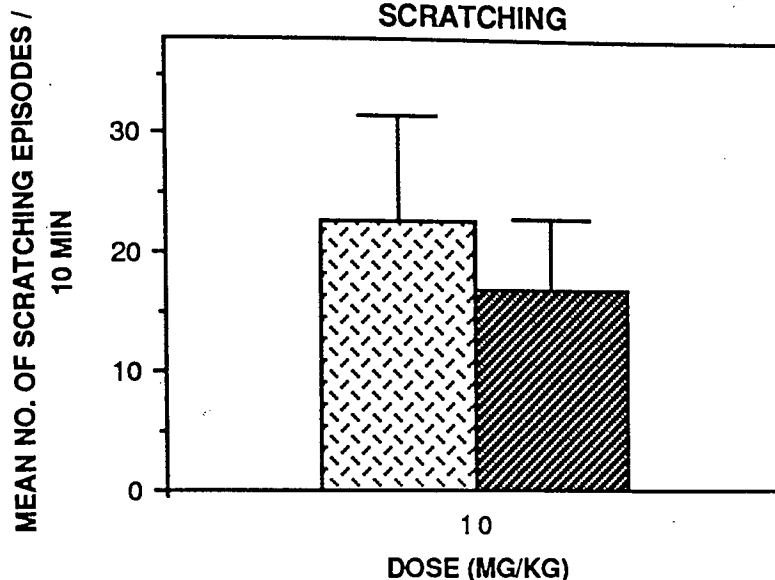
FIG. 2.23

☐ VEHICLE
▨ PRENALTEROL

EFFECT OF PROCATEROL ON DOI-INDUCED SCRATCHING
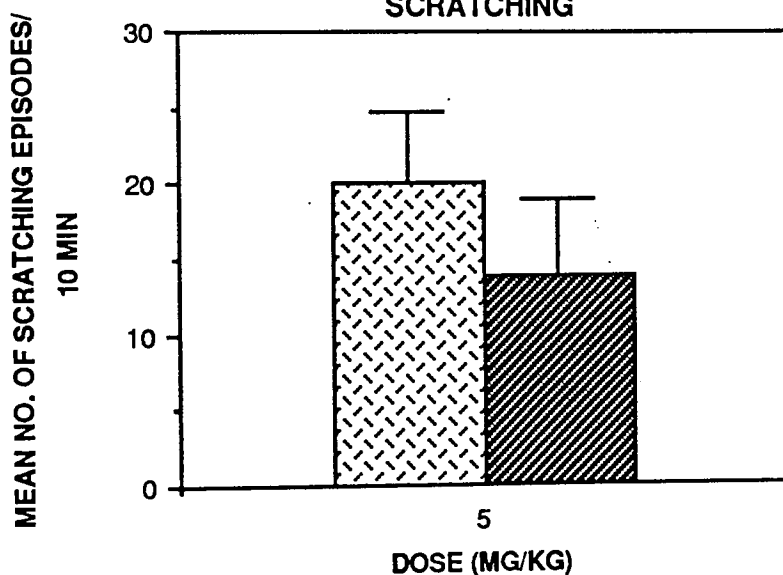
FIG. 2.24
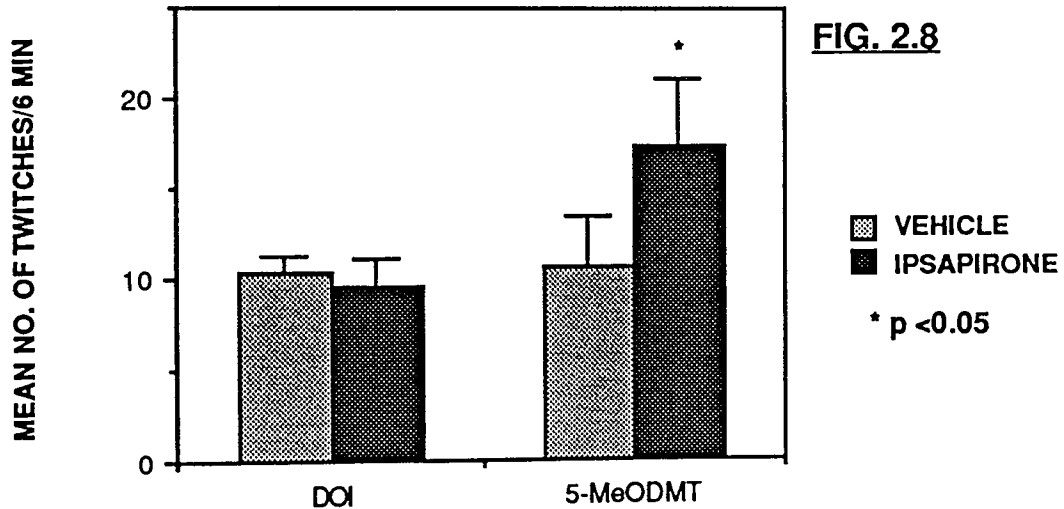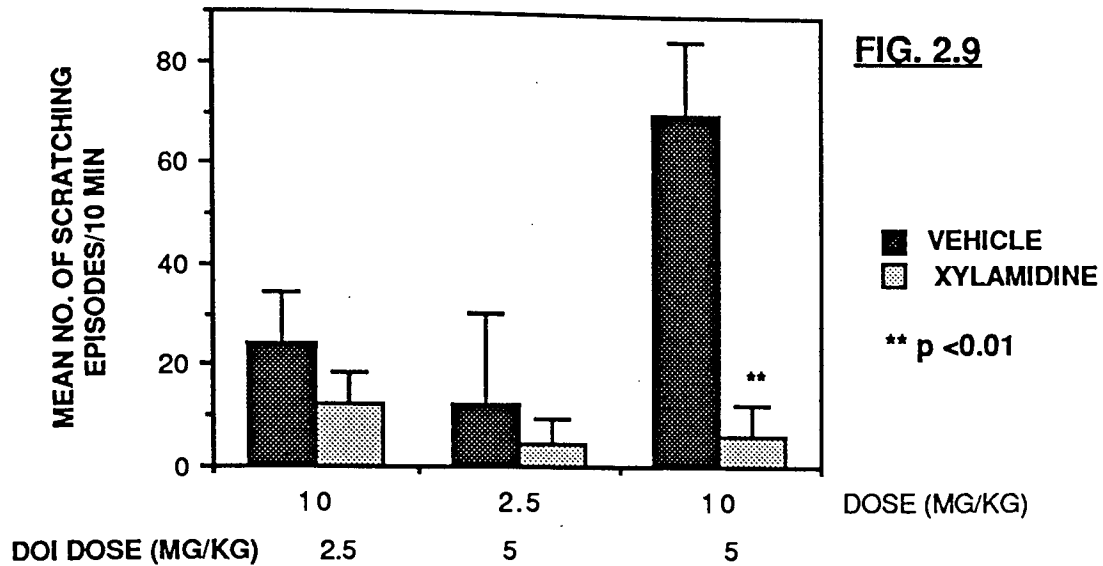
☐ VEHICLE
▨ PROCATEROL

All mice received (±)DOI (2.5mg/kg i.p.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 2.23 Control mice received vehicle (i.p.) and test mice prenalterol (i.p.).

Fig. 2.24 Control mice received vehicle (i.p.) and test mice procaterol (i.p.).

EFFECT OF DOI ON LOCOMOTOR ACTIVITY OF MICE (1)

FIG. 2.25

* p <0.05
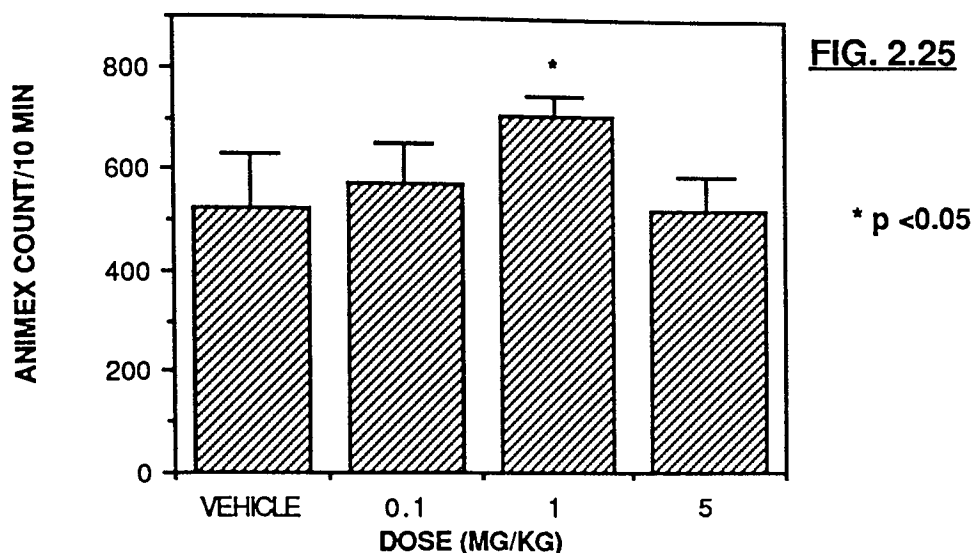
EFFECT OF DOI ON LOCOMOTOR ACTIVITY OF MICE (2)

FIG. 2.26
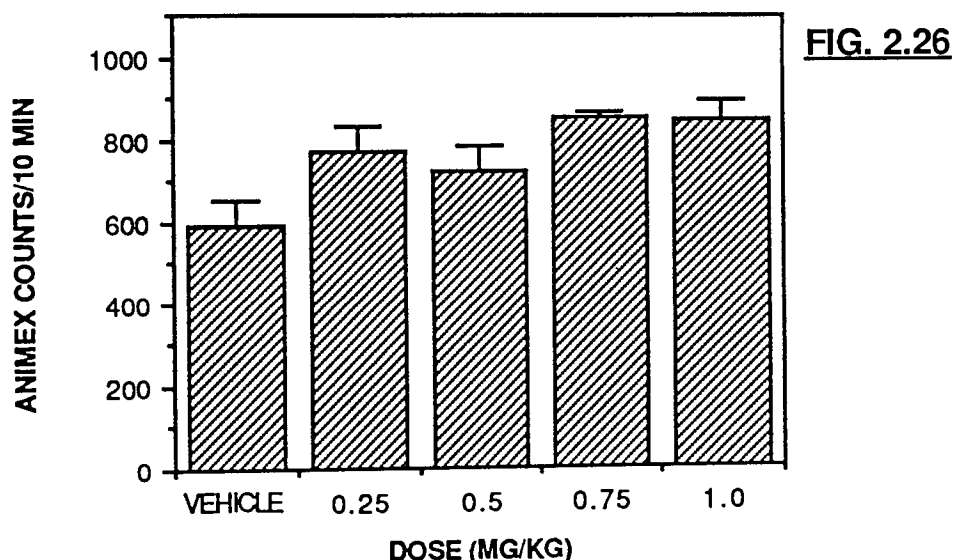
Control mice received vehicle (i.p.) and test mice one of the doses of (±)DOI. They were then placed back in their home cage, 10min later the locomotor activity of each mouse was measured using the Animex unit for 10min. All results are the means of at least 5 determinations and vertical bars represent s.e.m. Results were evaluated statistically using linear regression analysis on raw data.

Fig. 2.25   Induction of increased locomotor activity by (±)DOI (0-5mg/kg i.p.) was not dose-dependent (F = 0.54; df 1,19; p <0.48). However, an unpaired t-test revealed that test mice treated with (±)DOI 1mg/kg produced significantly higher locomotor activity counts (compared with vehicle treated control mice).

Fig. 2.26   Induction of increased locomotor activity by (±)DOI (0-1mg/kg i.p.) was found to be dose-dependent (F = 11.36; df 1,33; p <0.003).

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EFFECT OF RITANSERIN ON DOI-INDUCED INCREASE IN
LOCOMOTOR ACTIVITY

FIG. 2.27

VEHICLE
DOI

* p <0.05
(relative to
vehicle)
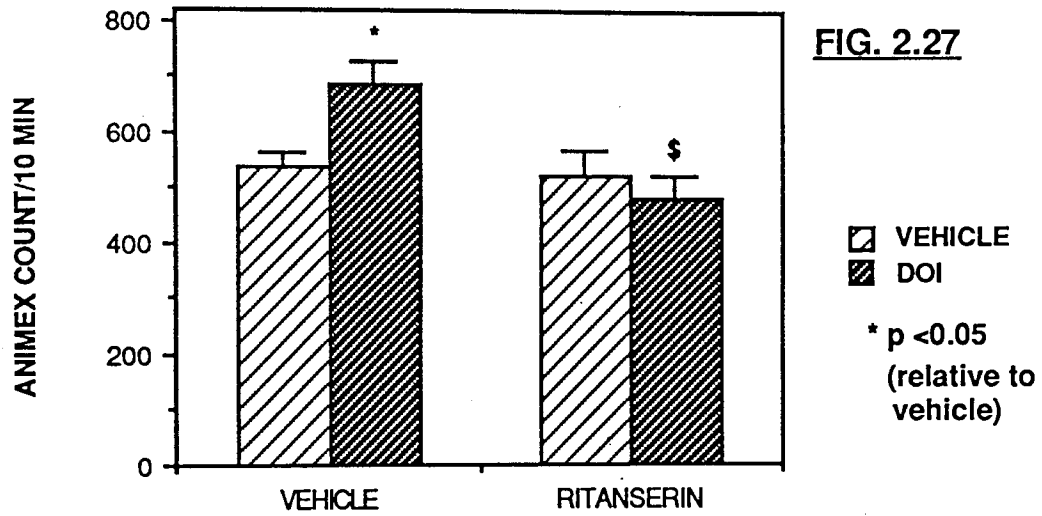
Fig. 2.27 Mice received either ritanserin (2mg/kg i.p.) or vehicle
(i.p.) 30min prior to (±)DOI (1mg/kg i.p.) or vehicle (i.p.).
Results are the means of 11 determinations and vertical bars
represent s.e.m.. Results were analysed using a two-way between
subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : F = 6.18; df 1,40; p <0.05. Interpretation :
Ritanserin significantly inhibited (±)DOI-induced increase in
locomotor activity.

EFFECT OF DOI ON NO. OF GROOMING BOUTS/15 MIN

F = 1.16; df 5,35; p >0.05
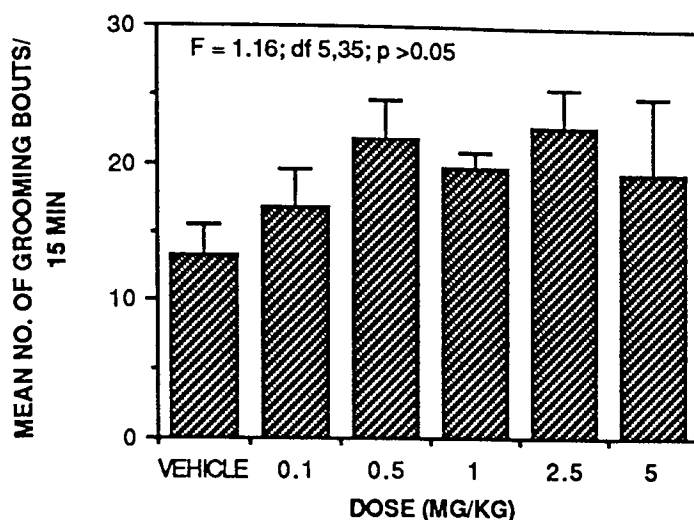
FIG. 2.28

EFFECT OF DOI ON TIME SPENT GROOMING/15 MIN

F = 2.21; df 5,35; p >0.05
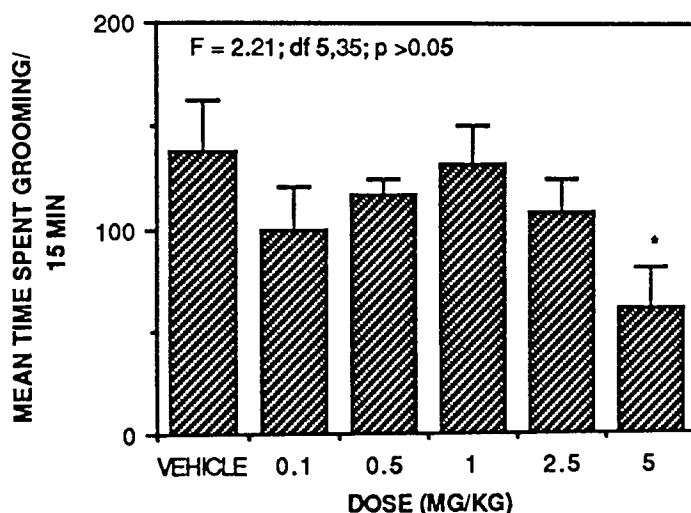
*p <0.05

Fig 2.28 Analysis of the effect of (±)DOI on on-going behaviour
(mean no. of grooming bouts and time spent grooming during the
first 15min after (±)DOI (0.1-5mg/kg i.p.) or vehicle (i.p.)
administration). Results were analysed statistically using a one-
way between subjects ANOVA on raw data (for the entire dose range).
For analysis of the effect of individual doses of (±)DOI, Duncan's
multiple-range test was used. * p <0.05 and ** p <0.01 (relative to
vehicle controls). All results are the means of 6 determinations
and vertical bars represent s.e.m..

EFFECT OF DOI ON NO. OF GROOMING BOUTS :
FOR THE 0 - 5 MIN PERIOD

$F = 4.69$; df 5,35; $p < 0.01$

FIG. 2.29

** $p < 0.01$

FOR THE 5 - 10 MIN PERIOD

$F = 1.71$; df 5,35; $p > 0.05$

FOR THE 10 - 15 MIN PERIOD

$F = 3.69$; df 5,35; $p < 0.05$

DOSE OF DOI (MG/KG)
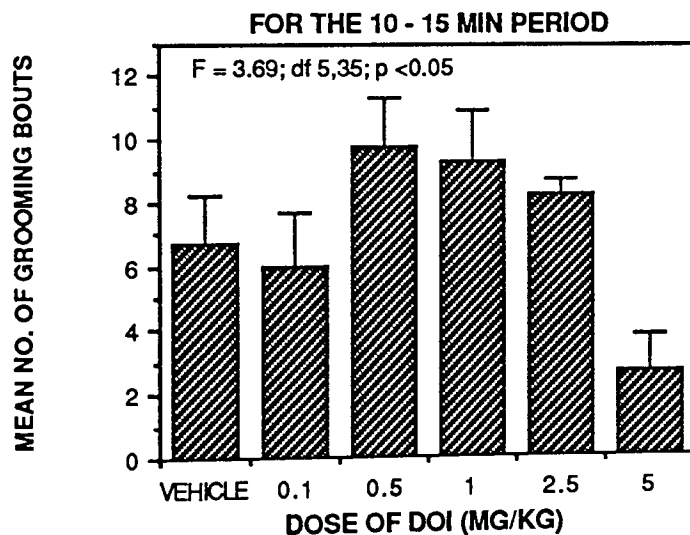
Fig. 2.29 Analysis of the effect of (±)DOI on on-going behaviour
(mean no. of grooming bouts in each 5min period (0-5, 5-10 and 10-
15min after (±)DOI (i.p.) or vehicle (i.p.) administration).
Results were analysed statistically using a one-way between
subjects ANOVA on raw data (for the entire dose range tested). For
analysis of the effect of individual doses of (±)DOI, Duncan's
multiple-range test was used. * p <0.05 and ** p <0.01 (relative to
vehicle controls). All results are the means of 6 determinations
and vertical bars represent s.e.m.

EFFECT OF DOI ON TIME SPENT GROOMING :

FOR THE 0 - 5 MIN PERIOD

FIG. 2.30

$F = 1.73; DF\ 5,35; p > 0.05$

MEAN TIME SPENT GROOMING

VEHICLE · 0.1 · 0.5 · 1 · 2.5 · 5

FOR THE 5 - 10 MIN PERIOD

$F = 1.14; DF\ 5,35; p > 0.05$

MEAN TIME SPENT GROOMING

VEHICLE · 0.1 · 0.5 · 1 · 2.5 · 5

FOR THE 10 - 15 MIN PERIOD

$F = 6.51; df\ 5,35; p < 0.01$

MEAN TIME SPENT GROOMING

VEHICLE · 0.1 · 0.5 · 1 · 2.5 · 5

DOSE OF DOI (MG/KG)
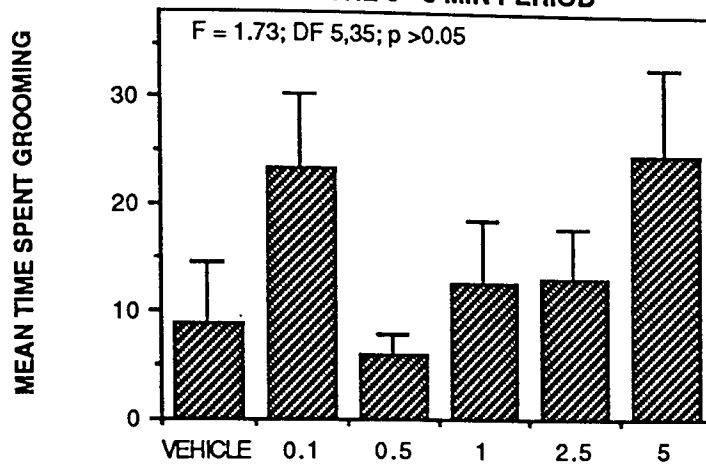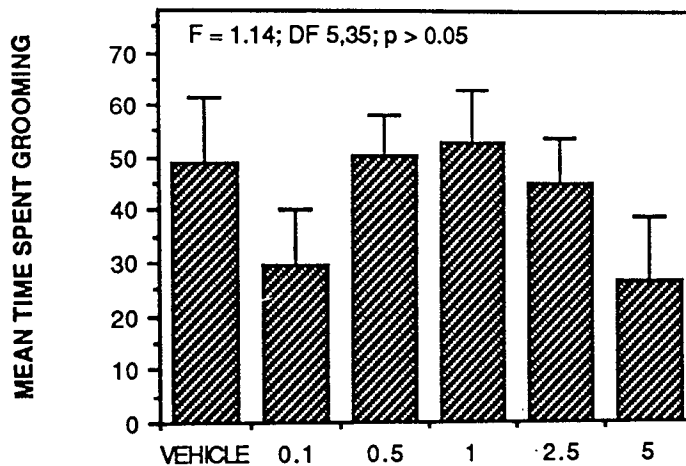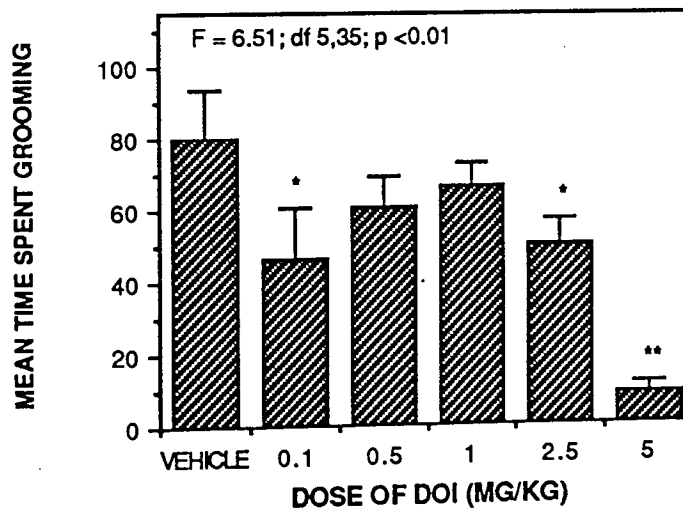
* p <0.05
** p <0.01

Fig. 2.30 Analysis of the effect of (±)DOI on on-going behaviour
(mean time spent grooming in each 5min period (0-5, 5-10 and 10-
15min after (±)DOI (i.p.) or vehicle (i.p.) administration).
Results were analysed statistically using a one-way between
subjects ANOVA on raw data (for the entire dose range tested). For
analysis of the effect of individual doses of (±)DOI, Duncan's
multiple-range test was used. * p <0.05 and ** p <0.01 (relative to
vehicle controls). All results are the means of 6 determinations
and vertical bars represent s.e.m..

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EFFECT OF DOI ON TIME SPENT/15 MIN IN :

FACE/PAW GROOMING

F = 2.24; df 5,35; p >0.05

FIG. 2.31

* p <0.05

PENILE GROOMING

F = 1.44; df 5,35; p >0.05

"LICK ELSEWHERE" (SEE TEXT) GROOMING

F = 3.94; df 5,35; p <0.01

* p <0.05
** p <0.01

DOSE (MG/KG)
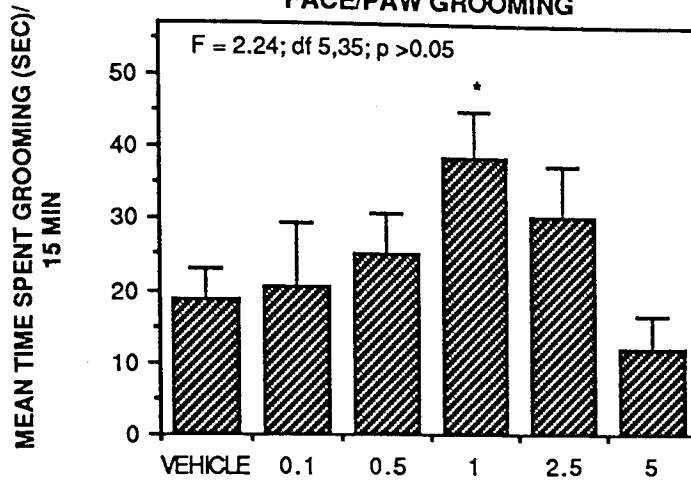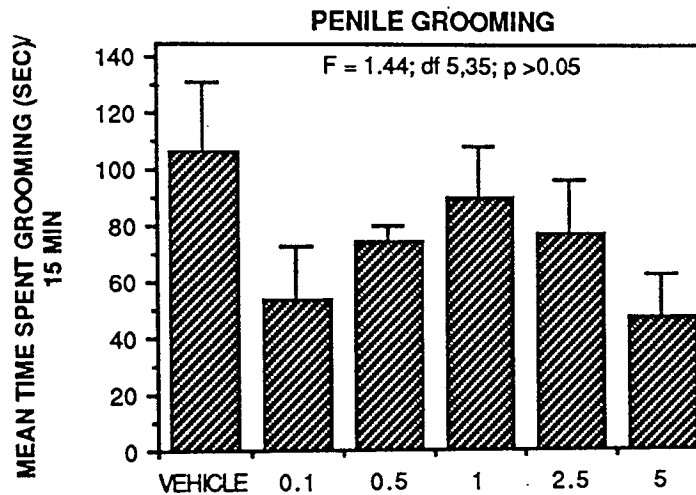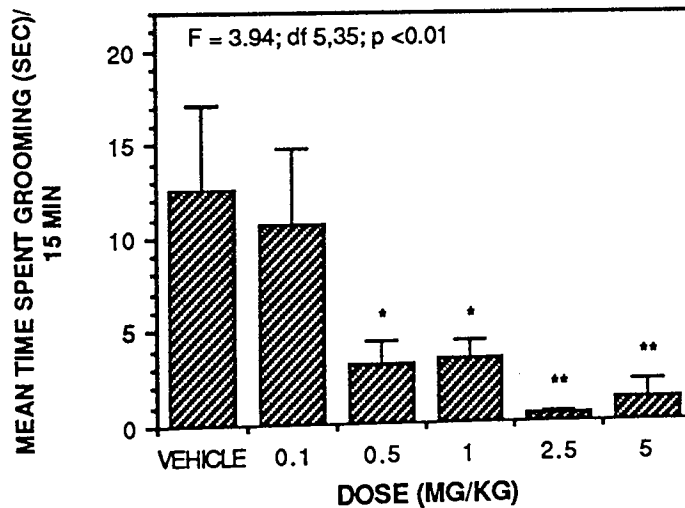
Fig. 2.31 Analysis of the effect of (±)DOI on on-going behaviour (mean time spent face/paw, lick elsewhere or penile grooming during the first 15min after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). Results are the means of 6 determinations and vertical bars represent s.e.m..

EFFECT OF DOI ON NO. OF GROOMING BOUTS/15 MIN OF :
FACE/PAW GROOMING

F = 1.44; df 5,35; p >0.05
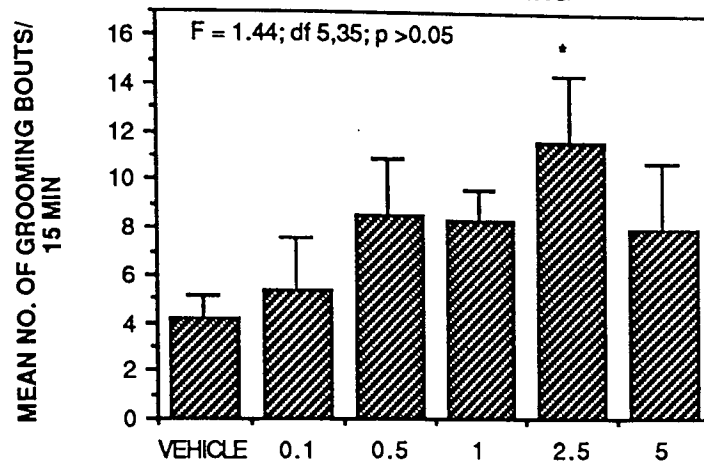
FIG. 2.32

* p <0.05

PENILE GROOMING

F = 1.01; df 5,35; p >0.05

"LICK ELSEWHERE" (SEE TEXT) GROOMING

F = 2.04; df 5,35; p >0.05
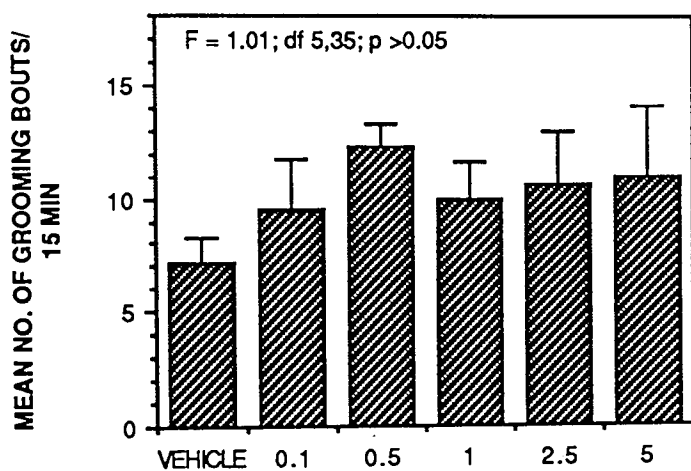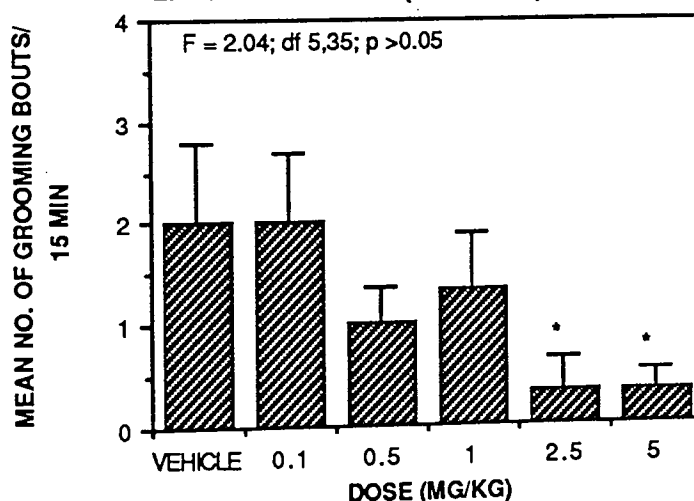
* p <0.05

Fig. 2.32   Analysis of the effect of (±)DOI on on-going behaviour (mean no. of face/paw, lick elsewhere or penile grooming bouts during first 15min after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). Results are the means of 6 determinations and vertical bars represent s.e.m.

EFFECT OF DOI ON FACE/PAW GROOMING :

FOR THE 0 - 5 MIN PERIOD

$F = 4.37; df 5,35; p < 0.01$

FIG. 2.33

** $p < 0.01$

FOR THE 5 - 10 MIN PERIOD

$F = 1.75; df 5,35; p > 0.05$

* $p < 0.05$

FOR THE 10 - 15 MIN PERIOD

$F = 1.72; df 5,35; p > 0.05$

DOSE (MG/KG)
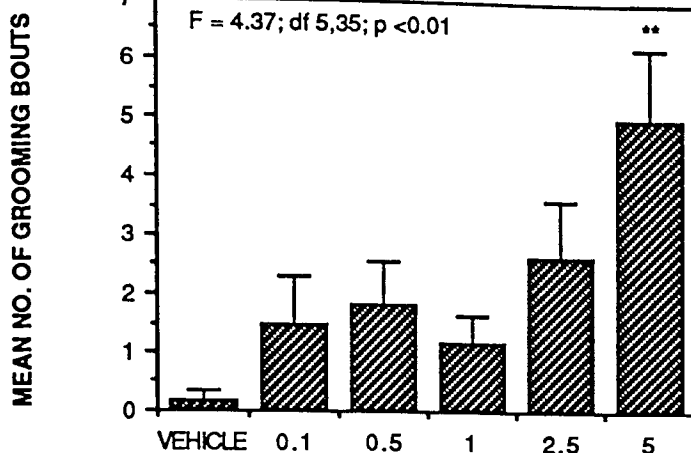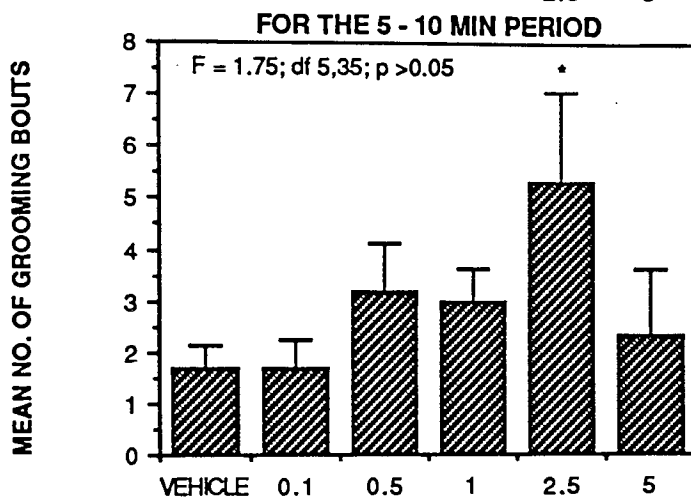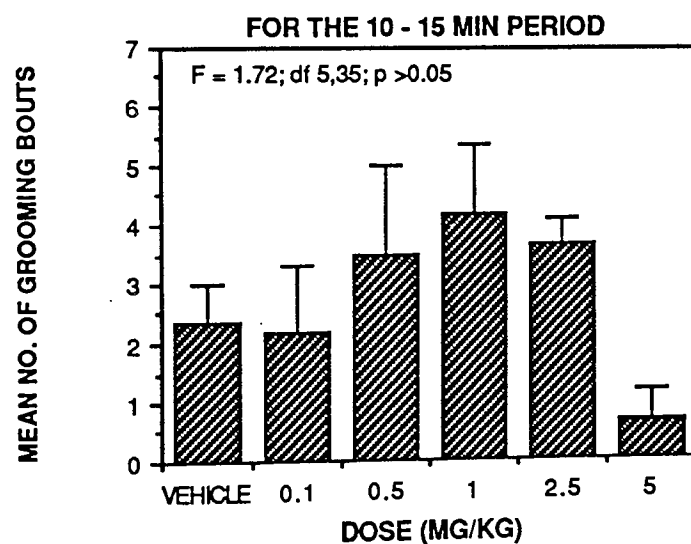
Fig. 2.33 Analysis of the effect of (±)DOI on on-going behaviour (mean no. of face/paw grooming bouts in each of the 5min periods examined (0-5, 5-10 and 10-15min) after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). All results are the means of 6 determinations and vertical bars represent s.e.m.

EFFECT OF DOI ON FACE/PAW GROOMING :

FOR THE 0 - 5 MIN PERIOD

$F = 1.08$; df 5,35; $p > 0.05$

FIG. 2.34

FOR THE 5 - 10 MIN PERIOD

$F = 1.71$; df 5,35; $p > 0.05$

FOR THE 10 - 15 MIN PERIOD

$F = 3.91$; df 5,35; $p < 0.01$
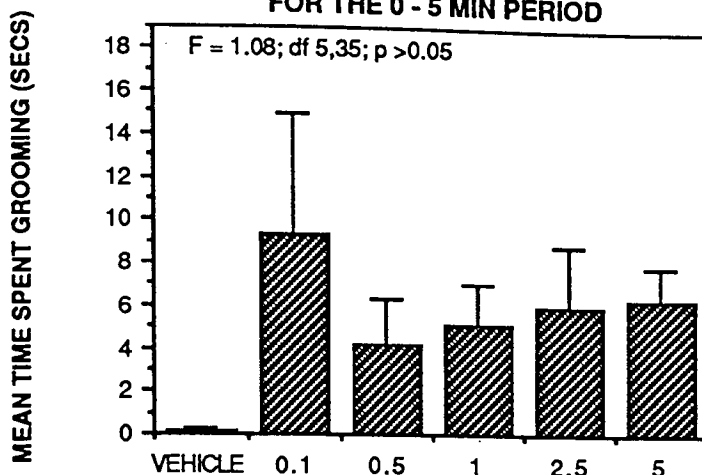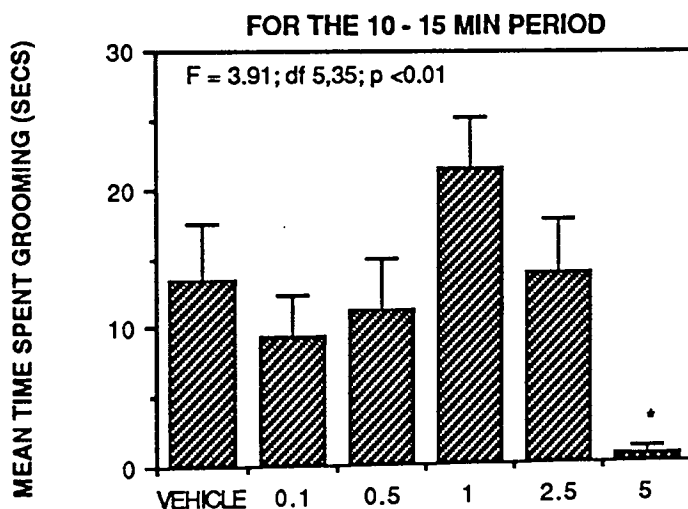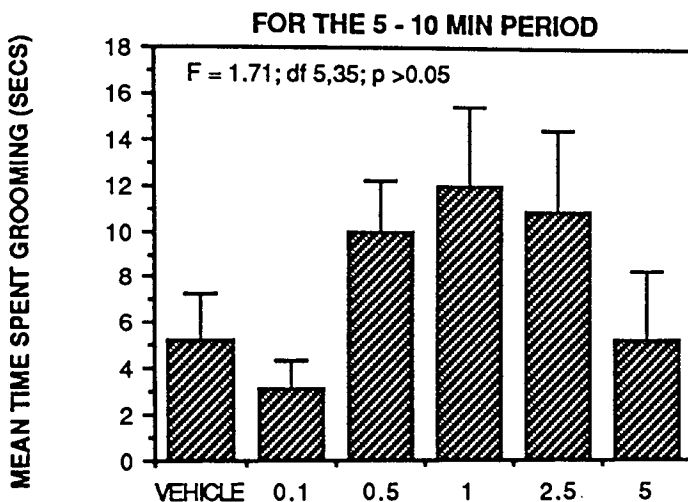
* $p < 0.05$

Fig 2.34   Analysis of the effect of (±)DOI on on-going behaviour (mean time spent face/paw grooming in each of the 5min periods examined (0-5, 5-10, 10-15min) after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * $p < 0.05$ and ** $p < 0.01$ (relative to vehicle controls). All results are the means of 6 determinations and vertical bars represent s.e.m.

EFFECT OF DOI ON PENILE GROOMING :
FOR THE 0 - 5 MIN PERIOD

FIG. 2.35

* p <0.05

FOR THE 5 - 10 MIN PERIOD

FOR THE 10 - 15 MIN PERIOD

* p <0.05

DOSE (MG/KG)
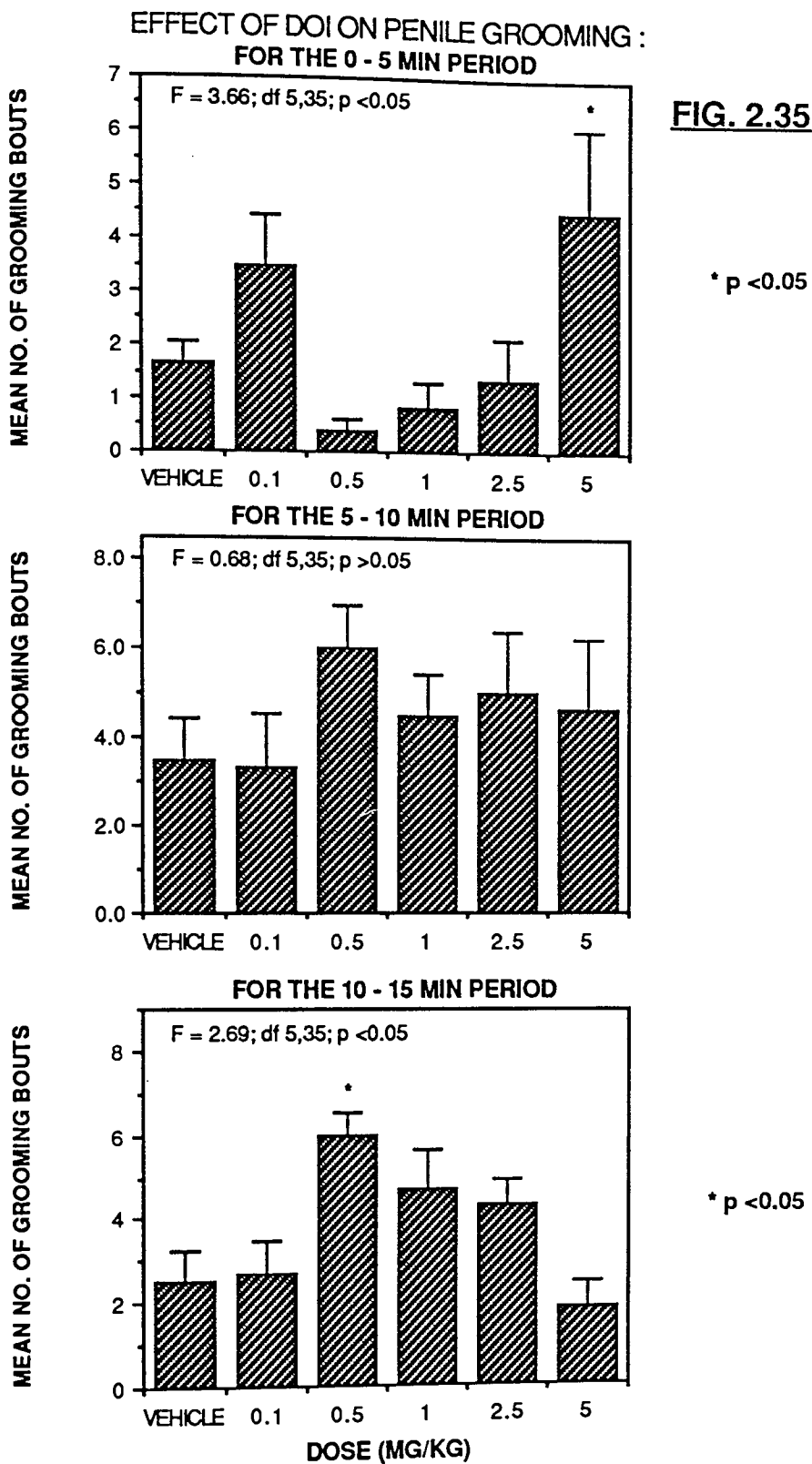
Fig. 2.35 Analysis of the effect of (±)DOI on on-going behaviour (mean no. of penile grooming bouts in each of the 5min periods examined (0-5, 5-10 and 10-15min) after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). All results are the means of 6 determinations and vertical bars represent s.e.m..

EFFECT OF DOI ON PENILE GROOMING :

FOR THE 0 - 5 MIN PERIOD

$F = 1.28; df\ 5,35; p > 0.05$

FIG. 2.36

MEAN TIME SPENT GROOMING (SEC) — X-axis: VEHICLE, 0.1, 0.5, 1, 2.5, 5

FOR THE 5 - 10 MIN PERIOD

$F = 1.27; df\ 5,35; p > 0.05$

MEAN TIME SPENT GROOMING (SEC) — X-axis: VEHICLE, 0.1, 0.5, 1, 2.5, 5

FOR THE 10 - 15 MIN PERIOD

$F = 2.69; df\ 5,35; p < 0.05$

** $p < 0.01$

MEAN TIME SPENT GROOMING (SEC) — X-axis: VEHICLE, 0.1, 0.5, 1, 2.5, 5

DOSE (MG/KG)
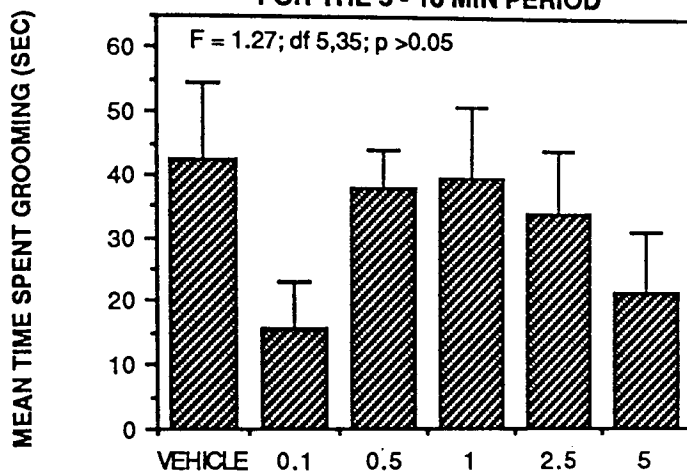
Fig. 2.36 Analysis of the effect of (±)DOI on on-going behaviour
(mean time spent penile grooming in each of the 5min periods
examined (0-5, 5-10 and 10-15min) after (±)DOI (i. p.) or vehicle
(i. p.) administration). Results were analysed statistically using a
one-way between subjects ANOVA on raw data (for the entire dose
range tested). For analysis of the effect of individual doses of
(±)DOI, Duncan's multiple-range test was used. * p <0. 05 and ** p
<0. 01 (relative to vehicle controls). All results are the means of
6 determinations and vertical bars represent s. e. m.).

EFFECT OF DOI ON LICK ELSEWHERE GROOMING :

FOR THE 0 - 5 MIN PERIOD

F = 1.62; df 5,35; p >0.05

FIG. 2.37

MEAN NO. OF GROOMING BOUTS

VEHICLE   0.1   0.5   1   2.5   5

* p <0.05

FOR THE 5 - 10 MIN PERIOD

F = 0.68; df 5,35; p >0.05

MEAN NO. OF GROOMING BOUTS

VEHICLE   0.1   0.5   1   2.5   5

FOR THE 10 - 15 MIN PERIOD

F = 2.68; df 5,35; p <0.05

MEAN NO. OF GROOMING BOUTS

VEHICLE   0.1   0.5   1   2.5   5

DOSE (MG/KG)
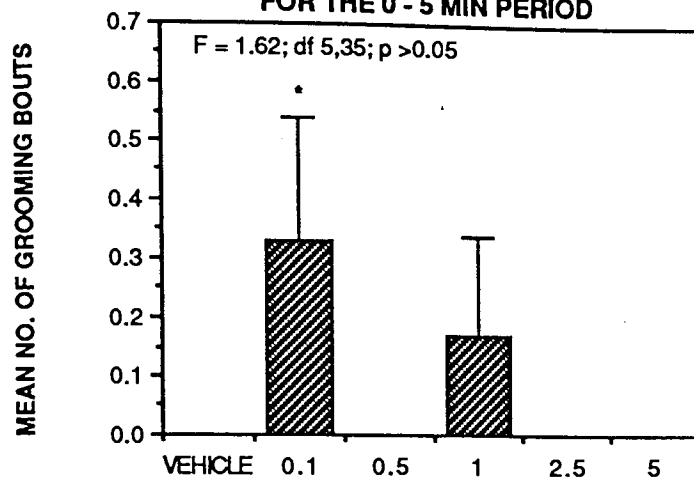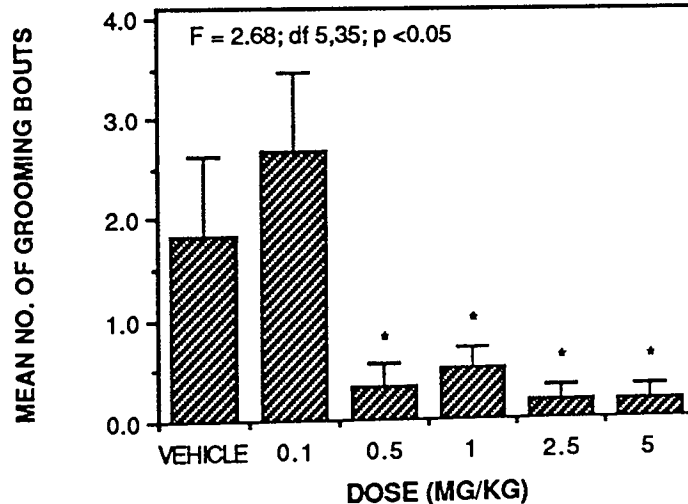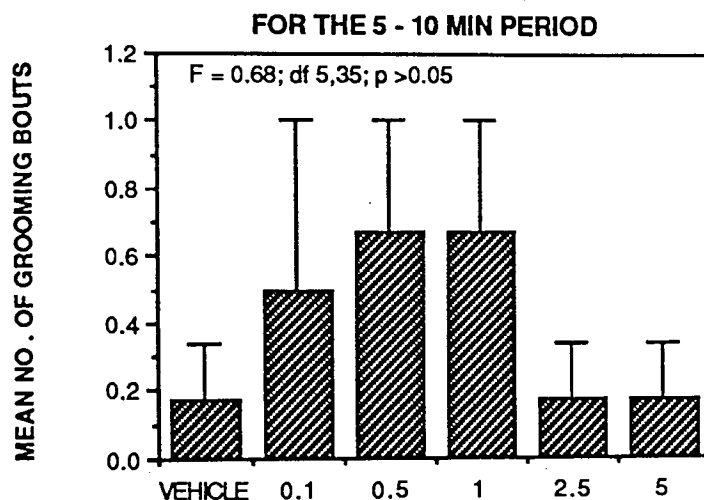
* p <0.05

Fig 2.37 Analysis of the effect of (±)DOI on on-going behaviour (mean no. of lick elsewhere grooming bouts in each of the 5min periods examined (0-5, 5-10 and 10-15min) after (±)DOI (i.p.) or vehicle (i.p.) administration). Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). All results are the means of 6 determinations and vertical bars represent s.e.m.

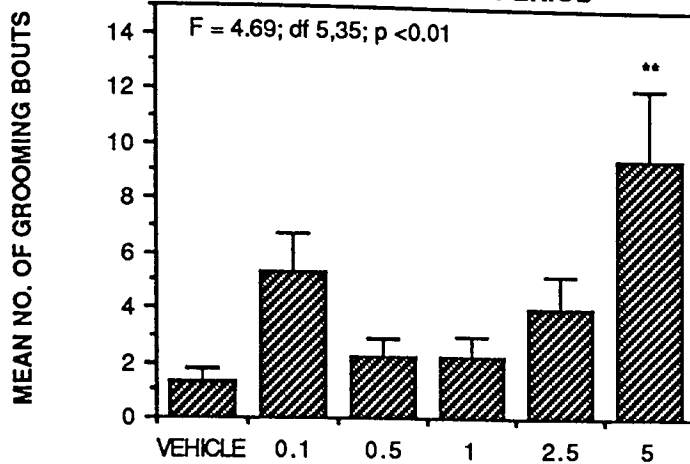# EFFECT OF DOI ON LICK ELSEWHERE GROOMING :
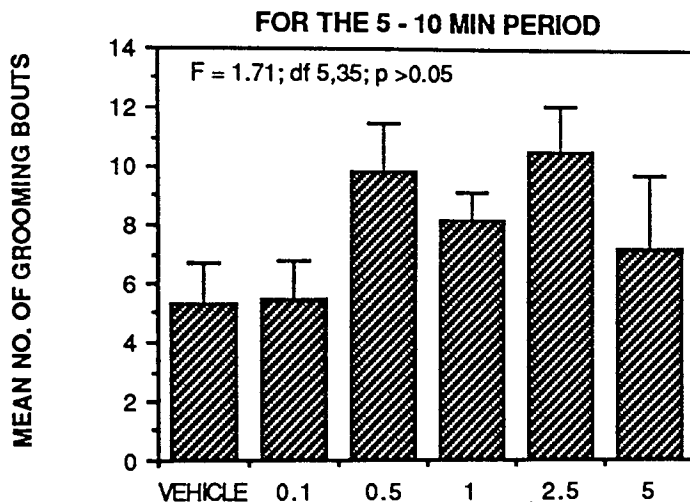
FOR THE 0 - 5 MIN PERIOD

FIG. 2.38

F = 1.44; df 5,35; p >0.05

FOR THE 5 - 10 MIN PERIOD

F = 0.50; df 5,35; p >0.05

FOR THE 10 - 15 MIN PERIOD

F = 3.42; df 5,35; p <0.05

* p <0.05
** p <0.01

DOSE (MG/KG)
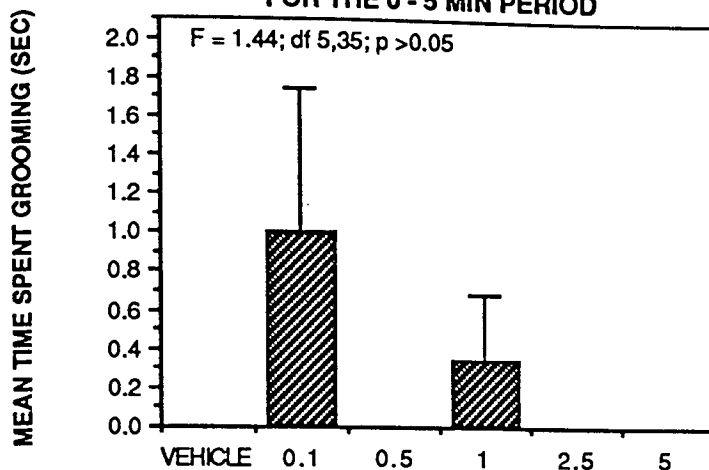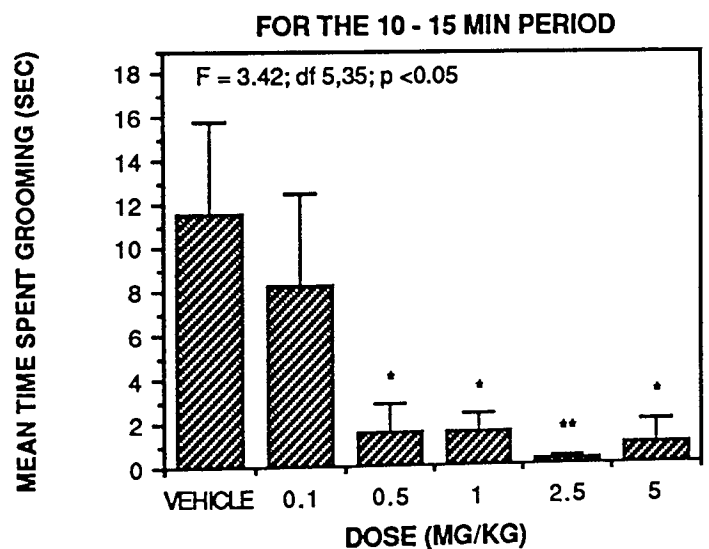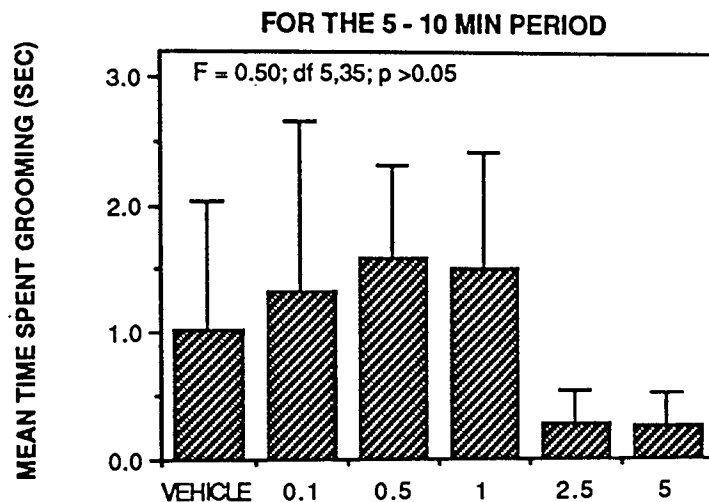
Fig. 2.38 Analysis of the effect of (±)DOI on on-going behaviour (mean time spent lick elsewhere grooming in each of the 5min periods examined (0-5, 5-10 and 10-15min) after (±)DOI (i.p.) or vehicle (i.p.) administration. Results were analysed statistically using a one-way between subjects ANOVA on raw data (for the entire dose range tested). For analysis of the effect of individual doses of (±)DOI, Duncan's multiple-range test was used. * p <0.05 and ** p <0.01 (relative to vehicle controls). All results are the means of 6 determinations and vertical bars represent s.e.m.

MESCALINE-INDUCED SCRATCHING

FIG. 2.39

** p <0.01
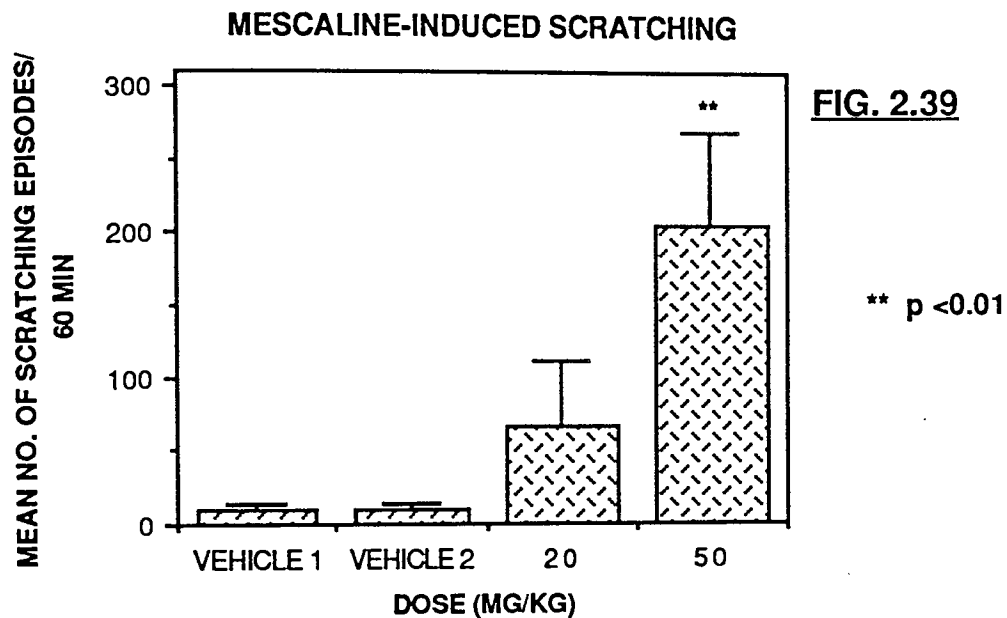
Fig. 2.39 Mice received either mescaline (i.p.) or vehicle (i.p.).
Scratching episodes were counted for 60min after injection. Vehicle
1 represents the control group for the 20mg/kg dose of mescaline,
and Vehicle 2 represents the control group for the 50mg/kg dose of
mescaline. Results were analysed statistically using an unpaired t-
test.

Table 2.2 Analysis of the effect of (±)DOI on on-going behaviour in rodents.

a) Effect of (±)DOI on mouse on-going behaviour intensity (15min after i.p. injection.

behaviour	dose (mg/kg)	0.2	1	10
			intensity score	
fighting		0	0	0
vocalisation		0	0	0
pain (too hypersensitive to be tested)				
straub tail		0	0	0
restlessness		0	0	2
twitches		2	4	6
convulsions		0	0	0
tremor		0	0	2
sniffing		0	4	4
fearfulness		0	2	4
respiration		0	0	0
grooming and scratching		0	4	6
ptosis		0	0	2
alertness		0	0	0
body sag		0	0	4
spontaneous activity		0	-2	-2
startle response		2	2	6
touch response		0	2	6
gait		0	0	0
passivity		0	-2	0
pinna reflex		0	2	4
corneal reflex		0	0	4
cyanosis		0	2	6

Behaviour intensity was scored on the scale +6 to -6 using the criteria of Irwin (1968) 15min after the injection of 0.2, 1 and 10mg/kg of (±)DOI i.p. in mice.

b) Effect of (±)DOI (5mg/kg i.p.) on rat on-going behaviour.

behaviour	time	5	15	30
			intensity score	
wet dog shakes		3	3	3
chewing		1	1	1
gnawing		1	1	1
flat body posture		1	2	2
fore limb tremor		0	1	1
fore paw treading		0	1	1

Intensity of 5-HT-mediated behaviour was scored on a scale of 0 to 3, at 5, 15 and 30min after i.p. injection of (±)DOI at 5mg/kg.

Table 2.3 Dose-response and time-course of (±)DOI-induced head-twitching in mice and twitching in rats.

dose-response

mice: dose (mg/kg i.p.)

dose (mg/kg i.p.)	mean no. of twitches/6min
0.1	7.4 (1.0)
0.25	13.0 (1.2)
0.5	16.2 (1.6)
1	29.3 (4.2)
5	26.7 (3.7)
10	21.9 (2.0)

rat: dose (mg/kg i.p.)

dose (mg/kg i.p.)	mean no. of twitches/15min
0.5	5.4 (1.7)
1	12.0 (1.1)
5	13.0 (1.1)

time-course : mice (1mg/kg i.p.)

min	vehicle	(±)DOI
2	0.4 (0.2)	0.6 (0.2)
4	0 (0)	2.8 (0.5)
6	0.4 (0.2)	3.6 (0.9)
8	1 (0.3)	5.2 (0.7)
10	0.8 (0.4)	5.6 (0.9)
12	1 (0.4)	3.2 (0.4)
14	0 (0)	2.2 (1.1)
16	0.8 (0.4)	2.8 (0.4)
18	0.2 (0.2)	2.8 (0.5)
20	0.4 (0.4)	2.4 (0.6)
22	0.2 (0.2)	1.6 (0.5)
24	0.2 (0.2)	2.2 (0.6)
26	0.6 (0.4)	1.4 (0.4)
28	0.2 (0.2)	1.6 (0.9)
30	0 (0)	0 (0)

Results are expressed as mean head-twitch or twitch counts (from at least 6 determinations). Bracketed figures represent s.e.m..

<u>Table 2.3 continued :</u>

time-course : rats (1.0mg/kg i.p.)
_____

min	(±)DOI		min	(±)DOI	
3	0	(0)	33	1.5	(0.3)
5	0.5	(0.3)	35	1.8	(0.3)
7	1.5	(0.3)	37	1	(0.5)
9	1.8	(0.3)	39	1.8	(0.3)
11	1.5	(0.3)	41	1	(0.4)
13	1.5	(0.3)	43	1	(0.4)
15	1.3	(0.3)	45	1.5	(0.3)
17	1.3	(0.5)	47	1	(0)
19	1	(0.4)	49	1.8	(0.5)
21	1.8	(0.5)	51	1.8	(0.5)
23	0.8	(0.5)	53	1.8	(0.3)
25	1.5	(0.3)	55	2.3	(0.5)
27	0.8	(0.5)	57	1	(0)
29	0.8	(0.5)	59	1	(0)
31	1.3	(0.6)	61	1	(0.4)

Results are expressed as mean twitch counts (from at least 6 determinations). Bracketed figures represent s.e.m..

Table 2.4 Dose-response and time-course for (±)DOI-induced scratching in mice.

1)

dose (mg/kg i.p.)	mean no. of scratching episodes (30min)
1	19.2 (6.8)
2.5	76.6 (25.6)
5	157.3 (15.7)

2)

dose (mg/kg)	mean no. of scratching episodes (10min)
vehicle	0.63 (0.5)
0.1	0.67 (0.4)
0.5	1.2 (0.5)
1	11.2 (4.5)
2.5	9.0 (6.6)
4	26.0 (15.3)
5	36.5 (12.1)
10	48.6 (20.1)

time course (2.5mg/kg i.p.)

time (min)	mean	time (min)	mean
1	0 (0)	16	2 (1.3)
2	0 (0)	17	2 (1.0)
3	0.8 (0.6)	18	3.6 (1.8)
4	2.6 (1.4)	19	1.6 (0.7)
5	3.4 (2.7)	20	2.4 (0.9)
6	4.2 (2.7)	21	1.2 (0.6)
7	5.4 (2.2)	22	1 (0.5)
8	6.6 (2.8)	23	2.6 (1.4)
9	6.6 (2.6)	24	0 (0)
10	5.8 (1.8)	25	0.2 (0.2)
11	5.6 (1.9)	26	0.4 (0.2)
12	5.8 (2.4)	27	1.2 (0.6)
13	3.8 (1.2)	28	0.4 (0.4)
14	3.6 (1.0)	29	0 (0)
15	2.8 (1.2)	30	0.2 (0.2)

Results are expressed as mean no. of scratching episodes (from at least 5 determinations). Bracketed figures represent s.e.m..

Table 2.5 Effect of (±)DOI on locomotor activity of mice.

locomotor activity - dose response

1)	Dose (mg/kg)	Animex activity count (10-20min)	
	vehicle	524.6 (75.3)	
	0.1	572.4 (56.2)	
	1	709.2 (24.7)	p <0.05
	5	522.6 (46.2)	
2)	vehicle	593.4 (62.0)	
	0.25	770.7 (64.0)	p <0.05
	0.5	722.0 (60.8)	
	0.75	850.3 (18.5)	p <0.01
	1	843.1 (49.5)	p <0.01

Results are expressed as mean activity counts (for at least 5 determinations). Bracketed figures represent s.e.m.
Results were analysed statistically using linear regression analysis and one-way between subjects analysis of variance followed by Duncan's multiple range test.

In the case of 2) (±)DOI was found to produce dose-dependent increase (0-1mg/kg) in locomotor activity (p <0.003), whereas in the case of 1) a non-significant probability for the linear regression analysis of p <0.48 was found. Duncan's multiple range test indicated that in 1) (±)DOI 1mg/kg and 2) (±)DOI 0.25, 0.75 and 1mg/kg produced significant increases in locomotor activity compared with vehicle treated control mice.

<u>Table 2.6</u> <u>Effect of (±)DOI on total grooming behaviour of mice.</u>

Legend for Tables 2.6, 2.7, 2.8 and 2.9 :

Results are the means of 6 determinations. Bracketed figures represent s.e.m. Results were analysed statistically using one-way between subjects ANOVA followed by Duncan's multiple-range test. F values quoted (df 5,35 in all cases) indicate whether there is a significance between the 6 treatment groups (vehicle, 0.1, 0.5, 1, 2.5 and 5mg/kg (±)DOI) with respect to grooming scores. The results of Duncan's multiple-range test are illustrated as * and **, * = p <0.05 and ** = p <0.01 (relative to vehicle controls).

a) <u>Effect of (±)DOI on total grooming/15min.</u>

	bouts	time spent (sec)	
(±)DOI dose (mg/kg)			
vehicle	13.3 (2.2)	137.9 (24.5)	F (bouts)=1.16
0.1	16.8 (2.8)	99.1 (21.4)	NS
0.5	21.8 (2.8)	116.4 (7.6)	F (time)=2.21
1	19.7 (1.1)	131.9 (18.6)	NS
2.5	22.7 (2.8)	108.2 (15.6)	
5	19.3 (5.6)	60.7 (19.6) *	

b) <u>Effect of (±)DOI on total grooming in each 5min period (0-5, 5-10 and 10-15min) after (±)DOI administration.</u>

<u>Grooming bouts.</u>

min after (±)DOI or vehicle administration :

(±)DOI dose (mg/kg)	0-5min	5-10min	10-15min	
vehicle	1.3 (0.5)	5.3 (1.4)	6.7 (1.5)	F (0-5)= 4.69
0.1	5.3 (1.4)	5.5 (1.3)	6.0 (1.7)	p <0.01
0.5	2.2 (0.7)	9.8 (1.7)	9.8 (1.5)	F (5-10)=1.71
1	2.2 (0.8)	8.2 (0.9)	9.3 (1.6)	NS
2.5	4.0 (1.2)	10.5 (1.6)	8.2 (0.5)	F (10-15)=3.69
5	9.5 (2.6) **	7.2 (2.5)	2.7 (1.1)	p <0.05

<u>Table 2.6 continued :</u>

<u>Time spent grooming.</u>

min after (±)DOI or vehicle administration :

(±)DOI dose (mg/kg)	0-5min	5-10min	10-15min	
vehicle	8.9 (5.7)	48.7 (12.9)	80.3 (13.1)	F (0-5)=1.73
0.1	23.5 (6.9)	29.4 (11.0)	46.1 (14.4) *	NS
0.5	5.9 (1.9)	50.5 (7.7)	60.9 (8.7)	F(5-10)=1.14
1	12.6 (6.1)	52.9 (10.3)	66.4 (6.7)	NS
2.5	13.1 (4.9)	45.0 (8.6)	50.1 (6.9) *	F(10-15)=6.51
5	24.9 (8.0)	26.8 (12.0)	8.4 (2.4) **	p <0.01

Results are the means of 6 determinations. Bracketed figures represent s.e.m. Results were analysed statistically using one-way between ANOVA followed by Duncan's multiple-range test.
* p <0.05 and ** p <0.01 (both relative to vehicle controls).

Table 2.7 Effect of (±)DOI on face/paw grooming.

a) Effect of (±)DOI on face/paw grooming/15min.

	bouts	time spent (sec)	
(±)DOI dose (mg/kg)			
vehicle	4.2 (1.0)	18.8 (4.4)	F (bouts)=1.44
0.1	5.3 (2.2)	20.7 (8.6)	NS
0.5	8.5 (2.4)	25.1 (5.7)	F (time)=2.24
1	8.3 (1.3)	38.6 (6.3) *	NS
2.5	11.7 (2.8) *	30.5 (7.1)	
5	8.0 (2.8)	12.1 (4.3)	

b) Effect of (±)DOI on face/paw grooming in each 5min period (0-5, 5-10 and 10-15min) after (±)DOI administration.

Bouts.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	0.2 (0.2)	1.7 (0.5)	2.3 (0.7)	F(0-5)=4.37
0.1	1.5 (0.8)	1.7 (0.6)	2.2 (1.1)	p <0.01
0.5	1.8 (0.8)	3.2 (1.0)	3.5 (1.5)	F(5-10)=1.75
1	1.2 (0.5)	3.0 (0.6)	4.2 (1.2)	NS
2.5	2.7 (0.9)	5.3 (1.7) *	3.7 (0.4)	F(10-15)=1.72
5	5.0 (1.2) **	2.3 (1.3)	0.7 (0.5)	NS

Time spent.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	0.1 (0.1)	5.2 (2.1)	13.4 (4.2)	F(0-5)=1.08
0.1	9.3 (5.6)	3.1 (1.3)	9.3 (3.0)	NS
0.5	4.1 (2.1)	9.9 (2.3)	11.2 (3.7)	F(5-10)=1.71
1	5.2 (1.9)	12.0 (3.5)	21.5 (3.7)	NS
2.5	5.9 (2.9)	10.8 (3.7)	13.7 (4.0)	F(10-15)=3.91
5	6.3 (1.5)	5.2 (3.1)	0.6 (0.5) *	p <0.01

Table 2.8 Effect of (±)DOI on penile grooming.

a) Effect of (±)DOI on penile grooming/15min.

	bouts	time spent (sec)	
(±)DOI dose (mg/kg)			
vehicle	7.2 (1.1)	106.3 (25.3)	F(bouts)=1.01
0.1	9.5 (2.2)	54.1 (19.3)	NS
0.5	12.3 (1.0)	74.8 (5.4)	F(time)=1.44
1	10.0 (1.6)	89.9 (18.6)	NS
2.5	10.7 (2.3)	77.2 (19.1)	
5	11.0 (3.1)	47.3 (15.9)	

b) Effect of (±)DOI on penile grooming in each 5min period (0-5, 5-10 and 10-15min) after (±)DOI administration.

Bouts.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	1.7 (0.4)	3.5 (0.9)	2.5 (0.8)	F(0-5)=3.66
0.1	3.5 (1.0)	3.3 (1.2)	2.7 (0.8)	p <0.05
0.5	0.4 (0.2)	6.0 (1.0)	6.0 (0.5) *	F(5-10)=0.68
1	0.8 (0.5)	4.5 (1.0)	4.7 (1.0)	NS
2.5	1.3 (0.8)	5.0 (1.4)	4.3 (0.6)	F(10-150=4.62
5	4.5 (1.6) *	4.7 (1.5)	1.8 (0.7)	p <0.01

Time spent.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	8.7 (5.6)	42.5 (11.9)	55.3 (17.5)	F(0-5)=1.28
0.1	12.3 (5.1)	15.7 (7.3)	26.1 (12.3)	NS
0.5	1.7 (1.3)	38.1 (5.9)	34.9 (5.1)	F(5-10)=1.27
1	7.1 (4.8)	39.5 (11.5)	43.3 (7.0)	NS
2.5	7.2 (4.7)	33.9 (10.0)	36.2 (6.9)	F(10-15)=2.69
5	18.6 (7.0)	21.4 (9.8)	7.4 (2.6) *	p <0.05

Table 2.9 Effect of (±)DOI on lick elsewhere grooming.

a) Effect of (±)DOI on lick elsewhere grooming/15min.

	bouts	time spent (sec)	
(±)DOI dose (mg/kg)			
vehicle	2.0 (0.8)	12.6 (4.4)	F(bouts)=2.04
0.1	2.0 (0.7)	10.6 (4.1)	NS
0.5	1.0 (0.4)	3.1 (1.2) *	F(time)=3.94
1	1.3 (0.6)	3.4 (1.0) *	p <0.01
2.5	0.3 (0.3) *	0.4 (0.4) **	
5	0.3 (0.2) *	1.3 (1.0) **	

b) Effect of (±)DOI on lick elsewhere grooming in each 5min period
   (0-5, 5-10 and 10-15min) after (±)DOI administration.

Bouts.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	0 (0)	0.2 (0.2)	1.8 (0.8)	F(0-5)=1.62
0.1	0.3 (0.2) *	0.5 (0.5)	1.2 (0.5)	NS
0.5	0 (0)	0.7 (0.3)	0.3 (0.2) *	F(5-10)=0.68
1	0.2 (0.2)	0.7 (0.3)	0.5 (0.2) *	NS
2.5	0 (0)	0.2 (0.2)	0.2 (0.2) *	F(10-15)=2.68
5	0 (0)	0.2 (0.2)	0.2 (0.2) *	p <0.05

Time spent.

min after (±)DOI or vehicle administration :

	0-5min	5-10min	10-15min	
(±)DOI dose (mg/kg)				
vehicle	0 (0)	1.0 (1.0)	11.6 (4.3)	F(0-5)=1.44
0.1	1.0 (08)	1.3 (1.3)	8.3 (4.2)	NS
0.5	0 (0)	1.6 (0.7)	1.5 (0.7) *	F(5-10)=0.50
1	0.3 (0.3)	1.5 (0.9)	1.6 (0.8) *	NS
2.5	0 (0)	0.3 (0.3)	0.2 (0.2) **	F(10-15)=3.42
5	0 (0)	0.3 (0.3)	1.0 (1.0) *	p <0.05

Table 2.10   Effect of a variety of 5-HT-related agents on (±)DOI-
induced head-twitching.

1) ritanserin (r) 30min pre.

0.5mg/kg i.p.	v	10.7 (1.5)	p <0.05
	r	4.8 (0.3)	
1mg/kg i.p.	v	12.6 (3.5)	p <0.05
	r	1.8 (0.9)	
5mg/kg i.p.	v	8.0 (0.9)	p <0.01
	r	0.0 (0.00	

2) xylamidine (x) 10mg/kg i.p. 15min pre.

	v	5.9 (0.9)	NS
	x	8.4 (1.4)	

3) 8-OH-DPAT (d) 10min pre

0.2mg/kg i.p.	v	7.7 (1.3)	p <0.05
	d	4.5 (0.8)	
1mg/kg i.p.	v	10.0 (1.3)	p <0.01
	d	2.5 (0.9)	
2mg/kg i.p.	v	12.2 (1.5)	p <0.01
	d	1.0 (0.5)	

4) buspirone (b) 30min pre.

1mg/kg i.p.	v	11.0 (1.8)	NS
	b	8.6 (0.5)	
5mg/kg i.p.	v	9.5 (0.9)	p <0.05
	b	4.3 (0.9)	
10mg/kg i.p.	v	11.8 (2.6)	p <0.05
	b	1.8 (0.7)	

5) ipsapirone (i)   30min pre.

5mg/kg i.p.	v	10.3 (0.9)	NS
	p	9.6 (1.5)	
5-MeODMT (5mg/kg i.p.)	v	10.7 (2.7)	p <0.05
	p	17.3 (3.8)	

(±)DOI = 0.5mg/kg i.p. unless stated otherwise

Times "pre" refer to the time prior to (±)DOI or 5-MeODMT that either
the drug or vehicle were administered

Results are expressed as the mean head-twitch counts (from at least 5
determinations). Bracketed figures represent s.e.m..

NS = non significant - p >0.05.

Table 2.11 <u>Effect of idazoxan on inhibition of (±)DOI-induced head-</u>
<u>twitching by 8-OH-DPAT.</u>

Mice received either idazoxan (0.5mg/kg s.c.) or vehicle (s.c.) 30min
prior, and 8-OH-DPAT (1mg/kg i.p.) or vehicle (i.p.) 10min prior, to
(±)DOI (0.5mg/kg i.p.).

vehicle)--------vehicle	14.2 (2.3)	$F(A) = 1.67$ df 1,20 NS
)------8-OH-DPAT	4.4 (1.2)	$F(B) = 40.62$ df 1,20 $p < 0.01$
idazoxan)-------vehicle	15.7 (1.7)	$F(AB) = 0.38$ df 1,20 NS
)-----8-OH-DPAT	7.6 (1.2)	

$F(A)$ = idazoxan.
$F(B)$ = 8-OH-DPAT.
$F(AB)$= the interaction of idazoxan and 8-OH-DPAT with respect to
(±)DOI-induced head-twitching.

Results are expressed as mean head-twitch counts (from 11
determinations). Bracketed figures represent s.e.m..

The results were statistically analysed using a two-way between/within
ANOVA on the raw data followed by Tukey's U test (for unconfounded
means).

Tukey's u-test------v/v compared with v/8-OH-DPAT	$p < 0.01$
v/v compared with v/idazoxan	NS
v/8-OH-DPAT compared with idazoxan/8-OH-DPAT	NS
v/idazoxan compared with idazoxan/8-OH-DPAT	$p < 0.01$

The Tukey's U test results are represented as the treatments that the
mice received whose means were compared using this test.

NS = non significant - $p > 0.05$.

Table 2.12 Effect of a variety of agents on (±)DOI-induced
           scratching.

_____

1) ritanserin (r) 30min pre.
_____

    0.02mg/kg i.p.   v   37.0 (10.8)   NS
                        r   28.0   (9.8)
    0.2mg/kg i.p.   v   29.2   (8.2)   p <0.05
                        r   7.5   (3.7)
    2mg/kg i.p.   v   91.7   (9.3)   p <0.01
                        r   0.5   (0.3)
_____

2) ICI 169,369 (i) 30min pre.
_____

    2mg/kg i.p.   v   29.2   (4.2)   p <0.05
                        i   9.8   (3.9)
_____

                                        Dose of (±)DOI

3) xylamidine (x) 15min pre.   2.5mg/kg i.p.   5mg/kg i.p.
_____

    2.5mg/kg i.p.   v   12.3 (4.5) NS
                                                  x   18.0 (5.3)
    10mg/kg i.p.   v 24.3 (12.2) NS   v   70.2 (6.4) p <0.01
                                x 10.0 (6.4)   x   15.0 (6.9)
_____

4) 8-OH-DPAT (d) 10min pre.
_____

    0.01mg/kg i.p.   v   25.0   (9.4)   p <0.05
                        d   39.6   (9.4)
    0.1mg/kg i.p.   v   14.9   (4.9)   p <0.01
                        d   37.4   (9.6)
    1mg/kg i.p.   v   19.7   (7.4)   NS
                        d   37.0 (11.4)
_____

5) FLA-63 (f) 4hr pre.
_____

    40mg/kg i.p.   v   23.1   (5.4)   p <0.01
                        f   1.2   (0.5)
_____

6) clonidine (c) 30min pre.
_____

    0.05mg/kg i.p.   v   50.3 (12.0)   p <0.01
                        c   14.8   (5.2)
    0.1mg/kg i.p.   v   27.0   (9.3)   p <0.05
                        c   8.3   (3.1)
_____

Results are expressed as mean no. of scratching episodes (from at
least 6 determinations). NS = non significant (p >0.05).
Times "pre" refer to the time the drug or vehicle were administered
prior to (±)DOI.

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Table 2.12 continued :

```
_____

7) idazoxan (i) 30min pre.
_____

   0.5mg/kg i.p.        v      15.3  (3.0)       NS
                        i      13.8  (3.1)
   1mg/kg i.p.          v      34.5 (14.0)       NS
                        i      26.5 (10.9)
_____

8) prenalterol (pe) 15min pre.
_____

   10mg/kg i.p.         v      22.7  (8.8)       NS
                        pe     17.0  (5.9)
_____

9) procaterol (pc) 15min pre.
_____

   5mg/kg i.p.          v      20.1  (4.7)       NS
                        pc     13.9  (5.0)
_____

10) naloxone (n) 15min pre.
_____

   2mg/kg i.p.          v      26.5 (10.0)       NS
                        n      33.1  (5.7)
_____

11) terfenadine (t) 30min pre.
_____

   5mg/kg i.p.          v      37.8  (9.1)       NS
                        t      33.8 (11.7)
_____
```

Results are expressed as mean no. of scratching episodes (from at least 6 determinations).
NS = non significant (p >0.05).
Times "pre" refer to the time that the test drug or vehicle were administered prior to (±)DOI.

Table 2.13   Effect of ritanserin on (±)DOI-induced increase in
<u>locomotor</u> <u>activity.</u>

Mice received either ritanserin (2mg/kg i.p.) or vehicle (i.p.) 30min
prior to (±)DOI (1mg/kg i.p.) or vehicle (i.p.), then 10min later were
assessed for locomotor activity using an Animex activity monitor.

vehicle)--------vehicle   536.3 (27.5)   F(A) = 1.81   df 1,40 NS
)----------(±)DOI   688.8 (40.3)   F(B) = 7.67   df 1.40 p <0.01
ritanserin)-----vehicle   525.1 (43.3)   F(AB)= 6.19   df 1,40 p <0.05
)------(±)DOI   479.6 (43.8)

F(A) = ritanserin.
F(B) = (±)DOI.
F(AB)= interaction between ritanserin and (±)DOI with respect to
locomotor activity.

Results are expressed as mean Animex activity counts (from 11
determinations). Bracketed figures represent s.e.m..

The results were analysed statistically using a two-way between ANOVA
on the raw data followed by Tukey's U test (for unconfounded means).

Tukey's U test results---------v/v compared with v/(±)DOI   p <0.05
                      v/v compared with v/ritanserin   NS
             v/(±)DOI compared with v/ritanserin   p <0.01
    ritanserin/(±)DOI compared with v/ritanserin   NS

The Tukey's U test results are represented as the treatments that the
mice received whose means were compared using this test.

NS = non significant - p >0.05.

CHAPTER 3

THE EFFECT OF A VARIETY OF NORADRENERGIC DRUGS ON HEAD-
TWITCHING BY (±)DOI, 5-HTP, 5-MeODMT AND QUIPAZINE.

Chapter 3.

Introduction.

The work in this chapter was initiated after initial observations that beta2-agonists which had been previously demonstrated in our laboratory to produce potentiation of both head-twitching in mice and twitching in rats induced by every 5-HT-related inducer examined (5-HTP, 5-MeODMT and quipazine) (Handley and Singh 1984; Handley and Singh 1986a, b&c & Singh and Handley 1987) produced inhibition of head-twitch production induced by the 5-HT2/5-HT1c agonist, (±)DOI (present study see results section 2.3).

On the basis of this unexpected result, coupled with reports by other authors (e.g. Heal et al 1986 & Martin et al 1986) of inhibition being produced by beta2-agonists on 5-MeODMT-induced head-twitching, it was decided to compare the modulating effect of a variety of noradrenergic ligands on several different head-twitch inducers (5-MeODMT, 5-HTP, quipazine and (±)DOI) to determine the pattern of modulation of the head-twitch inducers by such agents. It was also thought that this work might help provide an explanation of the loss of potentiatory effect of the migraine triggers tyramine and beta-PEA on head-twitch production, as it has been postulated that this effect is expressed through beta-receptor activation (see Chapter 1).

Results.

The pretreatment times mentioned below refer to the number of minutes prior to the injection of the head-twitch-inducing agent that the test agents were injected. All injections were i.p. unless otherwise stated.

The doses of the head-twitch inducing agents and periods of observation of head-twitching used in this study (unless stated otherwise) are as follows : (±)DOI 0.5mg/kg i.p. (observation period

from the 4th to the 15th minute after (±)DOI injection), 5-HTP 200mg/kg i.p. (observation period from the 20th to the 29th minute after 5-HTP injection), 5-MeODMT 5mg/kg i.p. (observation period from the 3rd to the 14th minute after 5-MeODMT injection) and quipazine 2mg/kg i.p. (from the 3rd to the 12th minute after quipazine injection). As previously outlined in "Methods", experiments were performed using pairs of mice, one of which received the drug being tested for its effect on head-twitch production, the other vehicle as a control. Head-twitches were counted for alternate minutes between these two mice over the observation periods mentioned above. The doses of head-twitch-inducing agents and observation periods used were based on those employed by Handley and Singh (1986c) for 5-HTP, Singh et al (1986) & Singh and Handley (1987) for 5-MeODMT, and Heal et al (1986) for quipazine.

The doses of alpha1 (phenylephrine)- and alpha2 (clonidine)-adrenoceptor agonists, and alpha1 (prazosin)- and alpha2 (idazoxan)-adrenoceptor antagonists used in the work reported in this chapter are based on the ID50/ED50 doses observed by Handley and Brown (1982) for, respectively, inhibition/potentiation of 5-HT (i.c.v.)-induced head-twitching in mice. The doses of beta1 (dobutamine)- and beta2 (procaterol and salbutamol)- adrenoceptor agonists used in the work reported in this chapter are based on ED50 doses observed by Handley and Singh (1986c) for the potentiation of head-twitching induced by 5-HTP in mice. The doses of the beta2-agonist, clenbuterol used in the work reported in this chapter are based on the work of Heal et al (1986) and Martin et al (1986). The doses of beta1 (metoprolol)- and beta2 (ICI 118,551)-adrenoceptor antagonists used in the work described in this chapter are based on the doses which Handley and Singh (1986c) found to inhibit beta1- and beta2-adrenoceptor agonist-induced potentiation of 5-HTP-induced head-twitching in mice. The dose of FLA-63 used in the experiments described in this chapter is that which Singh et al (1986) had previously found to produce selective inhibition of CNS NA levels and to potentiate head-twitching induced by 5-MeODMT.

1. Effect of noradrenergic depleting agents on 5-HT-related head-
twitching in mice (and twitching in rats *).

1.1 Effect of FLA-63 (40mg/kg i.p. 30min and 4hr pretreatment) on 5-
MeODMT-induced head-twitching.

FLA-63 produced inhibition of 5-MeODMT-induced head-twitching with a
30min pretreatment time (Fig. 3.1 and Table 3.4) but potentiation with
a 4hr-pretreatment time (Fig. 3.2 and Table 3.1).

1.2 Effect of FLA-63 (40mg/kg i.p. 4hr pretreatment) on quipazine-, 5-
HTP- and (±)DOI-induced head-twitching.

FLA-63 (4hr pretreatment) produced inhibition of both quipazine- and
(±)DOI-induced head-twitching but potentiation of 5-HTP-induced head-
twitching (Fig. 3.2 and Table 3.1).

1.3 Effect of ICI 118,551 (2.5mg/kg s/c given 30min prior to 5-MeODMT)
on FLA-63 (40mg/kg i.p. 4hr pretreatment)-induced potentiation of 5-
MeODMT-induced head-twitching.

ICI 118,551 was found to significantly inhibit the potentiation of 5-
MeODMT-induced head-twitching induced by FLA-63 (F(AB), the
interaction term from the two-way between/within subjects analysis of
variance (ANOVA) = 6.74; df 1,16; $p < 0.05$). Analysis of the data using
Tukey's test for unconfounded means after the ANOVA indicated that
FLA-63 produced a significant ($p < 0.01$) enhancement of 5-MeODMT-
induced head-twitching but that ICI 118,551 had no effect ($p > 0.05$) on
5-MeODMT-induced head-twitching in its own right (see Fig. 3.3 and
Table 3.3).

1.4 <u>Effect</u> of <u>FLA-63</u> <u>(40mg/kg i.p. 4hr pretreatment)</u> on <u>the modulation</u> by <u>dobutamine (1.5mg/kg i.p. given 15min prior to 5-MeODMT)</u> of <u>5-MeODMT-induced head-twitching.</u>

The interaction term F(AB) of the two way between/within subjects ANOVA used to analyse this data was non-significant (F(AB) = 1.21; df 1,12; p >0.05) indicating no significant effect of FLA-63 on the modulation of 5-MeODMT-induced head-twitching by dobutamine. Analysis of the data using Tukey's test for unconfounded means after the ANOVA indicated that neither dobutamine nor FLA-63 produced significant effects (both p >0.05) on 5-MeODMT-induced head-twitching (see Fig. 3.4 and Table 3.5).

* 1.5 <u>Effect</u> of <u>FLA-63</u> <u>(40mg/kg i.p. 4hr pretreatment)</u> on <u>(±)DOI-induced twitching in rats.</u>

FLA-63 produced inhibition of (±)DOI (0.5mg/kg i.p.)-induced twitching behaviour in rats (Fig. 3.5 and Table 3.2). The method of observation and observation period for (±)DOI-induced twitching in rats has previously been outlined in "Methods" (see section 3.3/3.4).

1.6 <u>Effect</u> of <u>diethyldithiocarbamate (DDC) (400mg/kg i.p. 4hr</u> <u>pretreatment)</u> on <u>5-MeODMT-induced head-twitching.</u>

DDC produced potentiation of 5-MeODMT-induced head-twitching (Fig. 3.6 and Table 3.6).

1.7 <u>Effect</u> of <u>disulfiram (400mg/kg 4hr pretreatment)</u> on <u>5-MeODMT-induced head-twitching.</u>

Disulfiram produced no effect on 5-MeODMT-induced head-twitching in two separate experiments. When the data from these experiments was combined a significant potentiatory effect was found (see Fig. 3.7 and Table 3.6).

2. Effect of beta-adrenoceptor agonists and antagonists on 5-HT-related head-twitching in mice (and twitching in rats **).

2.1 Effect of beta1-agonist, dobutamine (15min pretreatment) on 5-MeODMT-, 5-HTP- and (±)DOI-induced head-twitching.

Dobutamine (1.5mg/kg i.p.) produced potentiation of (±)DOI- (Fig. 3.8) and 5-HTP (100mg/kg i.p. and carbidopa 9mg/kg s/c)- induced head-twitching but produced inhibition of 5-HTP (200mg/kg i.p. and carbidopa 9mg/kg s/c)-induced head-twitching (Fig. 3.9 and see Table 3.7). No effect was seen with 3mg/kg i.p. (Fig. 3.11) and in two individual experiments on the effect of 1.5mg/kg i.p. on 5-MeODMT-induced head-twitching (Fig 3.10 and see Table 3.7). However, combination of the results of these two experiments, where a trend towards potentiation was seen, produced a significant effect (Fig. 3.10 and Table 3.7).

2.2 Effect of the beta2-agonists, clenbuterol (30min pretreatment), procaterol (15min pretreatment) and salbutamol (30min pretreatment) on 5-MeODMT-induced head-twitching.

Clenbuterol (1mg/kg i.p.), salbutamol (0.5mg/kg i.p.) and procaterol (2mg/kg i.p.) produced inhibition of 5-MeODMT-induced head-twitching. Two higher doses of procaterol (5 & 10mg/kg i.p.) and one lower dose of salbutamol (0.25mg/kg i.p.) produced no effect on 5-MeODMT-induced head-twitching (see Fig. 3.13 and Table 3.10).

2.3 Effect of the beta2-agonists, procaterol (15min pretreatment) and clenbuterol (30min pretreatment) on (±)DOI-induced head-twitching.

Both clenbuterol (1 & 5mg/kg i.p.) and procaterol (1 & 5mg/kg i.p.) produced inhibition of (±)DOI (0.25mg/kg)-induced head-twitching (see Fig. 3.12 and Table 3.8).

2.4 Effect of the beta2-agonists, clenbuterol (30min pretreatment), procaterol (15min pretreatment) and salbutamol (30min pretreatment) on 5-HTP-induced head-twitching.

Clenbuterol (1mg/kg i.p.), procaterol (5mg/kg i.p.) and salbutamol (0.25mg/kg i.p.) all produced potentiation of 5-HTP-induced head-twitching (see Fig. 3.14 and Table 3.9).

2.5 Effect of clenbuterol (30min pretreatment) and procaterol (15min pretreatment) on quipazine-induced head-twitching.

Both clenbuterol (1mg/kg i.p.) and procaterol (5mg/kg i.p.) produced inhibition of quipazine-induced head-twitching (see Fig. 3.15 and Table 3.11).

** 2.6 Effect of procaterol (2mg/kg i.p. 15min pretreatment) on quipazine-induced twitching in rats.

Procaterol produced inhibition of quipazine-induced twitching in rats (Fig. 3.16 and Table 3.11).

2.7 Effect of the beta2-antagonist ICI 118,551 (2.5mg/kg i.p. 30min pretreatment) on 5-MeODMT-induced head-twitching.

ICI 118,551 produced no effect on 5-MeODMT-induced head-twitching (Fig. 3.17 and Table 3.12).

3. Effect of alpha-adrenoceptor agonists and antagonists on 5-HT-related head-twitching.

3.1 Effect of the alpha1-agonist, phenylephrine (1.25mg/kg i.p. 20min pretreatment) on (±)DOI- and 5-MeODMT-induced head-twitching.

Phenylephrine produced no effect on 5-MeODMT-induced head-twitching but potentiated (±)DOI-induced head-twitching (see Fig. 3.18 and Table 3.13).

3.2 <u>Effect of the alpha2-agonist clonidine (0.05mg/kg i.p. 30min pretreatment) on (±)DOI- and 5-MeODMT-induced head-twitching.</u>

Clonidine produced inhibition of both (±)DOI- and 5-MeODMT-induced head-twitching (see Fig. 3.19 and Table 3.15).

3.3 <u>Effect of the alpha2-antagonist, idazoxan (30min pretreatment) on (±)DOI- and 5-MeODMT-induced head-twitching.</u>

Idazoxan (0.25mg/kg i.p.) produced no effect on (±)DOI-induced head-twitching but potentiation of 5-MeODMT-induced head-twitching. Idazoxan (0.5mg/kg i.p.) produced potentiation of (±)DOI-induced head-twitching (see Fig. 3.20 and Table 3.16).

3.4 <u>Effect of the alpha1-antagonist, prazosin (15min pretreatment) on (±)DOI- and 5-MeODMT-induced head-twitching.</u>

Prazosin produced dose-dependent inhibition of both (±)DOI- and 5-MeODMT-induced head-twitching (see Fig. 3.21 and 3.22, respectively, and Table 3.14). In the case of (±)DOI, linear regression analysis revealed a slope/scatter F ratio with a value of 11.10 and a significant probability of $p < 0.004$ df1,20 indicating the dose-dependent nature of the inhibition. An ID50 of 2.11 [0.10 - 70.59] was found. In the case of 5-MeODMT, linear regression analysis revealed a slope/scatter F ratio of 9.16 and a significant probability of $p < 0.007$ df1,22 indicating the dose-dependent nature of the inhibition. An ID50 of 2.34 [0.073 - 173.0] was found.

Discussion.

The effect of beta2-agonists on head-twitching varied with the agent used to induce head-twitches. Evidence for the presence of facilitatory beta2-adrenoceptors has mainly accumulated from the large number of studies reporting potentiation of 5-HTP induced head-twitching following the administration of beta2-adrenoceptor agonists (Delini-Stula et al 1979; Ortmann et al 1981; Nimgaonkar et al 1983; Handley and Singh 1986c; Heal et al 1986 & Martin et al 1986). Since receptors of the beta2-subtype have been found on noradrenergic terminals and appear to be involved in facilitation of noradrenaline release (Westfall, 1977; Dhalof et al 1981 & see Misu and Kubo 1983), Handley and Singh (1986c) raised the possibility that the potentiation of 5-HTP head-twitching following beta2-agonists may not be direct, but indirect via release of noradrenaline onto postsynaptic facilitatory (with respect to head-twitching) beta1-receptors. This suggestion was based on their finding that selective beta1-antagonists inhibited both beta1- and beta2-agonist-induced potentiation of 5-HTP-induced head-twitching (in mice), whereas a selective beta2-antagonist would only inhibit the potentiation of 5-HTP-induced head-twitching produced by beta2-agonists, not beta1-agonists. They provided further evidence for such a proposal when it was found that lesions of the locus coeruleus inhibited the potentiation of quipazine-induced twitching (in rats) produced by beta2-agonists, suggesting that these receptors were situated presynaptically, on the neurones of the locus coeruleus, whereas the potentiation of quipazine-induced twitching in rats by beta1-agonists was enhanced by such lesioning.

The present findings with clenbuterol, procaterol and salbutamol agree with the previous findings of potentiation of 5-HTP-induced head-twitching.

The 5-HTP head-twitch model does not discriminate between a presynaptic action on 5-HT neurones or an effect of these agents "downstream" of postsynaptic 5-HT2 receptors. Moreover, since 5-HTP causes excess 5-HT synthesis and the "spilling over" from presynaptic

stores (Grahame-Smith 1971) this may signify a rather non-physiological release and doubts are thus raised as to the significance of modulation of this process. To add to the complexity, conversion of 5-HTP to 5-HT is mediated by L-amino acid decarboxylase, an enzyme ubiquitous in the brain as well as the periphery. Indeed, most studies with 5-HTP use carbidopa (as did the present study) to inhibit peripheral L-amino acid decarboxylase so as to minimise peripheral effects of 5-HT and maximise central effects (Modigh 1972). However, within the brain, conversion to 5-HT can also occur in neurones other than 5-HT neurones (Fuxe et al 1971); functioning here as a false transmitter. If these same neurones are involved in modulation of head-twitching, presence of 5-HT in these neurones might alter the modulatory role of these neurones and add to the difficulty in interpreting the nature of interactions.

In order to separate the presynaptic interactions from those "downstream" of 5-HT2 receptors a direct 5-HT receptor agonist is required. 5-MeODMT has been used as such an agent (Fuxe et al 1972), however, its activity at 5-HT1a and 5-HT1b receptors (Glennon 1987) make it less than ideal. (±)DOI was used in the present study since it has high affinity for 5-HT2 receptors and little affinity for 5-HT1a and 5-HT1b receptors (Glennon et al 1986; Titeler et al 1988 & Pierce and Peroutka 1989b). Both 5-MeODMT and (±)DOI also have high affinity for 5-HT1c receptors (Hoyer 1988b), the significance of this activity in terms of the modulation by noradrenergic ligands of 5-HT-related head-twitching is unknown.

The results produced in the present study indicate that when 5-MeODMT is used as a head-twitch inducer, the effect of beta2-agonists in our laboratory has changed from the potentiatory effect seen by Singh and Handley (1987) (using the beta2-agonist, procaterol) to inhibition (seen with the beta2-agonists, clenbuterol, procaterol and salbutamol) in the present study. An inhibitory effect of beta2-agonists on 5-MeODMT-induced head-twitching is in line with the work of Heal et al (1986) and Martin et al (1986). The effect of beta2-agonists on quipazine-induced shaking behaviour both in rats (twitches) and mice

(head-twitches) was also examined. Previous work in our laboratory had suggested that potentiation of quipazine-induced twitching in rats might be expected, as Handley and Singh (1986a) had found such an effect with procaterol and salbutamol. Once again, however, inhibition, of both quipazine-induced twitching in rats (using procaterol), and head-twitching in mice (using clenbuterol and procaterol) was observed. These results are in line with the work of Heal et al (1986) who found that clenbuterol inhibited quipazine-induced head-twitching in mice. The reason for the change seen in the effect of beta2-agonists on 5-MeODMT- and quipazine-induced shaking behaviour in our laboratory is unknown.

In line with the results for 5-MeODMT- and quipazine-induced head-twitching in the present study, beta2-agonists also inhibited ($\pm$)DOI induced head-twitching.

The reason for the differential modulatory effects of beta2-agonists on 5-HTP-, on the one hand, and ($\pm$)DOI/5-MeODMT/quipazine-induced head-twitching on the other is unknown. It would seem likely that the beta2-adrenoceptors mediating the inhibitory effect of beta2-agonists on 5-MeODMT-induced head-twitching are located postsynaptically with respect to the 5-HT2 receptors mediating head-twitch production since Martin et al (1986) found that the inhibitory effect of clenbuterol on 5-MeODMT-induced head-twitching in mice was not reduced by destruction of serotonergic neurones with 5,7-DHT. Although they did no biochemical assays to verify the lesions, in lesioned animals the response to 5-HTP was considerably reduced (-75%) whereas that to 5-MeODMT was enhanced (+130%) suggesting that serotonergic pathways were substantially damaged. In addition, it would seem unlikely that beta2-adrenoceptors located presynaptically with respect to the 5-HT2 receptors mediating head-twitching were involved in the inhibitory effect of beta2-agonists as such a presynaptic effect would be unlikely to effect direct agonist (($\pm$)DOI/5-MeODMT)-induced head-twitching.

The fact that 5-HTP-induced head-twitching was potentiated by beta2-agonists might be taken to suggest the presence of potentiatory beta2-adrenoceptors located presynaptically with reference to the 5-HT2 receptors mediating head-twitching, however, the existence of such receptors would seem unlikely as it might be expected that the effect of postsynaptic beta2-adrenoceptors, which appears to be predominantly inhibitory, would override a presynaptic one.

If it is postulated that presynaptic beta2-adrenoceptors do exist on 5-HT neurones, it may be that under certain circumstances, if their influence were strong enough then they could override an inhibitory postsynaptic beta2-adrenoceptor effect. The relative strength of the two beta2-adrenoceptor effects may thus be important in determining the outcome. Of the head-twitch inducing agents employed in this study, quipazine most closely resembles 5-HTP in the mechanism by which it induces head-twitching, as quipazine is thought, like 5-HTP, to have a presynaptic 5-HT releasing component in producing head-twitching. However, unlike 5-HTP, quipazine is also thought to have a direct agonist component to its shake-inducing mechanism. It may be this latter component that separates these two agents with respect to the modulation of their head-twitching by beta2-agonist. Possibly the relative strengths of the two beta2-adrenoceptor effects postulated above may be determined by the nature of the head-twitch inducer used i.e. when an agent such as 5-HTP which has a large presynaptic component to its head-twitch induction is used the potentiatory presynaptic beta2-adrenoceptor component predominates but where head-twitch induction has no presynaptic component ((±)DOI/5-MeOMDT) or both presynaptic 5-HT releasing and postsynaptic direct agonist components (quipazine) the influence of inhibitory beta2-adrenoceptors postsynaptic to the 5-HT2 receptors mediating head-twitching becomes overriding.

A beta1-agonist was also examined for its effect on head-twitching induced by a variety of 5-HT-related agents. Previously, work in our laboratory had found a generalized potentiatory effect of beta1-agonists with all the twitch-inducers examined i.e. 5-HTP (Handley and

Singh 1986c), 5-MeODMT (Singh and Handley 1987) (both in mice) and quipazine (in rats) (Handley and Singh 1986a).

The present work presents a more complicated picture. Dobutamine 1.5mg/kg potentiated 5-MeODMT-induced head-twitching, an effect that only became significant when the results from two experiments were combined, but is in line with previous findings. However, no effect was seen with a higher dose of 3mg/kg of dobutamine, possibly this related to an action of dobutamine on other receptors. Studies carried out in vivo have reported that dobutamine shows selectivity for beta1-adrenoceptors (e.g. Sonnenblick et al 1979). However, dobutamine isomers have also been reported to have a complex action on both alpha1-, beta1- and beta2-adrenoceptors in vascular smooth muscle (Ruffolo and Yaden 1983).

Dobutamine also produced potentiation of 5-HTP-induced head-twitching. This effect was found using a lower dose of 5-HTP (100mg/kg) than employed by Handley and Singh (1986c). Inhibition was seen when the dose of 5-HTP employed by Handley and Singh (1986c) (200mg/kg) was used. The reason for this difference is at present unclear, it may be suggestive of a dual action of beta1-agonists on 5-HTP-induced head-twitching, although the twitch rate in the 100mg/kg 5-HTP experiment was much lower than in the 200mg/kg experiment, so 'rate dependency' type effects can not be excluded.

The modulatory effect of dobutamine on head-twitching induced by (±)DOI, has also been examined although only at one dose (1.5mg/kg). As with the other head-twitch inducers potentiation was seen. Since (±)DOI is direct agonist with selectivity for 5-HT2(5-HT1c) receptors, this suggests that the beta1-receptors involved in the facilitatory response are located postsynaptically with relation to the 5-HT2 receptors involved in head-twitch production.

Neither the beta1-antagonist, metoprolol, nor the beta2-antagonist, ICI 118,551 produced any effect on 5-MeODMT-induced head-twitching. In contrast with the differing effects seen with beta2-agonists in the

present study, this is in line with previous results from this laboratory (see Handley and Singh 1986c; Singh et al (1986) and Singh and Handley 1987) and suggests there maybe 'not enough tone' in the NA systems under these experimental conditions for beta-adrenoceptor antagonists to modulate head-twitching.

The effect of alpha-adrenoceptor agonists and antagonists on 5-HT-related head-twitching was also examined. Clonidine has extensively been reported to decrease 5-HT-related shaking behaviour (Bednarczyk and Vetulani 1978; Vetulani et al 1980; Handley and Brown 1982 & Heal et al 1986) and this effect was seen again in the present study with (±)DOI- and 5-MeODMT-induced head-twitching. This effect is thought to be mediated by postsynaptic alpha2-adrenoceptors (Bednarczyk and Vetulani 1978 & Heal et al 1986) which are believed to be "downstream" from the 5-HT2 receptors mediating shaking behaviour since clonidine can inhibit shaking behaviour induced by direct 5-HT agonists such as 5-MeODMT (Heal et al 1986). However, the "dirty" nature of 5-MeODMT with respect to head-twitch production i.e. its relative lack of selectivity for 5-HT2-receptors, suggested that work with the more selective 5-HT2 agonist, (±)DOI would be useful confirmation of this interaction. As a result of the present study it can be said with some confidence that since the alpha2-agonist, clonidine also inhibited (±)DOI-induced head-twitching, the alpha2-receptors involved in this response are located postsynaptically with relation to the 5-HT2-receptors mediating head-twitching. Clonidine was also found to inhibit and the the alpha2-antagonist, idazoxan, potentiate 5-MeODMT-induced head-twitching in agreement with Heal et al (1986). In addition, idazoxan potentiated (±)DOI-induced head-twitching.

The effect of an alpha1-agonist and antagonist on (±)DOI- and 5-MeODMT-induced head-twitching were examined. Prazosin, the alpha1-antagonist produced inhibition of twitch production by both these agents with similar ID_{50} values ((±)DOI = 2.1 and 5-MeODMT = 2.34mg/kg i.p.). The inhibitory effect of prazosin on (±)DOI-induced head-twitching provides useful confirmation of the inhibitory action of alpha1-antagonists on 5-HT-related twitching using a more selective 5-

HT2 agonist than was previously available. Heal et al (1986) also found an inhibitory effect of peripheral prazosin on 5-MeODMT-induced head-twitching (at a similar dose - 3mg/kg), as did Handley and Brown (1982) (ID50 = 1.1mg/kg s.c.) using head-twitching induced by 5-HT (i.c.v.).

However, the same dose of an alpha1-agonist, phenylephrine given peripherally (i.p.) had no effect on 5-MeODMT-induced head-twitching but potentiated (±)DOI-induced head-twitching at the same dose. Heal et al (1986) found that centrally administered (i.c.v.) phenylephrine also had no effect on 5-MeODMT-induced head-twitching at low to moderate doses (0.625 and 2micrograms), although a high dose of 10micrograms totally inhibited this behaviour. They note however that at this high dose of phenylephrine the 5-MeODMT-mediated behavioural syndrome was markedly altered with a marked increase in locomotor activity and intense tremor. Handley and Brown (1982) using 5-HT-induced head-twitching also found an inhibitory effect of phenylephrine when given i.c.v. with all but the lowest dose of phenylephrine used (0.625micrograms) which produced potentiation. They also looked at the effect of peripherally administered (s.c.) phenylephrine and found a biphasic effect; doses of less than 2.0mg/kg produced potentiation while larger doses produced inhibition. The dose of phenylephrine in the present study was chosen as one which had produced potentiation when administered peripherally (s.c.) in the Handley and Brown (1982) study i.e. 1.25mg/kg i.p. and indeed potentiation of (±)DOI-induced head-twitching was seen with this dose. The reason for the difference in effect produced by phenylephrine on 5-MeODMT- and (±)DOI-induced head-twitching is unknown - possibly it is related to the greater selectivity of (±)DOI.

FLA-63 is a selective NA depleting agent (Svensson and Waldeck 1969 & see Singh et al 1986) which with the dose and pretreatment time used in these experiments has been shown to produce 90% reduction in central NA levels with no significant effect on central DA levels (Singh et al 1986). When the original experiments (Singh et al 1986) were done using 5-MeODMT as the head-twitch inducer, it was predicted

that this drug would cause inhibition of head-twitching because Handley and Brown (1982) concluded that the 5-HT-related head-twitch needs intact alpha1-adrenoceptors in order to occur since it is prevented by alpha1-antagonists (Handley and Brown 1982). The absence of inhibition of 5-MeODMT-induced head-twitching by FLA-63 was taken by Singh et al (1986) to suggest that very little NA is needed to activate the alpha1-adrenoceptors involved. The potentiation seen with 5-MeODMT-induced head-twitching was therefore unexpected (Singh et al (1986). In the present study the potentiatory effect of FLA-63 on 5-MeODMT was confirmed. In addition, FLA-63 was also found to potentiate 5-HTP-induced head-twitching. In contrast, however, (±)DOI- and quipazine-induced head-twitching was inhibited by FLA-63.

Potentiation was reported for reserpine of 5-HT (i.c.v.)- and 5-methoxytryptamine (i.v.)-induced head-twitching in mice by Nakamura and Fukushima (1978), but in this case the other monoamines would also be depleted (Carlsson et al 1957). Lesioning noradrenergic neurones with 6-hydroxydopamine (i.c.v.) failed to affect 5-HTP head-twitching in rats (Bednarczyk and Vetulani 1978). However the data of Bednarczyk and Vetulani (1978) is difficult to interpret, as no biochemical data (NA/DA levels in the lesioned and control animals) was presented in this paper. Heal et al (1986) found potentiation of head-twitching using verified 6-hydroxydopamine (i.c.v.) lesioning in mice with 5-MeODMT as the twitch-inducing agent. This potentiating effect was also found with peripherally administered (i.p.) DSP-4. A result that Eison et al (1988) repeated using quipazine as the head-twitch inducer in rats. The potentiation of 5-HT-related head-twitching seen with these two noradrenergic lesioning agents is in line with the effect produced by FLA-63 (Singh et al 1986). However, the time scale of these two effects is very different e.g. in the case of Eison et al (1988) no change was seen in quipazine-induced head-twitching 3 days after DSP-4 treatment, potentiation of quipazine-induced head-twitching occurred 10 days after DSP-4 treatment, whereas in the case of FLA-induced potentiation of 5-MeODMT-induced head-twitching the effect occurred 4 hours after treatment (Singh et al 1986). The DSP-4-induced potentiation was associated with an increase in beta-adrenergic

receptor number, i.e no change in number was seen 3 days after DSP-4 treatment, but after 10 days a significant increase in beta-adrenoceptors was observed, Eison et al (1988) suggested that this effect, probably coupled with beta-receptor supersensitivity (denervation supersensitivity), is responsible for the potentiation of quipazine-induced head-twitching by DSP-4 10 days after treatment.

In contrast Handley and Singh (1986a) reported that verified 6-OHDA lesions of the L.C. reduced quipazine-induced twitching in rats by about 30%. This is difficult to reconcile in particular with the potentiatory effect of DSP-4, as this agent is thought to produce the equivalent of an L.C. lesion. The length of time after the lesion may be important, Handley and Singh (1986a) used a minimum of 21 days whereas Eison et al (1988) observed potentiation of quipazine-induced in DSP-4 treated rats after 10 days. Similarly, Heal et al (1986) found potentiation of 5-MeODMT-induced head-twitching in mice by DSP-4- and 6-OHDA-induced lesions 10 and 14 days after injection of the lesioning agent. Possibly the greater time allowed in the study of Handley and Singh (1986a) gave greater scope for adaptive changes to occur.

Singh et al (1986) suggested that possible explanations for the facilitatory effect of FLA-63 were a reduction in alpha2-adrenoceptor occupation and/or the occurence of super-sensitivity response either in alpha1- or beta-adrenoceptors, all of which might be expected to facilitate head-twitching e.g. Handley and Brown (1982) & Handley and Singh (1986b&c). Metoprolol, a selective beta1-antagonist (Ablad et al 1973) was found to prevent the FLA-63-induced potentiation of 5-MeODMT-induced head-twitching indicating the involvement of beta-adrenoceptors (Singh et al 1986). Altered agonist availability can cause marked effects in beta-adrenoceptor sensitivity, at least in vitro, within the required time scale, an effect thought to be due to changes in the degree of coupling of the beta-adrenoceptor to the regulatory sub-unit of adenylate cyclase (see Harden 1983).

The present experiments on the effects of FLA-63 were carried out to determine whether the facilitatory effect previously seen in our laboratory with 5-MeODMT-induced head-twitching was also produced on other 5-HT-related inducing agents i.e. 5-HTP, quipazine and particularly, the recently available, (±)DOI. In addition, the effect of a beta2-antagonist, ICI 118,551 (O'Donnell and Wanstall 1980) on FLA-63-induced potentiation of 5-MeODMT-induced head-twitching was also examined to determine whether beta2-adrenoceptors might also be involved in the potentiatory effect of FLA-63. Such experiments were to be consolidated by measuring monoamine depletion by FLA-63. However, although arrangements were made to use HPLC equipment in the Biochemistry laboratories, a fault developed in it and repairs were not achieved by the laboratory concerned in time for this to be done.

The noradrenergic depleting agent, FLA-63 was found to produce markedly different effects depending on the 5-HT-related agent used to induce head-twitching. Thus head-twitching induced by both the 5-HT precursor, 5-HTP, and the direct 5-HT agonist 5-MeODMT were potentiated whereas head-twitching by the other two 5-HT agonists examined, (±)DOI and quipazine, was inhibited (see Figs. 3.2, 3.3 & 3.5 and Tables 3.1, 3.2 & 3.3). Although head-twitch rates by the four agents did not exactly match, the difference in effect was not due to rate dependency since 5-HTP produced the highest rates alone and was potentiated.

The reason for the difference between FLA-63 effects on 5-HTP & 5-MeODMT on the one hand, and quipazine & (±)DOI on the other is, at present, unclear. A difference might be expected between 5-HTP, with the presynaptic component of its activity and the direct 5-HT agonists eg (±)DOI, 5-MeODMT, as appears to be seen with the beta2-agonists, at least in some groups' hands. This is, however, not the explanation as head-twitching induced by both the direct 5-HT agonist, 5-MeODMT, and the 5-HT precursor, 5-HTP were potentiated.

Another explanation is that the differential effects of FLA-63 on 5-HT-related head-twitching are based on the ability of the head-twitch

inducing agents to cause the release of NA. Tricklebank (1985) has raised doubts that 5-HT2 receptors are involved in mediating 5-HTP-induced head-shaking since, in contrast to mescaline-induced head-shaking, the ED50 for inhibition of 5-HTP-induced shaking by ritanserin was 100-fold more than that for the displacement of 5-HT2 binding by ritanserin. Indeed, a noradrenergic component to the head-twitch inducing capacity of 5-HTP has been postulated by Meert et al (1988). Antagonist activity at 5-HT2 receptors was found to be essential to reduce head-twitching but should be associated with activity at dopamine2 or alpha1-receptors for complete blockade. Exogenous 5-HTP is taken up into both 5-HT and catecholaminergic neurones where the newly synthesised 5-HT from 5-HTP displaces both DA and NA (Fuxe et al 1971). Awazi and Goldberg (1978), Everett (1979) and Van Praag (1983) have shown that systemic injections of 5-HTP increase not only the synthesis of 5-HT but also the turnover of DA and NA. On this basis it might be suggested that 5-HTP may cause the release of any remaining NA in the noradrenergic neurones onto postsynaptic beta-receptors made supersensitive by the NA depletion produced by FLA-63. 5-MeODMT may also have this effect as it has been shown to increase NA turnover (Fuxe et al 1972) and it binds with highest affinity to 5-HT1a receptors (Glennon 1987), an effect that has been associated with NA release with the 5-HT1a agonist, 8-OH-DPAT (Heal et al 1989a&B). Neither quipazine or (±)DOI have been reported to release NA, and (±)DOI shows negligible affinity for alpha1-, alpha2- and beta-noradrenergic receptors (Pierce and Peroutka 1989b).

In order to confirm the potentiation of 5-MeODMT-induced head-twitching produced by NA depletion, two other NA depleting agents, disulfiram and DDC were examined. It would have been interesting to look at the effect of DSP-4 i.p. over the time scale used for FLA-63 i.e. after a few hours rather than days, but unfortunately the cost was beyond the budget for the project. Potentiation of 5-MeODMT-induced head-twitch was observed (Fig. 3.6 & Table 3.6) with the dopamine beta-hydroxylase inhibitor DDC, the active metabolite of disulfiram (Goldstein et al 1964). A dose of DDC of 400mg/kg (4hr pretreatment) was chosen which was expected to produce potent

depletion of NA levels but minimise the increase in dopamine levels which have been demonstrated with this agent (Collins 1965; Carlsson et al 1966 & Maj and Vetulani 1970).

The dopamine beta-hydroxylase inhibitor, disulfiram (Musacchio et al 1964) which has also been shown to produce potent depletion in central NA levels in rats with the dose chosen 400mg/kg 4hr pretreatment (Musacchio et al 1966) produced potentiation of 5-MeODMT-induced head-twitching (Fig. 3.7 and Table 3.6), although the effect was less marked than with the other agents requiring the combination of data from two experiments to achieve statistical significance.

The effect of DDC and disulfiram on 5-MeODMT-induced head-twitching would not appear to be mediated by an effect on 5-HT metabolism, as neither of these compounds affect brain 5-HT levels at the doses used in this study (Maj et al 1970).

An attempt was made to confirm that supersensitive beta-adrenergic receptors were responsible for the potentiating effect of FLA-63 on 5-MeODMT-induced head-twitching. It was reasoned that if supersensitive beta1-adrenergic receptors were involved, FLA-63 should increase the potentiatory effect of dobutamine on 5-MeODMT. Unfortunately, in this experiment neither FLA-63 nor dobutamine were found to significantly potentiate 5-MeODMT-induced head-twitching and the interaction of FLA-63 and dobutamine was found to be non-significant. In retrospect due to the importance of this experiment in establishing the mechanism by which FLA-63 produces its potentiatory effect, it should have been repeated using different doses of dobutamine to find one which produced potentiation of 5-MeODMT-induced head-twitching, or tried another beta1-agonist such as prenalterol. However, this experiment was one of those carried out at the time (as previously mentioned in Chapter 1) when it was discovered that the efficacy of compounds which had previously produced strong modulatory effects on 5-MeODMT-induced head-twitching was markedly reduced or absent.

The data of Singh et al (1986) indicated that beta1-adrenoceptors were involved in the potentiatory effect of FLA-63 on 5-MeODMT-induced head-twitching. To extend this finding, the effect of a beta2-selective antagonist ICI 118,551 (O'Donnell and Wanstall 1980) was examined and clearly prevented FLA-63-induced potentiation without having any effect alone on 5-MeODMT-induced head-twitching (Fig. 3.3). This suggests that beta2-adrenoceptors are also involved in the potentiatory effect of FLA-63. This is not in line with the inhibitory effect of beta2-agonists on 5-MeODMT-induced head-twitching reported here and by others. It would be consistent with the potentiatory effect of beta2-agonists on 5-MeODMT-induced head-twitching found by Singh and Handley 1987. The possibility that ICI 118,551 at the dose used (2.5mg/kg) might act on beta1-adrenoceptors must be considered, since beta-adrenoceptor ligands do not in general reach a high degree of selectivity between beta1- and beta2-adrenoceptors (see Handley and Singh 1986c). This would seem unlikely, however, as the dose of ICI 118,551 chosen in the present study was shown by Singh and Handley (1987) to act selectively at beta2-adrenoceptors since it abolished potentiation induced by a beta2-agonist (procaterol) without affecting the response to a beta1-agonist (dobutamine). At present no explanation is available for this anomaly.

EFFECT OF FLA-63 ON 5-MeODMT-INDUCED HEAD-TWITCHING
(30 MIN PRETREATMENT)

FIG. 3.1

VEHICLE
FLA-63

* p <0.05

MEAN NO. OF TWITCHES/6 MIN

DOSE (MG/KG)

40
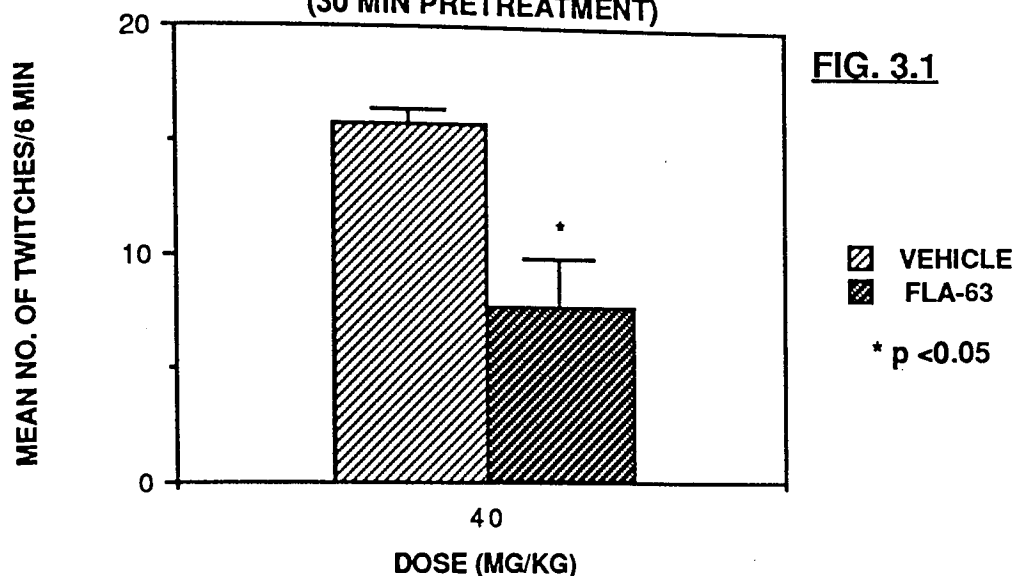
EFFECT OF FLA-63 ON HEAD-TWITCHING INDUCED
BY A VARIETY OF 5-HT-RELATED INDUCERS

FIG. 3.2

VEHICLE
FLA-63

* p <0.05
** p <0.01

MEAN NO. OF TWITCHES

5-HTP   5-MEO   QUIPAZINE   DOI

Pairs of mice were assigned to control or test conditions and
observed in parallel. Results are the means of at least 6
determinations and vertical bars represent s. e. m.

Fig. 3. 1 Mice received either FLA-63 (40mg/kg i. p.) or vehicle
(i. p.) 30min prior to 5-MeODMT (5mg/kg i. p.).

Fig. 3. 2 Mice received either FLA-63 (40mg/kg i. p.) or vehicle
(i. p.) 4hr prior to either 5-HTP (200mg/kg i. p.) and carbidopa
(9mg/kg s. c.), 5-MeODMT (5mg/kg i. p.), quipazine (2mg/kg i. p.) or
(±)DOI (0. 5mg/kg i. p.).

EFFECT OF ICI 118,551 ON FLA-63-INDUCED POTENTIATION OF 5-MeODMT-INDUCED HEAD-TWITCHING
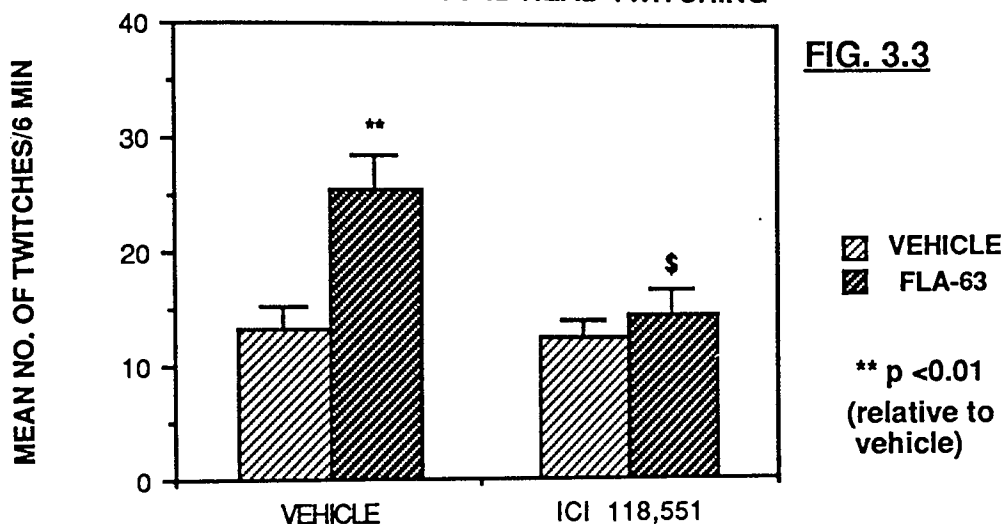
FIG. 3.3

Fig. 3.3   Mice received either FLA-63 (40mg/kg i.p.) or vehicle (i.p.) 4hr prior, and ICI 118,551 (2.5mg/kg s.c.) or vehicle (s.c.) 30min prior, to 5-MeODMT (5mg/kg i.p.).

Results are the means of 9 determinations and vertical bars represent s.e.m. Results were analysed using a two-way between/within subjects ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : $F(AB) = 6.74$; df 1,16; $p < 0.05$. Interpretation : ICI 118,551 significantly inhibits FLA-63-induced potentiation of 5-MeODMT-induced head-twitching.

EFFECT OF FLA-63 ON DOBUTAMINE-INFLUENCED
5-MeODMT-INDUCED HEAD-TWITCHING
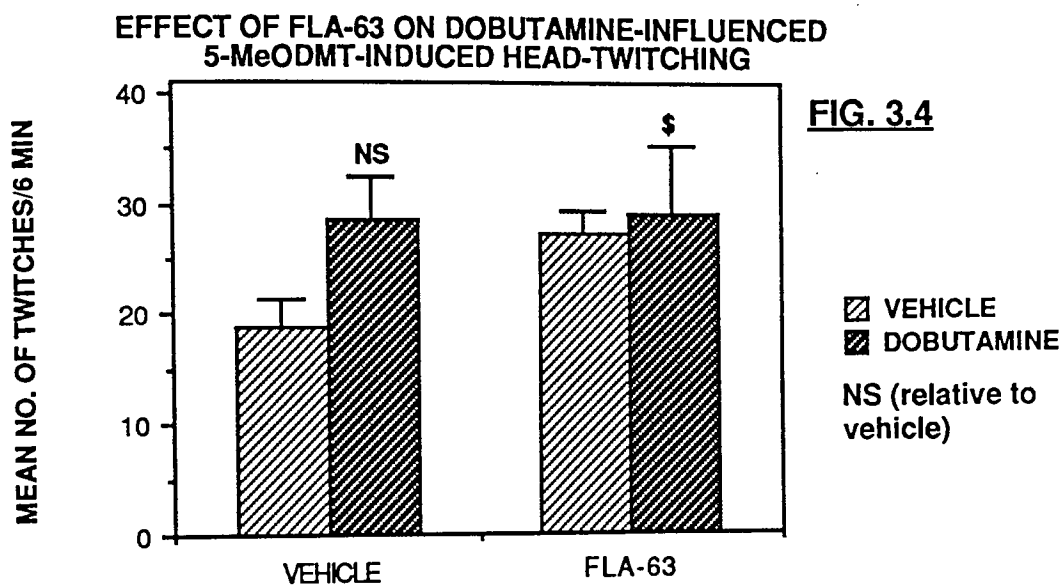
FIG. 3.4

Fig. 3.4   Mice received either FLA-63 (40mg/kg i.p.) or vehicle (i.p.) 4hr prior, and dobutamine (1.5mg/kg i.p.) or vehicle (i.p.) 15min prior, to 5-MeODMT (5mg/kg i.p.).

Results are the means of 7 determinations and vertical bars represent s.e.m.. Results were analysed using a two-way between/within ANOVA followed by Tukey's U test (for unconfounded means).

$ ANOVA result : F(AB) = 1.21; df 1,12; p >0.05. Interpretation : there is no interaction between FLA-63 and dobutamine with respect to 5-MeODMT-induced head-twitching.

EFFECT OF FLA-63 ON DOI-INDUCED TWITCHING IN RATS

FIG. 3.5

- ▨ VEHICLE
- ▨ FLA-63

** p <0.01
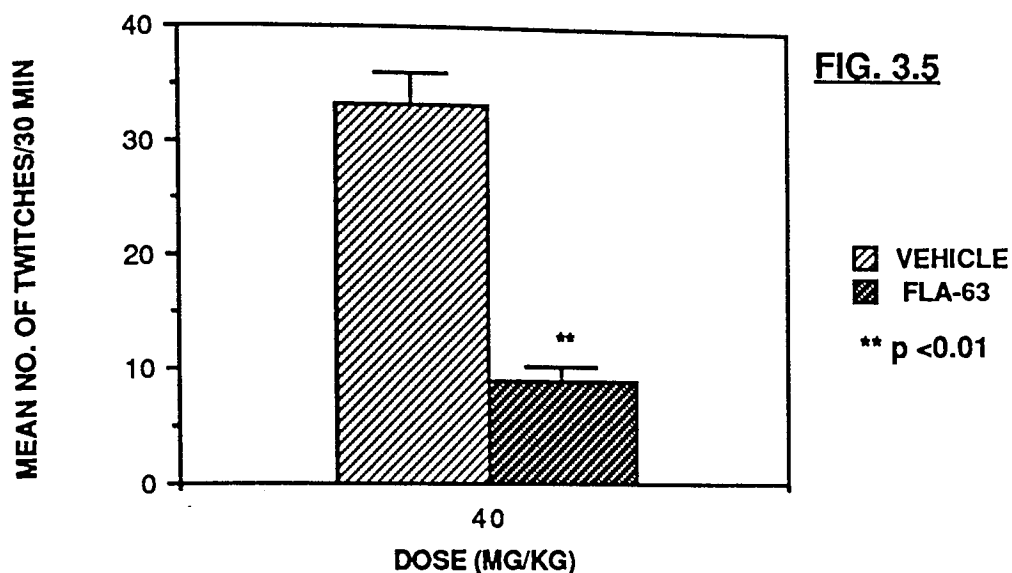
EFFECT OF DDC ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 3.6

- ▨ VEHICLE
- ■ DDC

* p <0.05
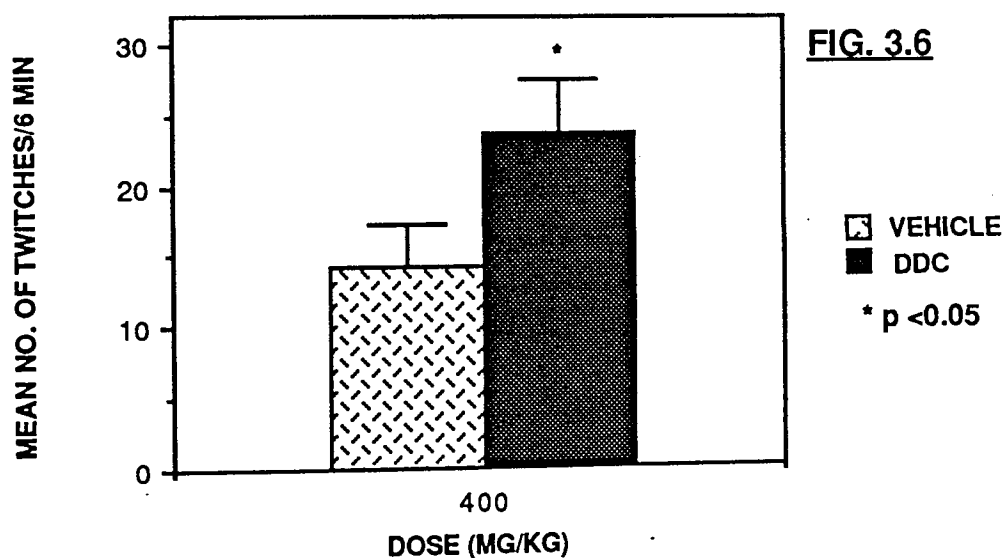
Pairs of mice or rats were assigned at random to control or test conditions and observed in parallel. All results are the means of at least 6 determinations and vertical bars represent s. e. m.

Fig. 3.5 Control rats received vehicle (i. p.) and test rats FLA-63, 4hr prior to (±)DOI (0. 5mg/kg i. p.).

Fig. 3.6 Control mice received vehicle (i. p.) and test mice DDC (i. p.), 4hr prior to 5-MeODMT (5mg/kg i. p.).

EFFECT OF DISULFIRAM ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 3.7

■ VEHICLE
▨ DISULFIRAM

* p <0.05
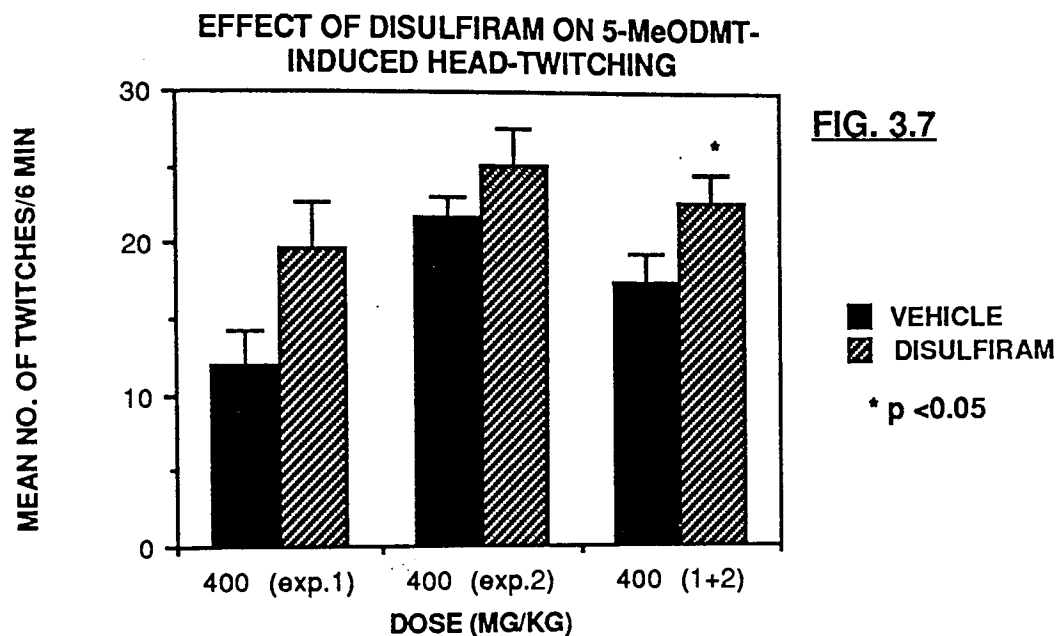
Fig. 3.7 All mice received 5-MeODMT (5mg/kg i.p.). Pairs of mice were assigned to control or test conditions and observed in parallel. The results shown are for two individual experiments (exp.1 and exp.2) and the combination of the two sets of results (1+2). Control mice received vehicle (i.p.) and test mice disulfiram (i.p.). Results are the means of at least 6 determinations and vertical bars represent s.e.m.

EFFECT OF DOBUTAMINE ON DOI-INDUCED HEAD-TWITCHING

FIG. 3.8

VEHICLE
DOBUTAMINE
* p <0.05
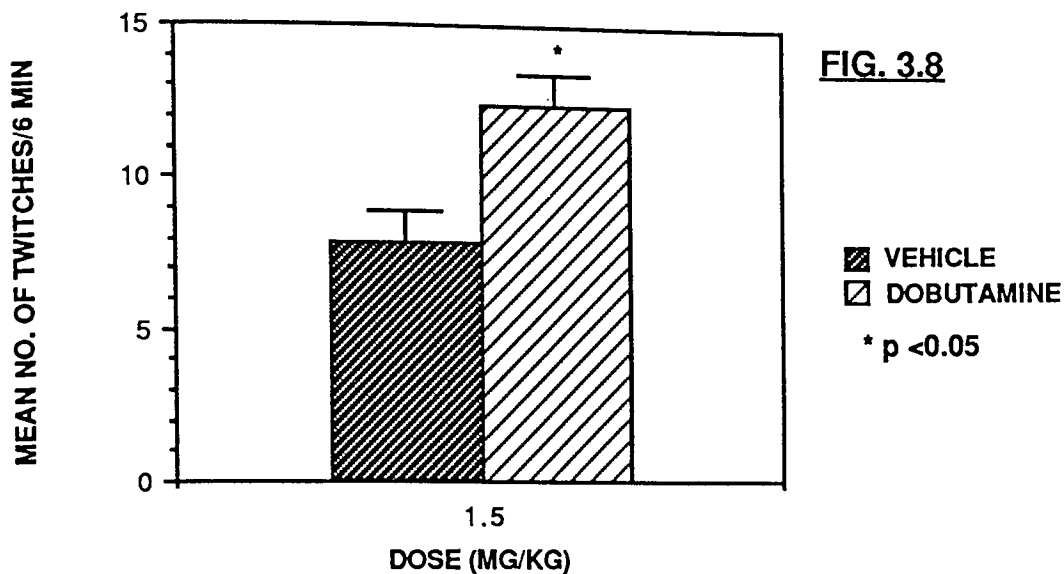
EFFECT OF DOBUTAMINE ON 5-HTP-INDUCED-HEAD-TWITCHING
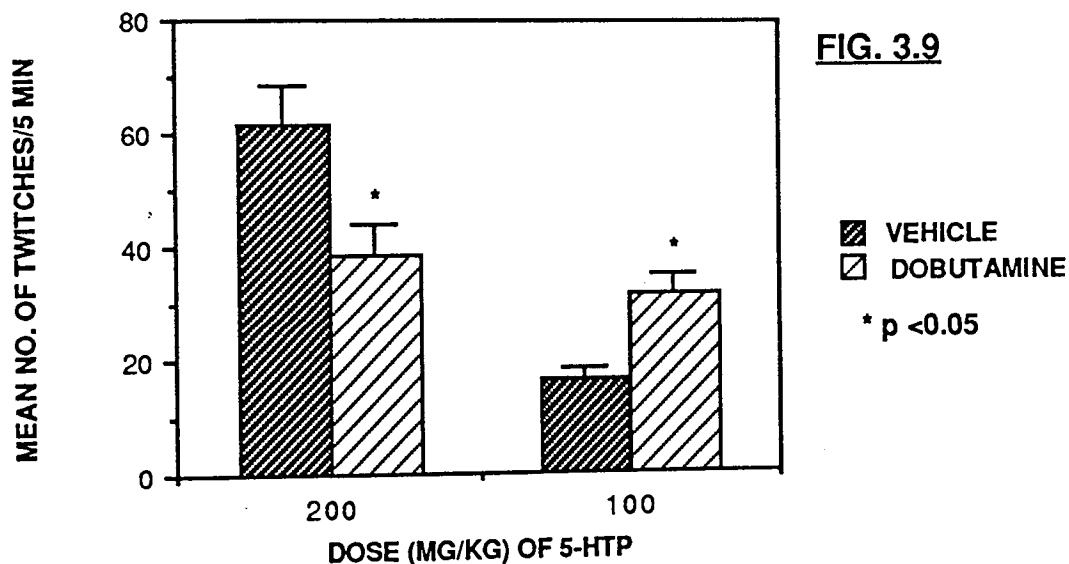
FIG. 3.9

VEHICLE
DOBUTAMINE
* p <0.05

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

Fig. 3.8 Control mice received vehicle (i.p.) and test mice dobutamine (i.p.) 15min prior to (±)DOI (0.5mg/kg i.p.).

Fig. 3.9 Control mice received vehicle (i.p.) and test mice dobutamine (1.5mg/kg i.p.) 15min prior to 5-HTP (100 or 200mg/kg i.p.) and carbidopa (9mg/kg s.c.).

EFFECT OF DOBUTAMINE ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 3.10

VEHICLE
DOBUTAMINE

* p <0.05

MEAN NO. OF TWITCHES/6 MIN

DOSE (MG/KG)

1.5 (exp.1) 1.5 (exp.2) 1.5 (1+2)

EFFECT OF DOBUTAMINE ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 3.11

VEHICLE
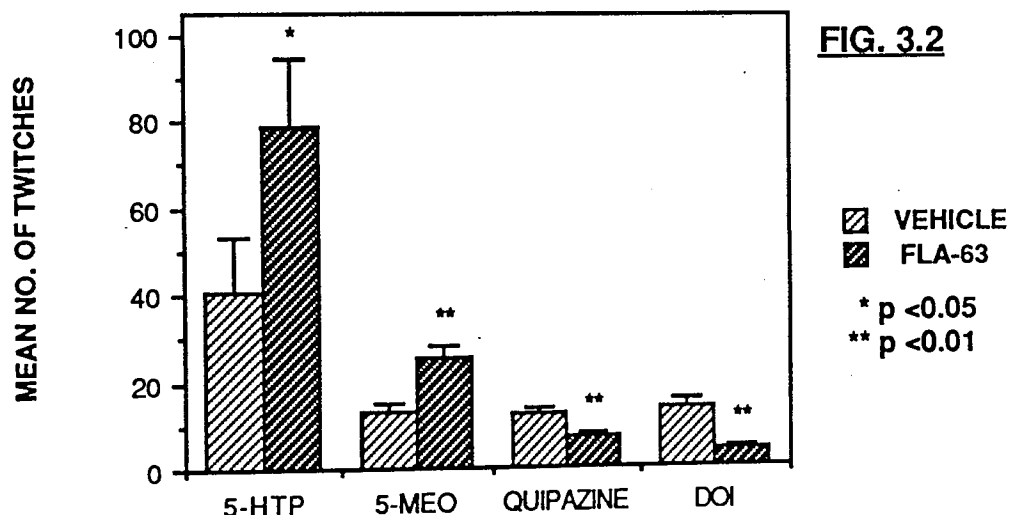DOBUTAMINE

MEAN NO. OF TWITCHES/6 MIN

DOSE (MG/KG)

3

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

Fig. 3.10 Control mice received vehicle (i.p.) and test mice dobutamine (i.p.) 15min prior to 5-MeODMT (5mg/kg i.p.). The results shown are for two individual experiments (exp.1 and exp.2) and the combination of the two sets of results (1+2).

Fig. 3.11 Control mice received vehicle (i.p.) and test mice dobutamine (i.p.) 15min prior to 5-MeODMT (5mg/kg i.p.).

EFFECT OF TWO BETA2-AGONISTS ON DOI-INDUCED HEAD-TWITCHING
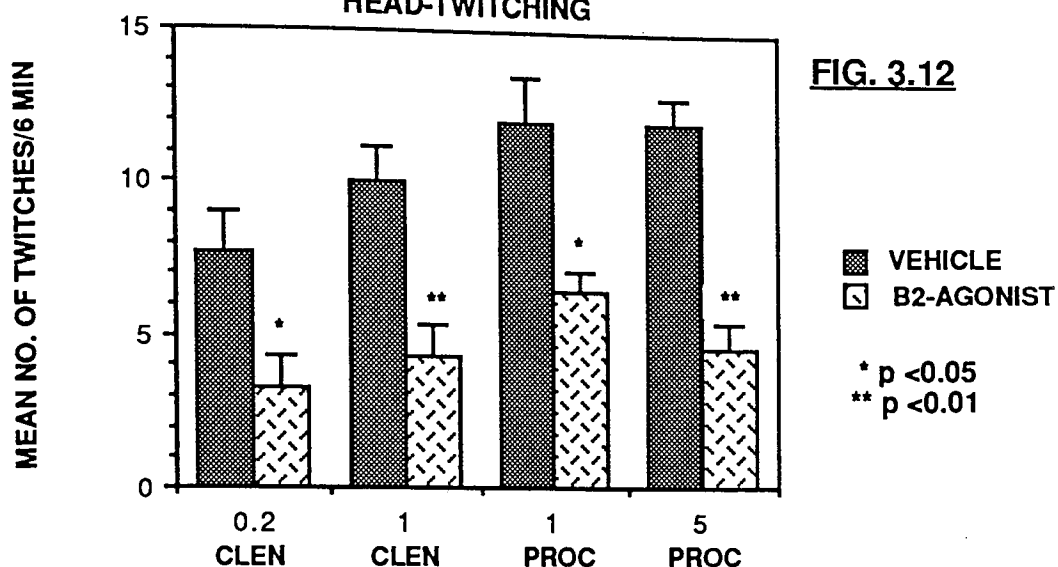
FIG. 3.12
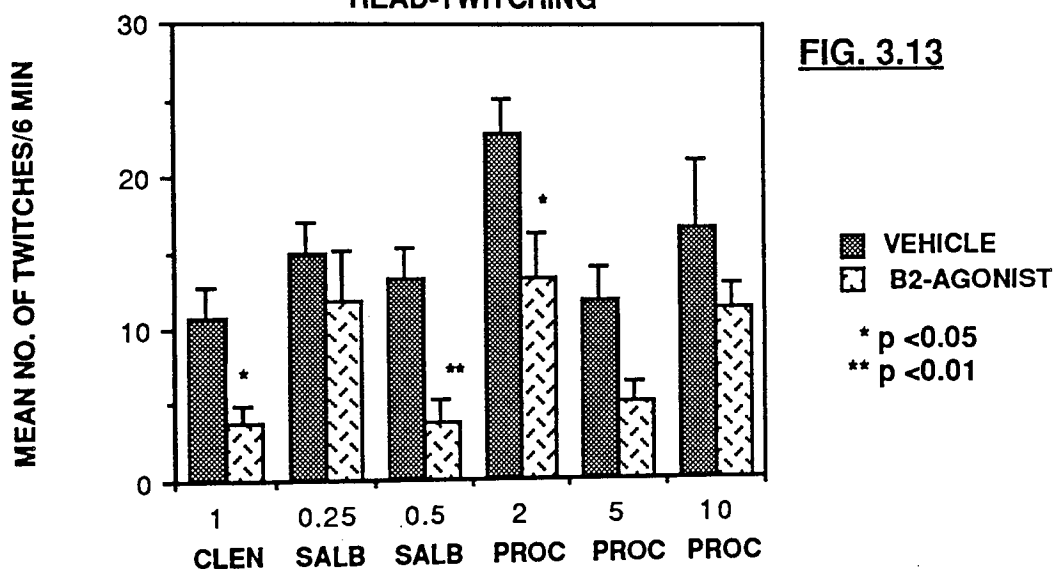
- ■ VEHICLE
- ▨ B2-AGONIST

* p <0.05
** p <0.01

EFFECT OF A VARIETY OF BETA2-AGONISTS ON 5-MeODMT-INDUCED HEAD-TWITCHING

FIG. 3.13

- ■ VEHICLE
- ▨ B2-AGONIST

* p <0.05
** p <0.01

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

CLEN = clenbuterol; PROC = procaterol; SALB = salbutamol.

Fig. 3.12 Control mice received vehicle (i.p.) and test mice a beta2-agonist (i.p.) prior to (±)DOI (0.25mg/kg i.p.).

Fig. 3.13 Control mice received vehicle (i.p.) and test mice a beta2-agonist prior to 5-MeODMT (5mg/kg i.p.).

EFFECT OF A VARIETY OF BETA2-AGONISTS ON 5-HTP-INDUCED HEAD-TWITCHING
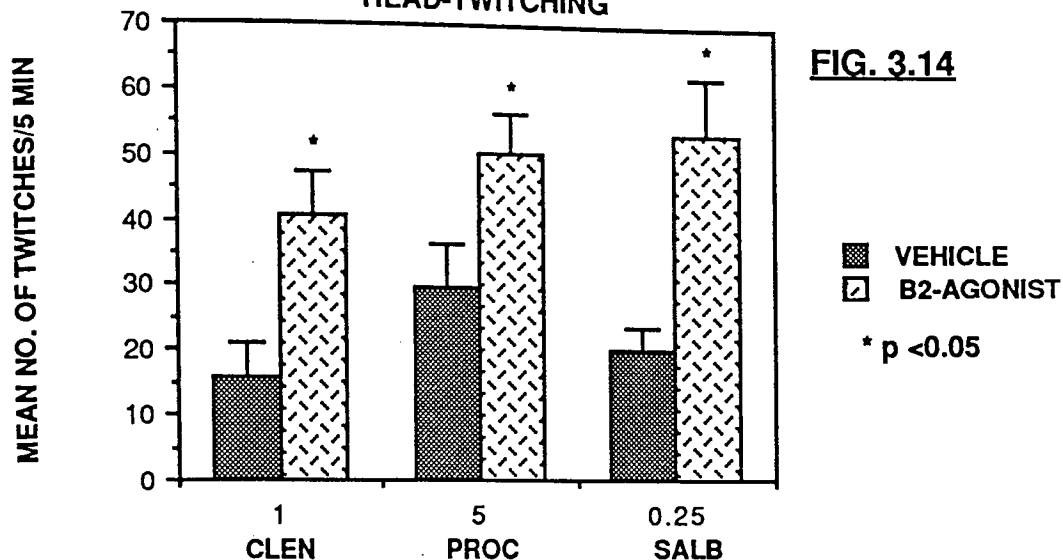
FIG. 3.14
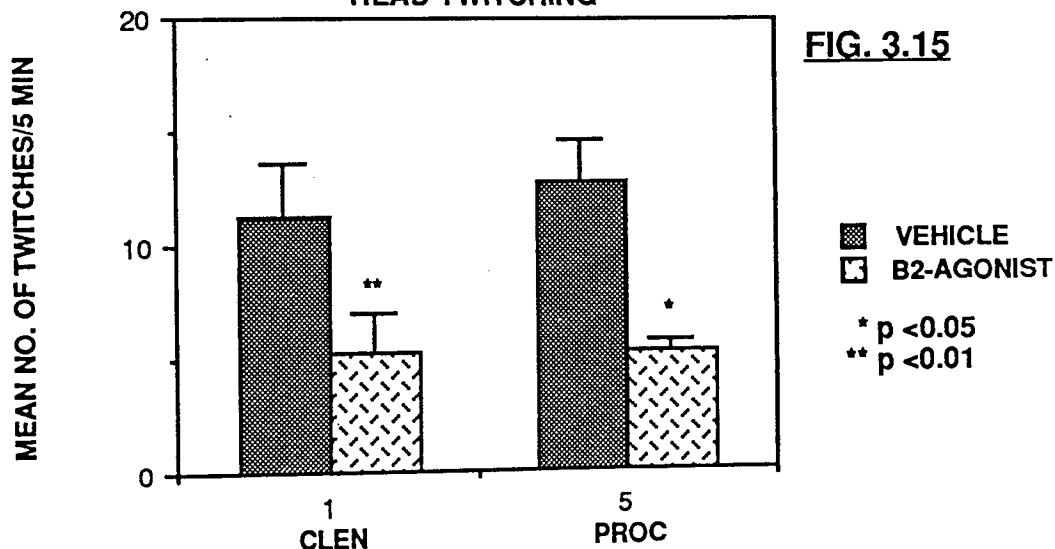
VEHICLE
B2-AGONIST

* p <0.05

EFFECT OF TWO BETA2-AGONISTS ON QUIPAZINE-INDUCED HEAD-TWITCHING

FIG. 3.15

VEHICLE
B2-AGONIST

* p <0.05
** p <0.01

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s. e. m.

CLEN = clenbuterol; PROC = procaterol; SALB = salbutamol.

Fig. 3.14 Control mice received vehicle (i. p.) and test mice a beta2-agonist (i. p.) prior to 5-HTP (200mg/kg i. p.) and carbidopa (9mg/kg s. c.).

Fig. 3.15 Control mice received vehicle (i. p.) and test mice a beta2-agonist (i. p.) prior to quipazine (2mg/kg i. p.).

EFFECT OF PROCATEROL ON QUIPAZINE-INDUCED HEAD-TWITCHING

FIG. 3.16

EFFECT OF ICI 118,551 ON 5-MeODMT-INDUCED HEAD-TWITCHING
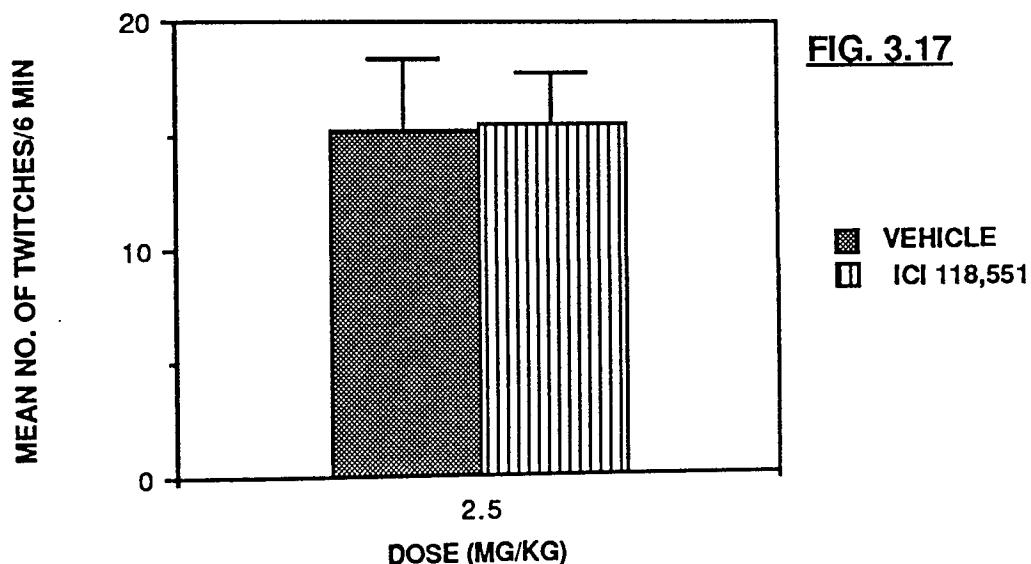
FIG. 3.17

Pairs of mice/rats were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

Fig. 3.16 Control mice/rats received vehicle (i.p.) and test mice/rats procaterol (5mg/kg for mice and 2mg/kg for rats) 15min prior to quipazine (2mg/kg i.p.).

Fig. 3.17 Control mice received vehicle (i.p.) and test mice ICI 118,551 (2.5mg/kg i.p.) 30min prior to 5-MeODMT (5mg/kg i.p.).

EFFECT OF PHENYLEPHRINE ON 5-MeODMT- AND DOI-INDUCED HEAD-TWITCHING

FIG. 3.18

■ VEHICLE
☐ PHENYL-
   EPHRINE

* p <0.05
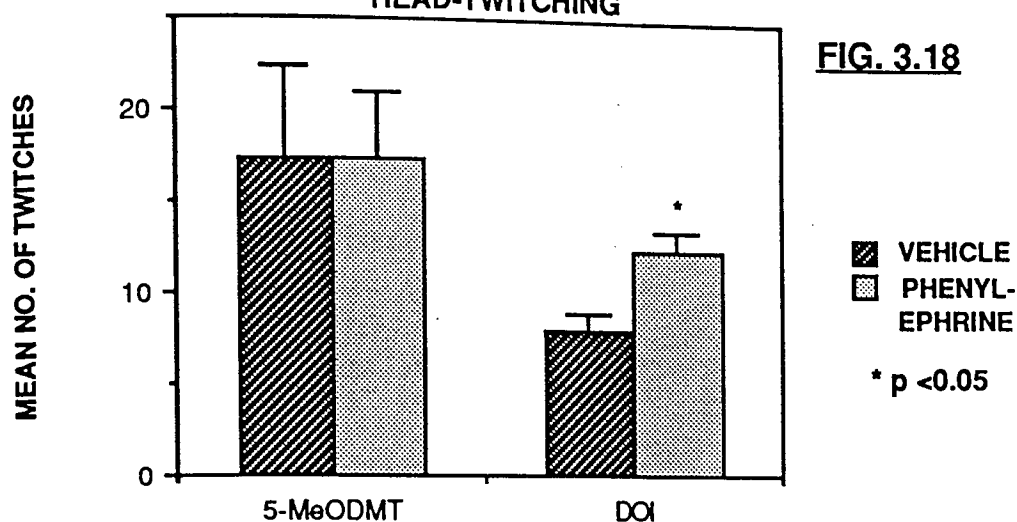
EFFECT OF CLONIDINE ON HEAD-TWITCHING INDUCED BY TWO DIFFERENT INDUCERS
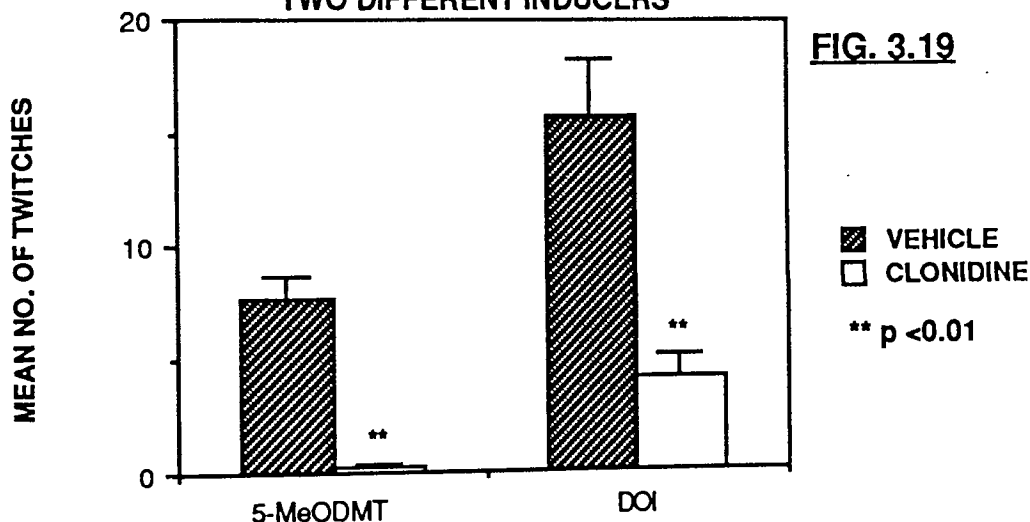
FIG. 3.19

■ VEHICLE
☐ CLONIDINE

** p <0.01

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

Fig. 3.18 Control mice received vehicle (i.p.) and test mice phenylephrine (1.25mg/kg i.p.) 20min prior to either 5-MeODMT (5mg/kg i.p.) or (±)DOI (0.5mg/kg i.p.).

Fig. 3.19 Control mice received vehicle (i.p.) and test mice clonidine (0.05mg/kg i.p.) 30min prior to either 5-MeODMT (5mg/kg i.p.) or (±)DOI (0.5mg/kg i.p.).

EFFECT OF IDAZOXAN ON HEAD-TWITCHING INDUCED BY TWO DIFFERENT INDUCERS
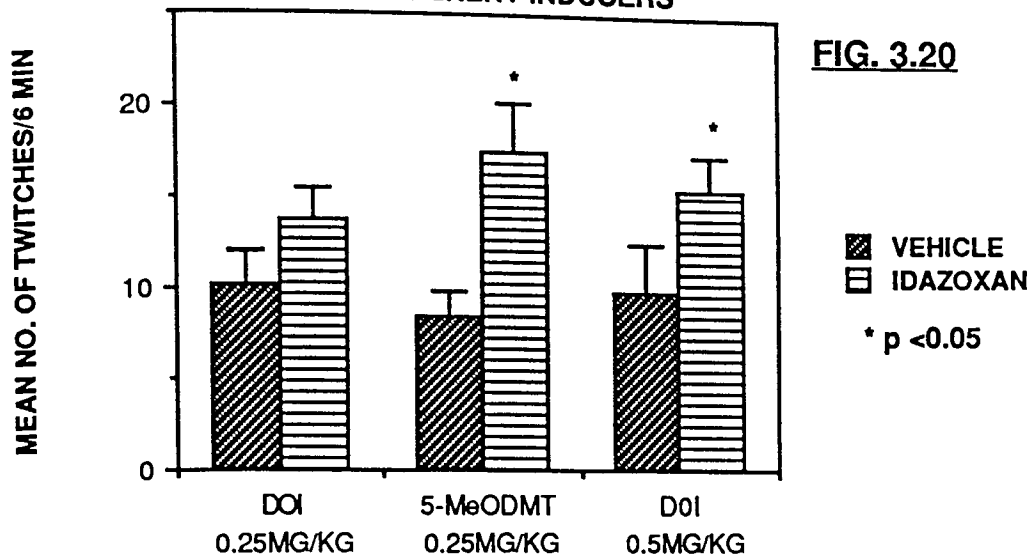
FIG. 3.20

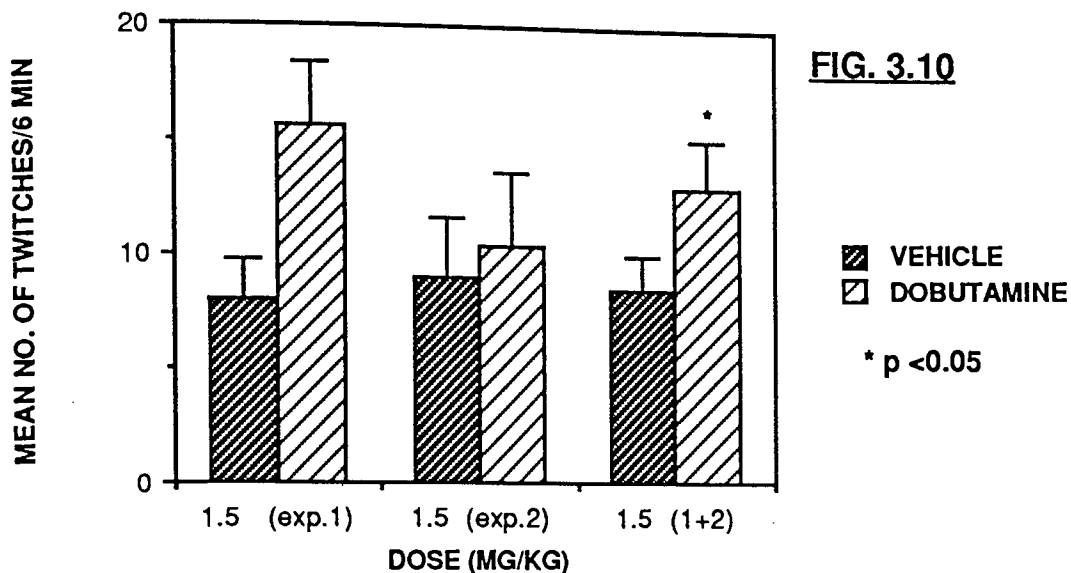## EFFECT OF PRAZOSIN ON DOI-INDUCED HEAD-TWITCHING
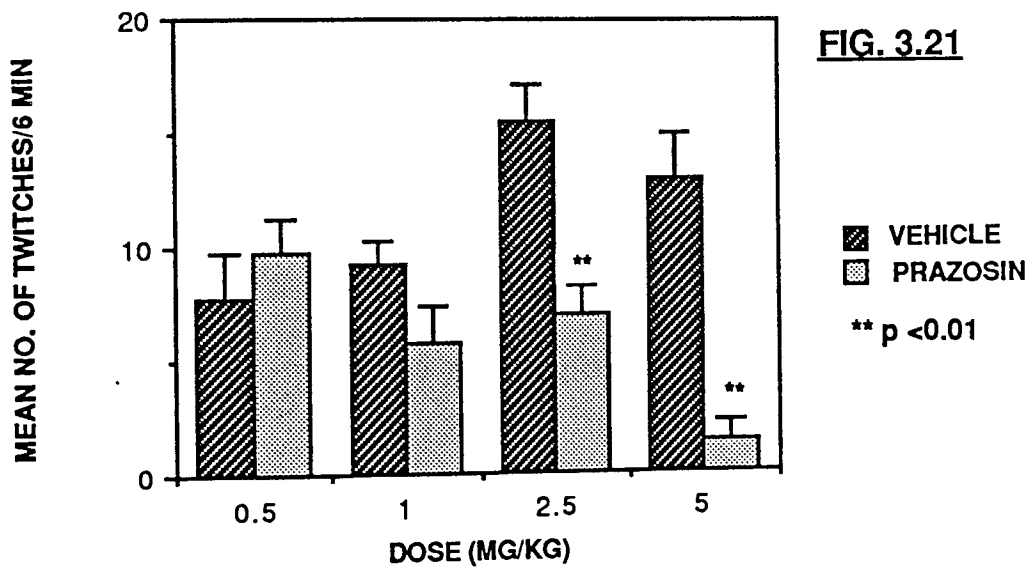
FIG. 3.21

Pairs of mice were assigned at random to control or test conditions and observed in parallel. Results are the means of at least 6 determinations and vertical bars represent s.e.m..

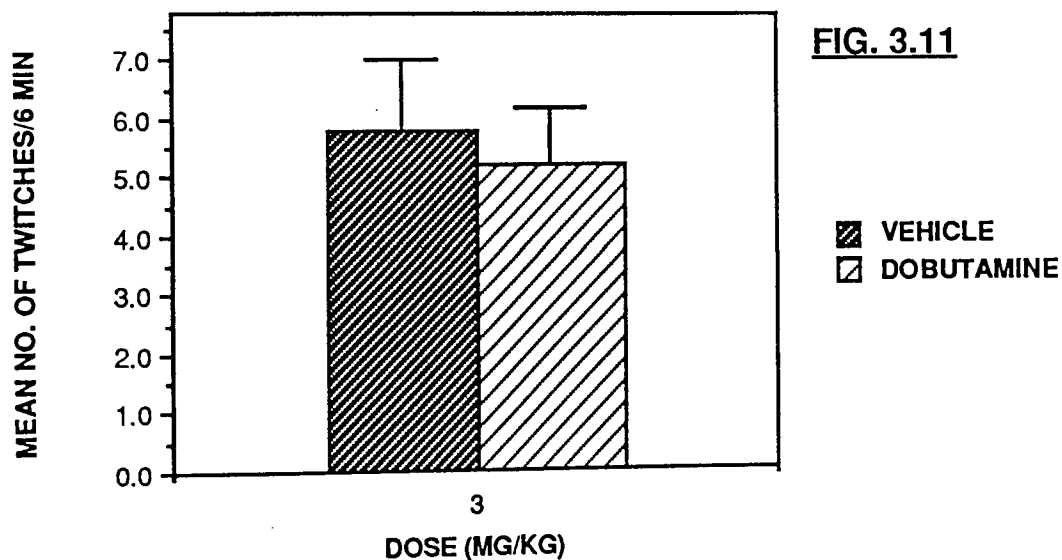Fig. 3.20 Control mice received vehicle (i.p.) and test mice idazoxan (0.25 or 0.5mg/kg i.p.) 30min prior to (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.).

Fig. 3.21 Control mice received vehicle (i.p.) and test mice prazosin (i.p.) 15min prior to (±)DOI (0.5mg/kg i.p.).

EFFECT OF PRAZOSIN ON 5-MeODMT-INDUCED HEAD-TWITCHING
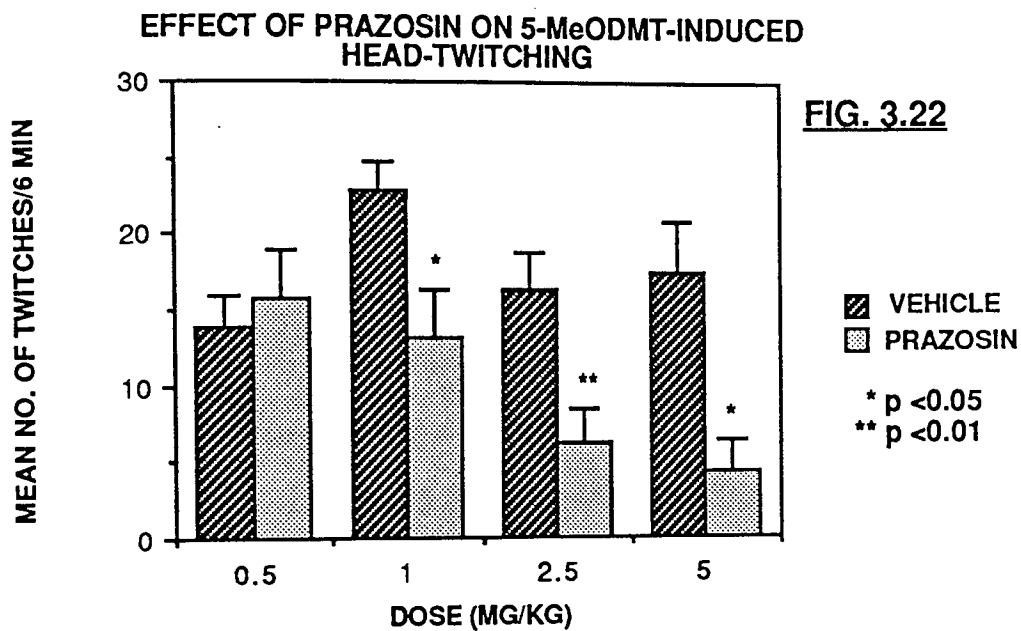
FIG. 3.22

Fig. 3.22 Pairs of mice were assigned at random to control or test conditions and observed in parallel. Control mice received vehicle (i.p.) and test mice prazosin (i.p.) 15min prior to 5-MeODMT (5mg/kg i.p.).

Table 3.1 Effect of FLA-63 on head-twitching induced by a variety of 5-HT-related head-twitch inducing agents in mice.

Mice received either FLA-63 (40mg/kg i.p.) or vehicle (i.p.) 4hr prior to one of the 5-HT-related head-twitch inducing agents ((±)DOI, 5-HTP, 5-MeODMT or quipazine).

FLA-63 (f)			
5-HTP (200mg/kg i.p.)+ carbidopa (9mg/kg s/c)	(±)DOI (0.5mg/kg i.p.)	5-MeODMT (5mg/kg i.p.)	quipazine (2mg/kg i.p.)
v 41.0 (12.2)	14.0 (1.8)	13.3 (2.0)	12.2 (1.8)
f 78.6 (15.7)	4.0 (0.9)	25.4 (3.1)	6.9 (1.3)
p <0.05	p <0.01	p <0.01 *	p <0.01

* results are part of the expt. described in table 3.3.

Table 3.2 Effect of FLA-63 on (±)DOI-induced twitching in rats.

FLA-63 4hr pre (±)DOI (0.5mg/kg i.p.) (40mg/kg i.p.) in rats
v 33.3 (2.8) p <0.01
f 9.0 (1.3)

Results are expressed as mean head-twitch/twitch count (from at least 6 determinations). Bracketed figures represent s.e.m.. Times "pre" refer to the time that the test drug or vehicle were administered prior to the head-twitch/twitch-inducing agent.

Table 3.3 Effect of ICI 118,551 on FLA-63-induced potentiation of 5-MeODMT- induced twitching.

Mice received either FLA-63 (40mg/kg i.p.) or vehicle (i.p.) 4hr prior, and ICI 118,551 (2.5mg/kg s.c.) or vehicle (s.c.) 30min prior, to 5-MeODMT (5mg/kg i.p.).

vehicle--------vehicle	13.3 (2.0)	$F(A) = 5.52$	df 1,16	$p < 0.05$
FLA-63---------vehicle	25.4 (3.1)	$F(B) = 12.65$	df 1,16	$p < 0.01$
vehicle----ICI 118,551	12.6 (1.3)	$F(AB) = 6.74$	df 1,16	$p < 0.05$
FLA-63-----ICI 118,551	14.4 (2.3)			

$F(A) = ICI\ 118,551$
$F(B) = FLA-63$
$F(AB) =$ the interaction of FLA-63 and ICI 118,551 with respect to 5-MeODMT-induced head-twitching.

Results are expressed as mean head-twitch counts (from 9 determinations).

The results were analysed statistically using a two-way between/within ANOVA on the raw data followed by Tukey's U test (for unconfounded means).

Tukey's U test------------v/v compared with v/FLA-63 $p < 0.01$
                v/v compared with v/ICI 118,551   NS
        v/FLA-63 compared with FLA-63/ICI 118,551 $p < 0.01$
        v/ICI 118,551 compared with FLA-63/ICI 118,551   NS

The Tukey's U test results are represented as the treatments that the mice received whose means were compared using this test.

Table 3.4 Effect of FLA-63 on 5-MeODMT-induced head-twitching.

FLA-63 (f) 40mg/kg i.p. 30min pre. v 5-MeODMT 5mg/kg i.p.

---

v	15.8 (0.6)	$p < 0.05$
f	7.7 (2.2)	

Results are expressed as mean head-twitch counts (from 6 determinations).
Bracketed figures represent s.e.m..

NS = non significant - $p > 0.05$.

Table 3.5 Effect of FLA-63 on the effect of dobutamine on 5-MeODMT-
          induced head-twitching.

vehicle)-------vehicle   18.7 (2.8)   F(A) = 1.17 df 1,12 NS
)-------dobutamine   28.6 (3.9)   F(B) = 2.45 df 1,12 NS
FLA-63)--------vehicle   27.4 (2.0)   F(AB)= 1.21 df 1,12 NS
)--------dobutamine 29.1 (6.2)

F(A) = FLA-63
F(B) = dobutamine
F(AB)= the interaction between FLA-63 and dobutamine with respect to
5-MeODMT-induced head-twitching.

Results are expressed as mean head-twitch counts (from 7
determinations).
The results were analysed statistically using a two-way between/within
ANOVA followed by Tukey's U test (for unconfounded means).

Tukey's u-test----------v/v compared with v/dobutamine   NS
                        v/v compared with v/FLA-63   NS
          v/dobutamine compared with FLA-63/dobutamine   NS
          v/FLA-63 compared with FLA-63/dobutamine   NS

The Tukey's U test results are represented as the treatments that the
mice received whose means were compared using this test.

Table 3.6 Effect of disulfiram and DDC on 5-MeODMT-induced head-
           twitching.

Mice received either disulfiram/DDC (400mg/kg i.p.) or vehicle (i.p.)
4hr prior to 5-MeODMT (5mg/kg i.p.).

1) disulfiram (d)	2) disulfiram	3) disulfiram	4) DDC (dd)
v 12.0 (2.2)	21.8 (1.3)	17.4 (1.9)	v 14.2 (3.2)
d 19.6 (3.0)	25.2 (2.4)	22.6 (2.0)	dd 23.7 (3.9)
NS	NS	p <0.05	p <0.05

Experiments 1) and 2) were two different experiments observing the
effect of disulfiram on 5-MeODMT-induced head-twitching, both show a
trend towards potentiation of head-twitching but were not significant
in their own right, combination of the results - 3) - produced a
significant result - disulfiram potentiated 5-MeODMT-induced head-
twitching.

Results are expressed as mean head-twitch counts (from at least 5
determinations). Bracketed figures represent s.e.m..

NS = non significant - p >0.05.

<u>Table 3.7</u> <u>Effect of dobutamine on (±)DOI-, 5-HTP- and 5-MeODMT-induced head-twitching.</u>

Mice received either dobutamine (1.5mg/kg or 3mg/kg i.p.) or vehicle (i.p.) 15min prior to either (±)DOI (0.5mg/kg i.p.), 5-HTP (100 or 200mg/kg i.p.) and carbidopa (9mg/kg s.c.) or 5-MeODMT (5mg/kg i.p.).

dobutamine (d)(mg/kg)		1.5	1.5	1.5	3
(±)DOI	v	7.9 (1.0)			
	d	12.4 (1.0)			
		p <0.05			
5-HTP (100mg/kg)	v	16.3 (2.3)			
	d	31.6 (3.30			
		p <0.05			
5-HTP (200mg/kg)	v	66.1 (6.8)			
	d	38.4 (5.5)			
		p <0.05			
5-MeODMT	v	1) 8.0 (1.7)	2) 9.0 (2.6)	3) 8.5 (1.5)	5.8 (1.2)
	d	15.6 (2.8)	10.4 (3.2)	13.0 (2.2)	5.2 (1.0)
		NS	NS	p <0.05	NS

In the case of 5-MeODMT, experiments 1) and 2) were separate experiments on the effect of dobutamine on head-twitching induced by this agent which suggested a potentiatory effect of dobutamine but were not significant in their own right. The combination of these two sets of results - 3) - was found to be significant.

<u>Table 3.8</u> <u>Effect of a two beta2-agonists on (±)DOI-induced head-twitching.</u>

Mice received either clenbuterol (i.p.) or vehicle (i.p.) 30min, or procaterol (i.p.) or vehicle (i.p.) 15min, prior to (±)DOI (0.25mg/kg i.p.)

	CLEN	CLEN	PROC	PROC
mg/kg	0.2	1	1	5
vehicle	7.7 (1.3)	10.0 (1.2)	12.0 (1.5)	12.0 (0.8)
beta2-	3.3 (1.0)	4.3 (1.0)	6.5 (0.6)	4.6 (0.8)
agonist	p <0.05	p <0.01	p <0.05	p <0.01

CLEN = clenbuterol, PRO = procaterol

Results are expressed as mean head-twitch counts (from at least 5 determinations). Bracketed figures represent s.e.m..
NS = non significant - p >0.05.

Table 3.9 <u>Effect of a variety of beta2-agonists on 5-HTP-induced head-twitching.</u>

Mice received either clenbuterol (i. p.) or vehicle (i. p.) 30min prior, or procaterol (i. p.) or vehicle (i. p.) 15min prior, or salbutamol (i. p.) or vehicle (i. p.) 30min prior, to 5-HTP (200mg/kg i. p.) and carbidopa (9mg/kg s. c.).

	CLEN	PROC	SALB
mg/kg	1	5	0. 25
vehicle	15. 6 (5. 6)	29. 8 (6. 3)	20. 3 (3. 2)
beta2-agonist	40. 9 (6. 3)	50. 4 (6. 0)	53. 3 (8. 9)
	p <0. 05	p <0. 05	p <0. 05

Table 3.10 <u>Effect of a variety of beta2-agonists on 5-MeODMT-induced head-twitching.</u>

Mice received either clenbuterol (i. p.) or vehicle (i. p.) 30min prior, or procaterol (i. p.) or vehicle (i. p.) 15min prior, or salbutamol (i. p.) or vehicle (i. p.) 30min prior, to 5-MeODMT (5mg/kg i. p.).

	CLEN	PRO	PRO	PRO	SALB	SALB
mg/kg	1	2	5	10	0. 25	0. 5
vehicle	10. 7 (2. 1)	22. 8 (2. 3)	11. 8 (2. 2)	16. 7 (4. 5)	15. 0 (2. 0)	13. 3 (2. 0)
beta2-agonist	3. 7 (1. 2)	13. 4 (3. 0)	5. 0 (1. 4)	11. 3 (1. 7)	11. 9 (3. 2)	3. 8 (1. 4)
	p <0. 05	p <0. 05	NS	NS	NS	p <0. 01

CLEN = clenbuterol, PRO = procaterol, SALB = salbutamol.

Results are expressed as mean head-twitch counts (from at least 5 determinations). Bracketed figures represent s. e. m. .

NS = non significant - p >0. 05.

Table 3.11   Effect of two beta2-agonists on quipazine-induced head-
             twitching in mice and twitching in rats.

Mice received either clenbuterol (i.p.) or vehicle (i.p.) 30min prior
to quipazine (2mg/kg i.p.). Mice or rats received procaterol (i.p.) or
vehicle (i.p.) 15min prior, to quipazine (2mg/kg (mice) or 2.5mg/kg
(rats), i.p.).

	CLEN (mice)	PRO (mice)	PRO (rats)
mg/kg	1	5	2
vehicle	11.3 (2.3)	12.7 (1.9)	54.6 (8.5)
beta2-	5.3 (1.7)	5.2 (0.6)	28.6 (1.9)
agonist	p <0.01	p <0.05	p <0.05

CLEN = clenbuterol, PROC = procaterol.

Table 3.12   Effect of ICI 118,551 on 5-MeODMT-induced head-twitching.

Mice received either ICI 118,551 (2.5mg/kg i.p.) 30min prior to 5-
MeODMT (5mg/kg i.p.).

vehicle	15.3 (3.1)	NS
ICI 118,551	15.5 (2.2)	

Table 3.13   Effect of phenylephrine on (±)DOI- and 5-MeODMT-induced
             head-twitching.

Mice received either phenylephrine (1.25mg/kg i.p.) or vehicle (i.p.)
20min prior to (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.)

	(±)DOI	5-MeODMT
vehicle	7.9 (1.0)	17.3 (5.0)
phenylephrine	12.4 (1.0)	17.2 (3.6)
	p <0.05	NS

Results are expressed as mean head-twitch counts (from at least 6
determinations). Bracketed figures represent s.e.m..
NS - non significant - p >0.05.

Table 3.14 Effect of prazosin on (±)DOI- and 5-MeODMT-induced head-twitching.

Mice received either prazosin (i.p.) or vehicle (i.p.) 15 min prior to (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.).

prazosin (p) (mg/kg)		(±)DOI			5-MeODMT		
0.5	v	7.8	(1.9)	NS	13.8	(2.1)	NS
	p	9.8	(1.4)		15.7	(3.3)	
1	v	9.2	(1.0)	NS	22.9	(1.8)	p <0.05
	p	5.7	(1.7)		13.1	(3.2)	
2.5	v	15.5	(1.6)	p <0.01	16.4	(2.4)	p <0.01
	p	7.0	(1.2)		6.2	(2.3)	
5	v	13.0	(2.0)	p <0.01	17.4	(3.4)	p <0.05
	p	1.4	(0.8)		4.4	(2.0)	

Table 3.15 Effect of clonidine on (±)DOI- and 5-MeODMT-induced head-twitching.

Mice received either clonidine (0.05mg/kg i.p.) or vehicle (i.p.) 30min prior to either (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.).

	(±)DOI	5-MeODMT
vehicle	15.7 (2.6)	7.7 (1.0)
clonidine	4.2 (0.9)	0.2 (0.2)
	p <0.01	p <0.01

Results are expressed as mean head-twitch counts (from at least 5 determinations). Bracketed figures represent s.e.m..
NS = non significant - p >0.05.

Table 3.16 Effect of idazoxan on (±)DOI- and 5-MeODMT-induced head-
twitching.

Mice received either idazoxan (i.p.) or vehicle (i.p.) 30min prior to
either (±)DOI (0.5mg/kg i.p.) or 5-MeODMT (5mg/kg i.p.).

idazoxan (i) mg/kg		(±)DOI		5-MeODMT	
0.25	v	10.2 (1.9)	NS	8.5 (1.3)	p <0.05
	i	13.7 (1.7)		17.5 (2.6)	
0.5	v	9.8 (2.7)	p <0.05		
	i	15.5 (1.8)			

Results are expressed as mean head-twitch counts (from at least 6
determinations). Bracketed figures represent s.e.m..
NS = non significant - p >0.05.

<u>Discussion.</u>

The general aim of the work undertaken in this thesis was to examine alterations in the on-going behaviour of rodents induced by 5-HT receptor activation (principally the induction of shaking behaviour) and the modulation by agents acting at 5-HT and other transmitter systems (principally the noradrenergic system) of such alterations in on-going behaviour.

In this discussion, I shall first consider the implications of these results, in terms of the use of shaking behaviour as an animal model that may be useful in the preclinical screening and discovery of systemically-active antimigraine agents.

The parallels between the pharmacology of migraine and shaking behaviour have been outlined in the Introduction. A model was proposed that migraine triggers, or those drugs which worsen migraine, might be expected to potentiate or induce shaking behaviour while the antimigraine agents might be expected to inhibit this behaviour. It was suggested in addition that the migraine triggers, tyramine and beta-PEA, might be expected to initially produce an inhibitory effect on 5-HT-related head-twitching followed by potentiation on the basis of their inhibitory effect on L.C. firing (Lundberg et al 1985).

Initially, both beta-PEA and tyramine produced such a pattern, inhibition of 5-MeODMT-induced head-twitching (0.5-5hr after administration) followed by potentiation (15-48hr after administration). In both cases, it was possible to inhibit the potentiation produced by these migraine triggers using one of the beta-adrenoceptor antagonists which has demonstrated efficacy in migraine prophylaxis - metoprolol (Weerasuriya et al 1982). These initial encouraging results supported the hypothesis. However, at a later stage the effects of beta-PEA and tyramine on 5-MeODMT-induced head-twitching could not be replicated. This lack of a consistently

repeatable effect suggests that, at best, the study on the effect of migraine triggers on 5-MeODMT-induced head-twitching is inconclusive.

Since this work was initiated, the most recent development in the treatment of migraine has been the revelation that selective agonists at a previously uncharacterised subtype (see Saxena and Ferrari 1989) of the 5-HT1 receptor (AH-25086 and GR 43175 (sumatriptan)) appear to be highly effective in aborting acute migraine attacks (Brand et al 1987; Doenicke et al 1987; 1988; Ferrari et al 1990 & Lance and Olesen 1990). It was unfortunately not possible to obtain a sample of either AH-25086 or GR 43175 during the course of this study, otherwise their effect on 5-MeODMT-induced head-twitching would have been examined. However, a variety of other anti-migraine agents were examined for their effect on 5-HT-related head-twitching. Some produced the expected inhibition (cyproheptadine, pitzotifen, methysergide, verapamil and nitrendipine), the rest (aspirin, ergotamine, metoprolol, nadolol and (±)pindolol) produced no effect on 5-MeODMT-induced head-twitching. These results suggest that it is unlikely that 5-MeODMT-induced head-twitching could be used as a model for the preclinical screening of antimigraine agents as it would not have shown up several clinically active antimigraine agents.

In chapter 1, it was suggested that the antimigraine drugs, cyproheptadine, methysergide & pitzotifen inhibited 5-MeODMT-induced head-twitching through 5-HT2 receptor antagonism, an action which may also be their mechanism of activity as antimigraine agents (see Introduction section 18.2b). Fozard and Gray (1989), however, proposed that the antimigraine activity of these agents was more likely to be related to their 5-HT1c receptor activity (Hoyer 1988a), since :

1) mCPP, a 5-HT1b/5-HT1c agonist (Hoyer 1989 & Hoyer and Schoeffter 1989), produces migraine-like headches in humans (Brewerton et al 1988) apparently through 5-HT1c receptor activation since 5-HT1b receptors are rodent-specific (Hoyer and Middlemiss 1989).

2) mCPP has only weak affinity for 5-HT2 receptors (Hoyer 1989) and is an antagonist at these sites (Cohen and Fuller 1983 & Cohen et al 1987) and

3) ketanserin, an antagonist which has good selectivity for 5-HT2 over 5-HT1c sites (e.g. Hoyer 1988a) appears, at least in a preliminary report, to be only weakly active as a migraine prophylactic agent (Winther 1985).

It is unlikely that 5-HT1c receptor antagonism is also responsible for the activity of other antimigraine agents such as the beta-adrenoceptor antagonists and the calcium antagonists since clinical effective agents in both these catagories have been shown to possess little affinity for 5-HT1 receptors (beta-antagonists - Weerasuriya et al 1982; Green et al 1983; Hoyer 1988a & Peroutka 1988; calcium antagonists - Adachi and Shoji 1986; Peroutka 1988 & Green et al 1990).

The 5-HT3 antagonists, in general, had no effect on 5-MeODMT-induced head-twitching, suggesting a) that there is no involvement of 5-HT3 receptors in the mediation of shaking behaviour, b) that there is unlikely to be interaction between 5-HT3 and 5-HT2 systems in the CNS, at least those involved in the control of head-twitch behaviour, and c) if these agents, as a group, turn out to be anti-migraine drugs, that 5-HT-related shaking behaviour will not provide an appropriate screening method for anti-migraine agents.

The apparent loss of effect of the migraine triggers on 5-MeODMT-induced head-twitching is difficult to explain. At the same time as the loss of effect of the migraine triggers was noticed, a change was also observed in the effect produced by beta2-agonists on 5-HT-related shaking behaviour in comparison with previous work in this laboratory. As outlined in Chapter 3, beta2-agonists, at doses which had facilitated 5-MeODMT- and quipazine-induced shaking behaviour (Handley and Singh 1986a & Singh and Handley 1987), produced an inhibitory effect in the present study. It is interesting to speculate whether

these two changes are related. An inhibitory effect of beta2-agonists on 5-MeODMT- and quipazine-induced shaking behaviour has been confirmed by two other laboratories (Heal et al 1986 & Martin et al 1986), in contrast, no work has been published that suggests a potentiatory effect of beta2-agonists on 5-MeODMT- or quipazine-induced shaking behaviour. However, another worker in our laboratory who did some work on the effect of beta2-agonists on 5-MeODMT-induced head-twitching in between Singh and the present study found both inhibition and potentiation, the effect depending on the agent and head-twitch inducer used and the dose of agonist, thus inhibition was seen with procaterol, whereas salbutamol produced potentiation or no effect (C. Thanki - personal communication).

A speculative explanation which draws together the apparent change in firm effects of beta2-agonists seen between groups of workers including the present study and, just possibly, the apparent disappearence of the effects of the migraine triggers on 5-MeODMT-induced head-twitching, is related to the suggested involvement of presynaptic beta2-adrenoceptors in the facilitation of NA release (Westfall 1977; Dhalof et al 1981 & see Misu and Kubo 1983). Handley and Singh (1986c) raised the possibility that the potentiation of 5-HTP head-twitch following beta2-adrenoceptor agonists (an effect also found in the present study see Chapter 3 results section 2.4) may not be direct but indirect via the release of NA onto postsynaptic, predominantly potentiatory receptors such as beta1- and alpha1-adrenoceptors. If this is the case it suggests that the effect produced by beta2-agonists on 5-HT-related head-twitching would be governed by the nature of the postsynaptic receptors stimulated by the resulting increase in NA release. It should be noted at this point that, in addition to the potentiatory beta1- and alpha1-adrenoceptors, there are postsynaptic alpha2-adrenoceptors which have an inhibitory effect on 5-HT-related shaking behaviour (Heal et al 1986). In addition, there is evidence that the sensitivity of all these postsynaptic adrenoceptors can vary e.g. it was proposed that the potentiatory effect of FLA-63 on 5-MeODMT-induced head-twitching was a product of increased sensitivity of beta1-adrenoceptors (see Chapter 3

discussion). Other studies have shown that beta1-adrenoceptor (see Introduction section 8.3 & O'Donnell and Wanstall 1987), alpha1- and alpha2-adrenoceptor number (Sulser 1984) can be altered by appropriate stimuli. It may thus be that the effect of beta2-agonists on 5-HT-related head-twitching may be determined by the proportion and functional status (i.e. facilitatory or inhibitory) of the subpopulation of noradrenergic synapses activated at the time of investigation.

If the effects produced by the migraine triggers initially were real, and, as postulated, produced through an adrenoceptor effect, then the theory stated in the previous paragraphs might also provide some explanation of the apparent loss of effect seen with the migraine triggers on 5-MeODMT-induced head-twitching, if the balance of postsynaptic adrenoceptor activation was altered, for reasons as yet unknown, from that under which the original experiments were performed. One possible explanation for such a change might be an alteration in the level of stress that the animals were subjected to. Stress alters the utilization of central neurotransmitters, in particular that of NA and 5-HT, and also alters behaviour (see Adell et al 1988). Obviously the degrees of stress the animals are under in experiments such as those described by Adell et al (1988) are extreme in comparison with anything that an experimental mouse is likely to be exposed to under 'normal' laboratory conditions, but possibly slight changes would be sufficient to change the receptor balance in favour of no effect for the migraine triggers on 5-MeODMT-induced head-twitching or inhibition for the effect of beta2-adrenoceptor agonists.

In Chapter 2, the behavioural profile of (±)DOI, a prototypic selective 5-HT2 agonist, was examined. At the time this study was initiated the high affinity of (±)DOI for the 5-HT1c site and the 'DOB binding site' was not known. The relevance of this binding to the effects of (±)DOI on on-going behaviour observed in the present study is not, at present, clear. As mentioned in Chapter 2 there is evidence to suggest that the shaking behaviour in rats and hyperlocomotion in mice induced by (±)DOI are mediated by 5-HT2 receptor activation.

Indeed our data with (±)DOI suggests a role for 5-HT2 receptors in the modulation of locomotor activity, at least in a novel environment. (±)DOI-induced scratching in mice would also seem more likely to be 5-HT2 rather than 5-HT1c receptor-mediated as agents possessing agonist activity at 5-HT1c receptors such as mCPP have not been reported to induce scratching in mice.

The 'DOB binding site' (Titeler et al 1985; Lyon et al 1987 & Wang et al 1988) has been recently named as the 5-HT2a site (with the 5-HT2b site corresponding to the 'classic' 5-HT2 receptor) (Pierce and Peroutka 1989a). (±)DOI has been shown to bind to this site with considerably higher potency than at the 5-HT2b site (IC50 5-HT2a = 0.4nM; 5-HT2b = 32nM)(Pierce and Peroutka 1989b). Little is known about the physiological significance of this site, but it may be that 5-HT2a activity plays a part in some of the behavioural effects of (±)DOI, possibly excessive scratching behaviour since it is apparently not produced by several other agents with 5-HT2 agonist activity e.g. d-LSD, and this agent has been reported to have a 10-fold lower affinity for the 5-HT2a binding site than (±)DOI (Pierce and Peroutka 1989b). (±)DOI-induced excessive scratching was blocked by ritanserin indicating that 5-HT2/5-HT1c receptors are likely to be involved in its mediation, however, it is not yet apparent to what extent agents such as ritanserin bind to the 5-HT2a site.

A number of differences in the modulation of (±)DOI-induced head-twitching and scratching were revealed in Chapters 2 and 3 suggesting that, even if both these behaviours are both mediated by 5-HT2 receptors, it would seem likely that they are controlled by different pathways. There appears to be a noradrenergic modulatory effect on both behaviours, as both were inhibited by the NA depleting agent, FLA-63 (Svensson and Waldeck 1969) and the alpha2-agonist, clonidine. However, the two behaviours were modulated differently by beta-adrenoceptor agonists and an alpha2-antagonist. These agents had no effect on (±)DOI-induced scratching, but powerfully affected (±)DOI-induced head-twitching (see Table 4.1), suggesting that different serotonergic pathways are involved in the noradrenergic modulation of

these two behaviours. Additionally, 8-OH-DPAT produced opposite effects on the two responses, inhibiting head twitching but potentiating scratching induced by (±)DOI (Heaton and Handley 1989) suggesting that 5-HT1a receptors can be both facilitatory and inhibitory depending on the behaviour involved.

The effects produced by 8-OH-DPAT on (±)DOI-induced head-twitching and scratching indicate the possibility of a functional relationship between 5-HT1a and 5-HT2 receptors. Such a relationship has also been suggested by a variety of other behavioural, biochemical and physiological work (see Chapter 2 discussion). It would appear that both pre- and postsynaptic 5-HT1a receptors might interact with 5-HT2 receptors. The inhibitory effect of 8-OH-DPAT on (±)DOI-induced head-shaking would seem likely to involve pre-synaptic 5-HT1a receptors on the basis of the work of Yocca et al (1990) and the recently demonstrated ability of the 5-HT depleting agent, p-chlorophenylalanine (PCPA), to block this inhibitory effect (Handley and Dursun 1991). However, the effect of (±)DOI and the 5-HT2 antagonists on the syndrome induced by 8-OH-DPAT would appear to be an effect on postsynaptic 5-HT1a receptors as the syndrome induced by 8-OH-DPAT is thought to be mediated through postsynaptic 5-HT1a receptors (Trulson et al 1976; Deakin and Green 1978; Hjorth et al 1982; Tricklebank 1985 & Tricklebank et al 1985a&b). In addition Araneda and Andrade (1988) have demonstrated an interaction between postsynaptic 5-HT2 and 5-HT1a receptors on the same cell in rat prefrontal cortex. The location of the 5-HT1a receptors which modulate (±)DOI-induced scratching is not known.

a) agents having the same effect on all head-twitch inducers tested.

Modulatory effect on shaking frequency.

	(±)DOI	quipazine	5-MeODMT	5-HTP
ritanserin	− (*)	− (7)		− (4)
alpha1-antagonist	− (*)		− (*, 5)	
alpha2-agonist	− (*)		− (*, 5)	− (8)
alpha2-antagonist	+ (*)		+ (*, 5)	
beta1-agonist	+ (*)	+ (9)	+ (*, 11)	+/− (*, 12)

b) agents having differing effects on the head-twitch inducers tested.

	(±)DOI	quipazine	5-MeODMT	5-HTP
8-OH-DPAT	− (*)	− (1)	o (1, 2)	− (4)
Ipsapirone	o (*)	− (1)	+ (*, 2, 3)	o (2, 3)
buspirone	− (*)	− (1)	o (2)	o (2)
FLA-63	− (*)	− (*)	+ (*, 6)	+ (*)
alpha1-agonist	+ (*)		o/− (*, 5)	
beta2-agonists	− (*)	+/− (*, 5, 9)	+/− (*, 5, 10, 11)	+ (*, 5, 10, 12)

o = no effect. + = potentiation. − = inhibition. * = present study.
1. Yocca et al (1990). 2. Moser (1990). 3. Goodwin et al (1986). 4.
Goodwin and Green (1985). 5. Heal et al (1986). 6. Singh et al (1986).
7. Backus et al (1990). 8. Bednarczyk and Vetulani (1978). 9. Handley
and Singh (1986a). 10. Martin et al (1986). 11. Singh and Handley
(1987). 12. Handley and Singh (1986c).

If the modulation of four head-twitch inducers by the agents in Table
4.1 is compared, although not all drugs were tested on all the head-
twitch inducers, it appears that quipazine and (±)DOI are the most
similarly modulated, being virtually identical in this respect,
followed by 5-HTP and then 5-MeODMT. The modulation of this latter
agent, as illustrated by Table 4.1b, being markedly different from

that of (±)DOI and quipazine. If the theories suggested in Chapters 2 & 3 and previously in this discussion are correct, it is the 5-HT1a activity of 5-MeODMT (not possessed by (±)DOI and quipazine), the 5-HT 'overflow' mechanism by which it is suggested 5-HTP induces head-twitching and the proposed noradrenergic component to 5-HTP-induced head-twitching which are responsible for the differences in modulation between these head-twitch inducers, shown in Table 4.1b. Unfortunately such theories do not explain why certain drugs, in contrast, produce the same effect on all the head-twitchers examined (Table 4.1a). Such differences in modulation do, however, suggest that a head-twitching agent with high selectivity for 5-HT2 receptors should perhaps be used for the examination of neurotransmitter interactions so that the data produced can be easily interpreted.

A variety of future work was suggested by results achieved in this thesis, including :

1) An investigation of the possibility that substance P is involved in scratching induced by (±)DOI and whether there is a serotonergic component to scratching induced by substance P.

2) Further work to evaluate the effect of (±)DOI on grooming comprising initially an examination of the effect of saline injections to determine if an increase in grooming like that seen in rats (Rodriguez Echandia et al 1983) was found in mice. If this was the case then it would necessitate using another form of (±)DOI administration which did not cause such an effect. One possibility might be oral administration.

3) An investigation of the parallels between (±)DOI-induced scratching in animals and pruritus in man to determine whether (±)DOI-induced scratching may be useful as a model for the preclinical screening of antipruritic agents.

4) An examination of the effect of other 5-HT1a agonists/partial agonists on (±)DOI-induced scratching to determine whether the

potentiatory effect seen with 8-OH-DPAT is specific to this agent. An examination of the effect of the non-selective beta-adrenoceptor/5-HT1a/5-HT1b antagonists on this potentiatory effect of 8-OH-DPAT would provide evidence as to whether the effect is being produced by stimulation of 5-HT1a receptors as 8-OH-DPAT has low affinity for 5-HT1b receptors (Hoyer 1988a). An investigation of the possibility of a dopaminergic component to this potentiatory effect by determining whether it is blocked by dopamine antagonists.

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