THE INVESTIGATION OF THE
ANTIPARKINSONIAN EFFECTS OF GLUTAMATE
RECEPTOR ANTAGONISTS

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ABSTRACT

1. This study investigated the locomotor effects of glutamate antagonists in drug-naive and reserpine-treated mice. The NMDA channel blockers, dextromethorphan, memantine, amantadine and phencyclidine (PCP), enhanced the locomotor activity of drug-naive mice. Two clear populations could be differentiated after the administration of 40 mg/kg dextromethorphan, i.e. non-responders and responders. We postulated that the responders were able to metabolise dextromethorphan to the more potent dextrorphan. Only high doses of memantine, PCP and ketamine reversed the akinesia induced by reserpine. Due to the induction of motor deficits, these drugs are not a viable option, at present, for use as monotherapy in PD. These drugs may be more beneficial as adjuncts to the dopamine-based therapy of PD. When interactions with the dopamine D₁ agonist, SKF 38393, the D₂ agonist, RU 24213, and the dopamine precursor, L-Dopa, were investigated in reserpine-treated mice, only dextromethorphan interacted positively with SKF 38393, while MK 801, the glutamate release blockers, lamotrigine and clonidine, the glycine site antagonist, (±) HA-966 and the competitive NMDA antagonist, CPP, interacted synergistically with L-Dopa. It is conceivable that subthreshold doses of the NMDA channel blockers are needed to potentiate the response to L-Dopa, as very low doses of MK 801 synergised with L-Dopa while higher doses induced motor deficits, which impeded locomotion. This curvilinear response is a hallmark of the glutamate antagonists, as low doses increase locomotion by possibly acting at the level of the striatum and/or the nucleus accumbens (NAc) and facilitating dopaminergic transmission by enhancing synthesis and/or release of dopamine, while high doses decrease locomotion by inducing motor deficits, which may result from the action of these drugs at sites outside the basal ganglia, such as the motor cortex.

2. Stereotaxic injections of the glutamate antagonists were then administered into the corpus striatum (CS) or the substantia nigra pars reticulata (SNr) of reserpine-treated rats to determine the site of action of these compounds. Only a subgroup of competitive
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NMDA receptor antagonists, CPP and CGP 40116, induced behavioural arousal from the CS whereas MK 801, PCP, the competitive NMDA receptor antagonist, AP-5, which was ineffective in the CS, and the AMPA receptor antagonist, NBQX in addition to CPP and CGP 40116 produced activity from the SNr. As there is a narrow window between doses which are beneficial and doses which induce motor deficits, careful titration of doses is necessary to separate out the beneficial effects from the detrimental effects. Our results indicate that both the CS and the SN are involved in the locomotor-stimulant as well as side-effect inducing properties of these drugs.

3. The effects of MK 801 were then determined in the cataleptic rat model of PD. Systemic MK 801 alleviated the catalepsy induced by systemic haloperidol and by intrastriatal or intraaccumbens haloperidol. MK 801 administered into the CS, NAc, SNr, subthalamic nucleus (STN) or the entopeduncular nucleus (EPN), attenuated the catalepsy induced by systemic haloperidol. However, systemic MK 801 was ineffective against the catalepsy induced by intrapallidal or intrathalamic muscimol. These results indicate that catalepsy induced by neuroleptics and the anticataleptic ability of MK 801 can arise from regions apart from the CS. The finding that MK 801 was ineffective against intracerebral muscimol-induced catalepsy supplements evidence for theories advanced by Chesselet and Delfs (1996) and Levy et al. (1997), stating that the hyperactivity of the STN may not depend directly on the hypoactivity of the GP, but instead the cortico-subthalamic glutamatergic pathway may be responsible.
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<p>| ABBREVIATIONS | \n|---|---|
| ACh | Acetylcholine |
| AADC | Aromatic L-amino acid decarboxylase |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate |
| ANOVA | Analysis of variance |
| AP-5 | 2-amino-5-phosphonopentanoic acid |
| AP-7 | 2-amino-7-phosphonoheptanoic acid |
| CGP 40116 | R-DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoate |
| CPP | 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate |
| D-CPPene | (−)-(R)-(E)-4-(3-phosphonoprop-2-etyl)piperazine-2-carboxylic acid |
| CS | Corpus Striatum |
| DMSO | Dimethylsulphoxide |
| DOPAC | 3,4-dihydroxyphenylacetic acid |
| EAA | Excitatory amino acids |
| EPN | Entopeduncular nucleus |
| GABA | γ-aminobutyric acid |
| GPi | Internal or medial globus pallidus |
| (±)HA-966 | (±)-3-amino-1-hydroxypyrrolidin-2-one |
| Glu | Glutamate |
| GPe | External or lateral globus pallidus |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>HVA</td>
<td>Homovanillic acid</td>
</tr>
<tr>
<td>i.p</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>L-3,4-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LTG</td>
<td>Lamotrigine</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine oxidase (of which there are 2 types, A and B)</td>
</tr>
<tr>
<td>MK 801</td>
<td>(+)-5-methyl-10,11-dihydro-[5H]-dibenzo[a,d] cyclo-hepten-5,10-imine</td>
</tr>
<tr>
<td>MPP⁺</td>
<td>1-methyl-4-phenylpyridinium ion</td>
</tr>
<tr>
<td>MPTP</td>
<td>N-methyl,4-phenyl-1,2,3,6-tetrahydropyridine</td>
</tr>
<tr>
<td>NAc</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NBQX</td>
<td>2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(f)-quinoxalinedione</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>RU 24213</td>
<td>N-n-propyl-N-phenyl-ethyl-p-(3-hydroxyphenyl)ethylamine</td>
</tr>
<tr>
<td>S.c.</td>
<td>Subcutaneous</td>
</tr>
</tbody>
</table>
SKF 38393  2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride

SN  Substantia nigra
SNc  Substantia nigra pars reticulata
SNr  Substantia nigra pars reticulata
STN  Subthalamic nucleus
VTA  Ventral tegmental area
VMT  Ventromedial thalamus
PUBLICATIONS

CHAPTER ONE
GENERAL INTRODUCTION
1.1. The aetiology and pathology of Parkinson's disease

Parkinson's disease (PD), first described by James Parkinson in 1817 who called it Paralysis agitans, is characterised by the degeneration of nigrostriatal pathways, but is later associated with pathology in non-nigral systems causing dysfunctions in multiple systems (Jellinger, 1989). Lewy inclusion bodies, intraneuronal bodies within the dopaminergic cells, and Lewy neurites are nearly always found during post-mortem examinations and are a hallmark finding in PD (Beizer, 1995; Forno, 1996).

This condition is characterised by rigidity, tremor, especially at rest, loss of postural reflexes and akinesia or bradykinesia (Marsden, 1984). Other symptoms of PD include a masklike face, with decreased blinking, micrographia, dysphagia, incontinence, decreased volume of voice and in the late stages, cognitive impairment. Autonomic dysfunction also occurs in PD, with symptoms like drooling, heat intolerance, orthostatic hypotension, seborrhea and sweating apparent. The symptoms only arise after an approximate 80% degeneration of the dopamine-containing neurones, especially of the striatum and the substantia nigra (SN) (Jellinger, 1986).

Hypokinesia or akinesia usually presents first in PD sufferers, as the inability to initiate and coordinate movement is associated with dopamine deficiency, while rigidity and tremor appear later as they involve the cholinergic, noradrenergic, \( \gamma \)-aminobutyric acidergic (GABAergic) and serotonergic systems. As there exists a balance between the cholinergic and dopaminergic systems and the glutamatergic and dopaminergic systems in the basal ganglia, loss of dopamine leads to a secondary hyperactivity of the cholinergic and glutamatergic systems, which then produces the symptoms of PD (Albin et al., 1989; see Beizer, 1995).

The causes of PD are unknown, although it may result from cerebral ischaemia, head trauma, virus encephalitis, cell death after exposure to manganese or neurotoxins or as a result of oxidative stress, which purports neurotoxicity caused by free radicals (Jenner, 1995; Jenner et al., 1992; Olanow, 1990). The link between free radicals, especially free iron, and the pathogenesis leading up to PD is controversial (Temlett et al., 1994). There is an increase of approximately 77% in the total iron content of the
substantia nigra pars compacta (SNC) in PD compared with control subjects (Temlett et al., 1994). The blood-brain barrier may be disturbed in the SN of parkinsonian patients due to the inflammatory processes which lead to the phagocytosis of damaged dopamine neurones and this can be induced by peripheral iron entering the SN and accumulating there therefore leading eventually to cell death (Gerlach et al., 1994). These findings have led Youdim et al. (1993) to suggest that an inflammatory process might be involved in the aetiology of PD. A genetic link has also been proposed after the discovery of several families with autosomal dominantly inherited parkinsonism (Duvoisin and Golbe, 1995; Duvoisin and Johnson, 1992; Spellman, 1962). Environmental factors may also contribute to the pathology of PD. This hypothesis is based on the report that MPTP in humans produced a parkinsonian condition, with symptoms closely resembling those of the progressive idiopathic disease. PD can also be drug-induced, i.e after exposure to agents that cause the depletion of dopamine, e.g. reserpine, methyldopa, antihistamines, or by exposure to neuroleptic drugs, such as haloperidol, which antagonise dopamine receptors (see Beizer, 1995).

A theory for the aetiology of idiopathic PD put forward recently, concerns cell death brought about by apoptosis of the nigrostriatal pathways, thereby causing loss of these dopaminergic neurones (Mogi et al., 1996). Apoptosis has been shown to be involved in cell death following MPTP treatment (Dipasquale et al., 1991). Evidence for this hypothesis appears to be mounting. Mogi et al. (1996) found that the levels of sFas, a soluble form of an apoptosis-signalling receptor molecule found on the surface of a number of cell types, were greatly increased in the nigrostriatal dopaminergic regions of parkinsonian patients when compared to age- and sex-matched controls. The authors go on to conclude that the presence of sFas could lead to the neurodegeneration seen in PD. PD patients have also been postulated to have altered functions of the immune system (Fiszer et al., 1991) and as sFas and cytokines are correlated, it is feasible that the cumulative interaction of cytokines on sFas may be involved in the pathogenesis of PD (Mogi et al., 1996).
1.2 The Neurochemistry of PD

Although the existence of the SN has been known since 1778, the pathology of PD was not associated with the SN until approximately the time that L-Dopa was used in its treatment (see Forno, 1996). As the most dramatic loss seen in PD is that of the dopamine cells in the SN and most research in PD has focused on the dopaminergic system, although work has also been done on the cholinergic system, which is hyperactive in PD. Lately, the glutamatergic system has also been investigated in the context of PD.

1.2.1 Dopamine in PD

The neurochemical changes in PD were only discovered in 1960 by Hornykiewicz (see Hornykiewicz and Kish, 1984). Hornykiewicz showed that dopamine levels in the SN and CS in post-mortem parkinsonian brains were markedly reduced. This was later correlated with the loss of nigrostriatal dopamine neurones. Symptoms of PD become apparent after 20-40% striatal dopamine is lost. Immediately after loss of dopamine neurones, compensatory mechanisms come into play. There is loss of dopamine transporter but increased turnover of dopamine and an up-regulation in the numbers of dopamine, primarily D\textsubscript{2}, receptors in parkinsonian conditions (see Cooper et al., 1991).

The loss of dopamine in PD suffers is heterogeneous, being greater in the putamen than in the caudate (Fahn et al., 1971) and in the putamen, the caudal loss is greater than the rostral with the reverse occurring in the caudate nucleus (Kish et al., 1987). These results are in correlation with the greatest dopaminergic loss occurring in the medial, caudal part of the SNc which mainly projects to the caudal putamen (Fahn et al., 1971).

The current therapy of PD is based on replacing the lost dopamine by administering the dopamine precursor, L-Dopa or by administering direct dopamine agonists, particularly D\textsubscript{2} agonists although recently specific D\textsubscript{1} agonists have also been shown to be beneficial (Kopin, 1993; Temlett et al., 1989).
1.2.1.1 Dopamine receptors

Dopamine receptors belong to either the D$_1$ receptor-like or the D$_2$ receptor-like families. Members of the D$_1$ receptor-like family include the D$_1$ (or D$_{1A}$) and the D$_5$ (or D$_{1B}$) (Deary et al., 1990; Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990) receptors and these receptors activate adenylyl cyclase and thereby, stimulate the production of cyclic AMP. The dopamine D$_2$ (which can be further subdivided into the D$_2$ short and D$_2$ long forms) (Bunzow et al., 1988), D$_3$ (Sokoloff et al., 1990) and D$_4$ (Van Tol et al., 1991) receptors all belong to the D$_2$ receptor-like family and these receptors produce their effects by inhibiting adenylyl cyclase or via other transduction systems (Kebabian and Calne, 1979). All the dopamine receptors belong to the seven transmembrane spanning G-protein linked receptor family.

The current research has concentrated mainly on dopamine D$_1$ and D$_2$ receptors and their agonists, which will be discussed next.

1.2.1.2 Dopamine agonists

The first selective D$_1$ receptor agonist synthesised and made available for use was SKF 38393 (Setler et al., 1978). Activity resides in the R isomer but the racemate is often used. SKF 38393 is 500 times more potent at D$_1$ receptors than at D$_2$ receptors (Arnt et al., 1992a, b), and this affinity ratio has not yet been surpassed. SKF 38393 is the agonist of choice when investigating the activity of the D$_1$ receptor, even though it is a partial agonist with low intrinsic activity in adenylyl cyclase assays and newer, more potent and behaviourally active agents are on the market. SKF 38393 is a weak behaviour-stimulant in normal animals but this activity is enhanced by eliminating endogenous dopamine, and hence reducing D$_1$ receptor tone, or by up-regulating the D$_1$ receptors or enhancing their coupling to adenylyl cyclase (Rinne et al., 1985). SKF 38393, therefore, has potent antiparkinsonian activity in rodents but this is not seen in primates or man (Braun et al., 1987; Close et al., 1985; Nomoto et al., 1988), possibly due to its low intrinsic activity and poor brain penetrability. However, other D$_1$ agonists have been found effective in primates and man (Temlett et al., 1989), indicating that D$_1$ receptors
have a role to play in motor control.

Further compounds have been identified on the basis of their activity in adenylyl cyclase assays. Examples of the analogues of SKF 38393 include SKF 82958 (3-N-allyl, 6-chloro derivative of SKF 38393) which is a full \( D_1 \) agonist (O'Boyle and Waddington, 1988; Pfeiffer et al., 1982), SKF 77434 (3-N-allyl SKF 38393) (Weinstock et al., 1985) and SKF 83959 (1-m-methylphenyl, 3-methyl, 6-chloro SKF 38393), which acts by inhibiting adenylyl cyclase (Arnt et al., 1992b). All these agents have been found to produce intense grooming in rats, which is a characteristic \( D_1 \)-mediated behaviour (Downes and Waddington, 1993). These data indicate that the assumption that all dopamine receptors act via activating adenylyl cyclase is too simplistic (Starr, 1995a).

Dihydrexidine, belonging to the phenanthridine family of compounds, (Mottola et al., 1992) produces behavioural stimulation akin to that of other \( D_1 \) agonists (Darney et al., 1981) and is also active in the rhesus monkey (Watts et al., 1993). Another phenanthridine, CY 208-243 ((-)-4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3ab]phenanthridine) (Markstein et al., 1988) has affinity for \( D_2 \), 5-HT, \( \alpha \)-adrenergic and opioid receptors (Markstein et al., 1988; Waddington and O'Boyle, 1989), but behaves like a typical \( D_1 \) agonist in parkinsonian animals (Abbott et al., 1991). CY 208-243 has been reported to be as effective as L-Dopa in producing an improvement in 8 idiopathic PD sufferers (Temlett et al., 1989).

Thienopyridines, such as SKF 89626 (4(3',4'-dihydroxyphenyl)-4,5,6,7-tetrahydrothieno-(2,3-c)pyridine) and SKF 89615 (7(3',4'-dihydroxyphenyl)-4,5,6,7-tetrahydrothieno-(3,2-c)pyridine), activate adenylyl cyclase (Andersen et al., 1987) but these drugs are weaker than SKF 38393 in inducing rotations in 6-OHDA lesioned rats, as they do not cross the blood-brain barrier readily. Isoquinolines and the aminotetralins have also been looked at but these are unselective for \( D_1 \) and \( D_2 \) receptors (Andersen and Jansen, 1990; Waddington and O'Boyle, 1989). A-77636 ((1R,3S)-3-(1'-adamantyl)-1-aminomethyl-3,4-dihydroxy-1H-2-benzopyran hydrochloride), a isochroman derivative, shows promise, as it has been reported to potently relieve the parkinsonism of MPTP-treated marmosets, with a prolonged duration of action when orally administered.
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(Kebabian et al., 1992). However, seizure-activity tends to be seen after the administration of these highly potent D\textsubscript{1} agonists.

The D\textsubscript{2} receptor preferring agonists are as varied, some being only slightly different from D\textsubscript{1} agonists (Andersen and Jansen, 1990; Waddington and O'Boyle, 1989). The clinically used antiparkinsonian agent, lisuride, is structurally similar to CY 208-243 but has 50-fold greater affinity at D\textsubscript{2} receptors than D\textsubscript{1} receptors (Andersen and Jansen, 1990). Bromocriptine, a highly potent D\textsubscript{2}-selective ergoline, with very little activity at D\textsubscript{1} receptors, is used clinically in the treatment of PD (see Beizer, 1995).

PHNO, a hydroxynaphthoxazine, is very active at D\textsubscript{2} receptors, as seen in behavioural studies (Martin et al., 1984). Other chemical groups, include the phenylethylamines, e.g. RU 24213 (N-n-propyl-N-phenylethyl-p-(3-hydroxy-phenyl)ethylamine), which is now used as a standard D\textsubscript{2} agonist in behavioural studies (Euvrard et al., 1980). LY 171555 (or quinpirole) (trans-(±)-4,4a,5,6,7,8a,9-octahydro-5-propyl-2H-pyrazole-(3,4-g)quinoline) is a partial ergoline with good brain penetrability with high affinity for the D\textsubscript{2} receptor (Tsurata et al., 1981). It has insignificant activity at D\textsubscript{1} or non-dopamine receptors, but after the cloning of the D\textsubscript{3} receptor, this agent has been found to have 100-fold greater affinity at the D\textsubscript{3} receptors as compared to D\textsubscript{2} receptors (Sokoloff et al., 1990). LY 171555 has been reported to be able to reverse the parkinsonism induced by MPTP treatment in marmosets (Nomoto et al., 1985, 1988) and this effect is most probably mediated via action of the excitatory D\textsubscript{2} receptors and not the inhibitory D\textsubscript{3} receptors.

L-dihydroxyphenylalanine (L-Dopa), the biological precursor of dopamine, can be considered an indirect mixed D\textsubscript{1}/D\textsubscript{2} agonist. The most common direct D\textsubscript{1}/D\textsubscript{2} agonist used in PD therapy is apomorphine (Seeman, 1981).

1.2.2 Other neurotransmitters involved in PD

Although the most dramatic reductions are in the levels of dopamine, with loss of dopamine cells in the mesolimbic, mesocortical and hypothalamic systems, there are also alterations in the levels of other neurotransmitters. Noradrenergic neurones are lost in the
locus coeruleus and there is also loss of serotoninergic neurones. The levels of somatostatin, neurotensin, substance P, enkephalin and cholecystokinin are also decreased in PD sufferers (see Cooper et al., 1991) while there is reported to be hyperactivity of cholinergic (Stoof et al., 1992; Starr, 1995a) and glutamatergic pathways, especially the corticostriatal and/or the subthalamic pathways (Albin et al., 1989; Bergman et al., 1990; Miller and DeLong, 1987; Riederer et al., 1992; Smith and Parent, 1988). Changes also occur in the receptor density and levels of other neurotransmitter, especially loss of benzodiazepene receptors in the mid and caudal parts of the putamen and a reduction in the muscarinic receptor density in the rostral putamen in PD, probably due to the excessive cholinergic activity (Griffiths et al., 1994).

Dopamine and glutamate have both been shown to regulate GABAergic activity by causing alterations in the expression of GAD, the rate-limiting enzyme in the synthesis of GABA. Alterations in GAD mRNA levels directly translate to alterations in GABAergic activity, synthesis and release (Lindefors et al., 1990). Dopamine, acting via D_2 receptors, reduces the levels of GAD mRNA while glutamate acting at NMDA receptors increases the levels of GAD mRNA in a number of brain regions including the striatum and the cortex (Qin et al., 1994). GAD mRNA levels were increased in the dorsolateral striatum and the GPi of MPTP-treated monkeys (Pedneault and Soghomonian, 1994). GABA levels are increased in conditions where there is a depletion of dopamine. The caudal striatum is the site where the greatest increase in GABA levels in PD is seen although the levels of glutamic acid decarboxylase (GAD), the enzyme which breaks glutamate down to GABA, are reduced (Lloyd and Hornykiewicz, 1973). GABA receptor density is decreased in PD with GABA receptor reductions seen in the mid and caudal putamen (Griffiths et al., 1994). A plausible reason for a reduction in GABA receptor density could be the loss of GABA receptors localised on the nigrostriatal dopamine axons in the striatum in PD (Griffiths et al., 1994).
1.2.3 Glutamate in PD

There is evidence showing that excessive stimulation of EAA receptors can result in cell death in the central nervous system. This neurotoxic response is probably due mainly to the excessive influx of Ca²⁺ ions through the NMDA receptor ion channel into the cells, which causes structural damage in the cells and finally leads to necrosis (Meldrum and Garthwaite, 1990). Glutamate excitotoxicity has been implicated in neurodegenerative disorders including ischaemia (Rothman and Olney, 1987), epilepsy (Croucher et al., 1982) as well as the movement disorders (Doble, 1995).

There exists a balance between the dopaminergic and glutamatergic systems in the basal ganglia and dopamine depletion causes hyperactivity in the glutamatergic pathways (Albin et al., 1989; Bergman et al., 1990; Miller and De Long, 1987; Mitchell et al., 1989; Smith and Parent, 1988). The loss of dopaminergic afferents, as occurs in PD, causes striatal cells to discharge abnormally, leading to overactivity in the neurones from the subthalamic nucleus (STN) to the G Pi (or EPN, in rats), which results in tonic inhibitory output to the motor thalamus (Albin et al., 1989; Alexander and Crutcher, 1990). Evidence from 2-deoxyglucose studies suggest this overactivity is due to increased glutamatergic activity in the STN (Mitchell et al., 1989), which sends glutamatergic projections to the G Pi (Smith and Parent, 1988).

1.2.3.1 Glutamate receptors and their localisation

Glutamate acts at two receptor types, namely the ionotropic and the metabotropic receptors. The ionotropic receptors consist of N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoxazole (AMPA) and kainate types. Binding studies have shown that most of the excitatory amino acid (EAA) receptors are localised in the cortex, basal ganglia and the sensory pathways. Using autoradiography, Ball et al. (1994) found in the human substantia nigra (SN), high concentrations of [³H]MK 801 and [³H]glycine binding sites, which represent NMDA receptors, and moderate densities of [³H]CNQX and [³H]kainate binding sites. There was a greater density of NMDA and kainate receptors and fewer AMPA receptors in the human SNc than in the SNr. Low levels of the NMDA,
AMP A and kainate receptors have been reported in the rat GPi, SN, ventral tegmental area (VTA) and the STN (Albin et al., 1989; 1992; Monaghan and Cotman, 1985). However, studies in the rat have shown that kainate receptors are relatively high in the caudate nucleus but the GP contains a low density of these receptors (Monaghan and Cotman, 1985). The highest density of glutamate receptors in the basal ganglia occurs in the caudate and putamen, nucleus accumbens (NAc) and the olfactory tubercle. It has been hypothesised that subpopulations of striatal EAA binding sites may be located on dopaminergic terminals as in unilaterally 6-OHDA lesioned rats the numbers of NMDA, AMPA, kainate and metabotropic receptors were decreased (Wüllner et al., 1994).

NMDA, AMPA and kainate receptors are all coupled to cation channels but the channels coupled to the NMDA receptors have a larger conductance and a higher permeability to calcium ions, Ca\(^{2+}\), than the channels coupled to AMPA and kainate receptors.

In the current research, we have concentrated mainly on the NMDA receptor with some work also done with an AMPA receptor antagonist. The NMDA receptor has four main regulatory sites; 1) the neurotransmitter or agonist binding site; 2) the strychnine-insensitive glycine binding site; 3) the polyamine site and 4) the site within the ion channel, but, in addition there are also sites for Mg\(^{2+}\) and a Zn\(^{2+}\) (see Figure 1.2). The agonist binding site forms a complex with the ion channel, which is permeable to Ca\(^{2+}\) and Na\(^{+}\) ions and is blocked by Mg\(^{2+}\) under resting conditions. The AMPA receptor-linked ion channel is permeable to Na\(^{+}\) ions (see Monaghan et al., 1989).

1.2.3.1.1 Molecular structure

The NMDA receptor is thought to be a pentameric structure and is made up of NMDAR1 and NMDAR2 subunits. There are four forms of the NMDAR2 (NMDAR2A-2D) subunit and eight variants of the NMDAR1 (NMDAR1A-1H) subunit. The presence of at least one NMDAR1 subunit is required to form a functional ion channel and the presence of an NMDAR2 subunit increases the current-carrying capacity of the channel (Nakanishi, 1992). These subunits have been shown to be heterogeneously distributed
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throughout the central nervous system (Kutsuwada et al., 1992). The NMDAR1A/NMDAR2A and the NMDAR1A/NMDAR2B are the most widespread forms of the NMDA receptor subtypes found in the brain while NMDAR1A/NMDAR2C and NMDAR1A/NMDAR2D are largely restricted to the cerebellum and the hindbrain of the rat. The NMDA receptors found in the lateral thalamus, which has a high density of NMDAR2A subunit mRNA, have a higher affinity for antagonists, such as CPP, and a lower affinity for agonists compared to the NMDA receptors in the medial striatum, a region with high levels of NMDAR2B mRNA (Buller et al., 1994; Standaert et al., 1994). The striatum expresses predominantly NMDAR1A and NMDAR2B while the STN and a small number of SNr neurones express NMDAR1F and NMDAR2D (Standaert et al., 1994).

Cloning studies have shown that AMPA receptors are made up of the GluR1-7 and KA1-2. The AMPA receptor is also thought to be a pentameric oligomer. The GluR1-4 subunits form the cation channel. Recombinant receptors formed by the Glu5-7 and the KA1 or KA2 subunits have high affinity for kainic acid and are thus, called kainate receptors (see Doble, 1995). The AMPA receptors in the SNr consist of the GluR1 and GluR2/3 subunits (Paquet and Smith, 1996).

There is some evidence that the NMDAR2B subunit is more sensitive to glutamate and to the enhancing effects of glycine and is likely to be overstimulated under pathological conditions (Kutsuwada et al., 1992; Lau and Huganir., 1995; Moon et al., 1994). More NMDAR2B subunits appear to be in an activated, i.e tyrosine phosphorylated, form in regions of the basal ganglia implicated in Parkinson's disease (Landwehreyer et al., 1995).

1.2.3.2 Glutamate antagonists

The classification, characterisation and cloning of glutamate receptors have provided a wealth of information, and have paved the way for pharmacological investigations of the various target sites. Agents used in these investigations, include compounds acting at the NMDA and AMPA receptors. Most of the research done in this field has focused on
Figure 1. A diagrammatic representation of the NMDA receptor and its regulatory sites. Listed are examples of the antagonists used to block these sites.
the NMDA and AMPA receptors, sidelining other glutamate receptors, therefore their relevance to PD is unknown.

Non-competitive NMDA antagonists, including the glycine and NMDA channel blockers, show some central activity in dopamine-depleted animals. Very little work has been done with the polyamine site antagonists, an example of which is ifenprodil. The development of the glycine site antagonists was outlined by Kemp and Leeson (1993), as glycine is thought to endogenously act as a co-agonist to glutamate. A number of glycine site antagonists, based on the structure of glycine, have been synthesised. (±) HA-966 ((R,S)-3-amino-1-hydroxypyrrolidin-2-one) (Fletcher and Lodge, 1988), a glycine site antagonist, has been hypothesised to be a potential antiparkinsonian agent. Glycine site antagonism is restricted to the (R)-(+) enantiomer while the (S)-(−) enantiomer is a sedative, although the mechanism behind this is unknown. Glycine site antagonists have the properties common to other NMDA antagonists, i.e. they are neuroprotective and anticonvulsant but appear not to be psychostimulant and are relatively free of debilitating side-effects (Kemp and Leeson, 1993).

The phencyclidine (PCP) site is located within the NMDA ion channel, and for the NMDA channel blockers to act, the channel must open (i.e. use-dependent antagonism). PCP is a dissociative anaesthetic but produces psychostimulation, which limits its effectiveness as a antiparkinsonian agent. The same disadvantages also apply to MK 801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)-cyclohepten-5,10-imine) (Wong et al., 1988). MK 801 and PCP are potent locomotor-stimulants in animals and they are thought to act by increasing the release of dopamine (Löschner and Höнак, 1992). MK 801 is often used as the standard glutamate antagonist and its antiparkinsonian potential has been comprehensively investigated.

Ketamine, a short-acting dissociative anaesthetic, is also a low-affinity NMDA channel blocker. Other NMDA channel blockers, include the 1-aminoadamantanes, memantine and amantadine (Kornhuber et al., 1991) and the commonly used antitussive, dextromethorphan, the dextrorotatory morphinan analogue of codeine (see Tortella et al., 1989). The anticholinergic agents, budipine and biperiden, have also been shown to block
NMDA channels (Jackisch et al., 1994). These compounds also have low toxicity due to faster rates of binding and detaching from the ion channel site (Lipton, 1993). The effectiveness of these drugs as NMDA antagonists is currently being correlated with their antiparkinsonian activity or lack of it.

A large number of drugs have been synthesised to act as competitive NMDA antagonists (Watkins et al., 1991). CPP (3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate) (Davies et al., 1986) and its unsaturated derivative D-CPPene are among the best known competitive NMDA antagonists. Structural modifications have produced CGS 19755 (Watkins et al., 1991) and the non-cyclic derivative CGP 37849 and the active isomer CGP 40116 (Schmutz et al., 1991) (R-DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoate). These agents are all centrally active when administered systemically but they do not show the apparent motor stimulation often seen after the administration of the channel blockers (Starr and Starr, 1993a, b, 1994a). AP-5 (2-amino-5-phosphonopentanoic acid) has poor brain penetrability but has been investigated in dopamine-depleted animals by intracerebral injection (Svensson and Carlsson, 1992).

Glutamate release blockers have also been investigated as potential antiparkinsonian agents, on the premise that if the antagonism of glutamate improves parkinsonism, so would a reduction in the release of endogenous glutamate, achieving the same goal of normalising the increased glutamatergic tone. Lamotrigine, a clinically used anticonvulsant, acts by suppressing the release of glutamate and aspartate by blocking voltage-gated Na⁺ channels (Fitton and Goa, 1995; Messenheimer, 1994; Meldrum and Leach, 1994). Lamotrigine does appear to possess antiparkinsonian activity but this is still under debate (see Löschmann et al., 1995; Zipp et al., 1993). Lamotrigine has less propensity to cause psychomotor impairment and even improved memory function in human volunteers, which is disimilar to the side-effects which occur after treatment with glutamate receptor antagonists (Fitton and Goa, 1995). However, add-on therapy with lamotrigine was associated with ataxia in epilepsy patients (Goa et al., 1993). Another glutamate release blocker, which has been investigated as a potential antiparkinsonian agent, is the adrenergic α₂ receptor agonist, clonidine.
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AMPA receptors are also thought to be involved in the pathophysiology of PD, especially as in most brain areas, they are co-expressed with NMDA receptors. One of the most effective AMPA antagonists is NBQX (2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(f)-quinoxaline-dione (Honoré et al., 1988), which is approximately 500 fold more selective for the AMPA receptor than for the NMDA receptor sites (Rogawski, 1993). NBQX, however, does not appear to be very potent in vivo and in primates, has a long latency of onset when systemically administered (Klockgether et al., 1991). Some of the AMPA antagonists also act at the glycine site, and thus the selectivity for AMPA receptors is increased when the glycine site is saturated (Starr, 1995a).

1.3 Animal models of PD

1.3.1 MPTP lesions

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been identified as a neurotoxin specific for the melanin-containing dopaminergic neurones of the SN of humans and non-human primates. The damage caused by MPTP, or more importantly the active 1-methyl-4-phenylpyridinium ion (MPP⁺) is considered to mimic human PD and MPTP can induce parkinsonism in humans. MPTP is usually administered into primates and the antiparkinsonian effects of various drugs can be studied on resulting repertoire of parkinsonian symptoms. MPTP reduces the levels of dopamine and its metabolites, DOPAC and HVA, markedly in the striatum (Bergman et al., 1990; Close et al., 1990; Crossman et al., 1989; Greenamyre, 1993; Mitchell et al., 1989; Kinemuchi et al., 1987; Klockgether et al., 1991; Löschmann et al., 1991; Zuddas et al., 1992). The mechanism of action of MPTP is only partly known. MPTP in astrocytes is transformed by MAO-B to the ion MPDP⁺ which then oxidises to the active ion MPP⁺. MPP⁺ is transported by a neuronal uptake carrier, to dopaminergic neurones (Javitch and Snyder, 1985; Javitch et al., 1985; Kinemuchi et al., 1987; Sonsalla et al., 1992). The striatal dopaminergic terminals are postulated to be the primary site of entry of MPP⁺ after systemic administration of MPTP in monkeys (Herkenham et al., 1991). Once in the terminals, MPP⁺ is transported to axons and cell bodies (Herkenham et al., 1991) and then into the
mitochondria where it accumulates and impairs respiratory complex I by inactivation of NADH dehydrogenase (Kinemuchi et al., 1987; Sonsalla et al., 1992). The resulting decrease in ATP production causes impaired functioning of Na⁺, K⁺-ATPases (Storey et al., 1992).

In mice, MPTP also causes dopaminergic lesions but much higher doses than those needed in primates are required to achieve this (Fuller, 1992; Heikkila and Sonsalla, 1992; Sonsalla et al., 1989, 1992). The peripheral administration of MPTP in rats has very little effect (Heikkila and Sonsalla, 1992; Kinemuchi et al., 1987), probably due to brain inaccessibility and different metabolic processes in the rat as compared to mice or primates (Kinemuchi et al., 1987). Intracerebral administration of MPP⁺, into the cell bodies of the dopaminergic neurones or the SNc or in the vicinity of dopaminergic nerve terminals or into the striatum causes extensive degeneration of dopaminergic neurones (Sayre et al., 1986; Turski et al., 1991). However, administration of MPP⁺ into the SNc or the striatum can also produce lesions in non-dopaminergic neurones, e.g. serotoninergic, GABAergic, substance P-containing neurones among others (Storey et al., 1992).

1.3.2 6-Hydroxy-dopamine (6-OHDA) lesions

This is a frequently used model and a selective lesion of dopaminergic neurones can be produced by the administration of 6-OHDA in the vicinity of these neurones. 6-OHDA enters dopaminergic neurones via a dopamine uptake carrier (if the noradrenaline uptake carrier is blocked by desmethylimipramine) when it is injected into the SNc or striatum (Zigmond et al., 1992). It then undergoes autooxidation which leads to the formation of toxic, free-oxygen radicals and quinones (Zigmond et al., 1992) which go on to produce degeneration of the dopaminergic neurones. The lesions manifest as akinesia and muscular rigidity (Duvoisin, 1976; Fuller, 1992). 6-OHDA is usually administered unilaterally and such a lesion results in the appearance of ipsilateral postural asymmetry which may constitute a model of unilateral PD (Duvoisin, 1976). Antiparkinsonian activity in this model is revealed by the lesioned animals exhibiting contralateral rotations (Ungerstedt,
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This model does not appear overtly parkinsonian but antiparkinsonian agents produce circling, thus providing an idea of the effectiveness of the drug being tested. This model can be used to investigate neuroprotective therapeutic intervention as well as determining the mechanisms involved in the pathophysiology and/or compensatory changes which occur after the onset of PD.

1.3.3 Amphetamines

Methamphetamine and amphetamine administered peripherally in very high doses produce lesions of the dopaminergic system in rats and mice (Fuller, 1992; Sonsalla et al., 1989, 1991, 1992). The primary site of action of these compounds are the striatal dopaminergic terminals (Fuller, 1992; Sonsalla et al., 1989, 1991, 1992). Animals exposed to the neurotoxic effects of methamphetamine or amphetamine show a reduction in tyrosine hydroxylase activity, in the level of dopamine and its metabolites, in the density of binding sites of the dopamine uptake carrier and in the number of dopaminergic terminals and axons in the striatum (Fuller, 1992; Sonsalla et al., 1989, 1991, 1992).

The process leading up to cell death caused by the amphetamines is unclear. It is assumed to involve the excessive carrier-mediated release of dopamine from presynaptic terminals, which is produced by these drugs (Fuller, 1992; Sonsalla et al., 1989; 1991: 1992). The toxic effect of methamphetamine appears to depend on dopamine synthesis, as it can be inhibited by the dopamine synthesis inhibitor, α-methylparatyrosine (α-MPT), and it can also be reduced by the blockade of dopamine receptors (see Ossowska, 1994). The excessive amounts of released dopamine might be subjected to toxic free radicals which then cause oxidative stress (Sonsalla et al., 1989, 1992).

1.3.4 Reserpine-induced akinesia

The model is fairly easy to set up and thus, is commonly used. Agents, such as reserpine are administered to temporarily impair the dopaminergic function. Reserpine causes the depletion of monoamines, including 5-HT, noradrenaline and dopamine in the striatum, olfactory tubercle and cerebral cortex. The advantages of using this model are
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that an approximate 95% depletion of dopamine can be achieved (Anden and Johnels, 1978; Bertler, 1961; Starr et al., 1987). Reserpine does not affect the synthesis and degradation of monoamines, it only disrupts storage (Curzon, 1990; LaHoste and Marshall, 1994; Starr et al., 1987). α-MPT can be co-administered with reserpine (Carlsson and Carlsson, 1989a, b; Kannari and Markstein, 1991; Klockgether and Turski, 1990; Klockgether et al., 1991; Maj et al., 1993b). Reserpine administered in rodents produces akinesia and rigidity within 3 hours of treatment but a near complete depletion of dopamine is seen approximately 20-24 h after treatment (Carlsson and Carlsson, 1989a, b; Kannari and Markstein, 1991; Klockgether and Turski, 1990; Klockgether et al., 1991; Maj et al., 1993b; Starr and Starr, 1993 a, b, 1994 a, b).

The criticisms of this model are that it produces wholesale depletions of all monoamines, not just dopamine; it does not involve the destruction of neurones; it is not specific to dopamine, and that; it is reversible, unlike the condition itself. Also, reserpine-treated mice tend to become hypothermic and must be kept in a temperature-controlled environment. This non-specificity of reserpine may indeed be advantageous as in PD, other neurotransmitter systems are also involved in the pathology of PD and the levels of neurotransmitters like noradrenaline, GABA and 5-HT are reduced in parkinsonian conditions. This model, also presents with two of the characteristic symptoms of PD, i.e muscular rigidity and akinesia (Carlsson and Carlsson, 1989b; Kannari and Markstein, 1991; Klockgether and Turski, 1990; Klockgether et al., 1991), and thus, the effects of agents against these two symptoms can be ascertained.

1.3.5 Neuroleptic-induced catalepsy

Striatal dopamine receptors are blocked by administering neuroleptics, such as haloperidol and fluphenazine. These drugs, usually belonging to the 'classical' family of neuroleptics, block dopamine D₂ receptors predominantly but they have some activity at D₁ receptors as well (Seeman and Grigoriadis, 1987). Bradykinesia, rigidity and tremor are characteristic of neuroleptic-induced parkinsonism. These symptoms are regarded as the animal equivalent of the human drug-induced syndrome (Ossowska et al., 1990;
Systemic or intracerebral administration of selective D₂ antagonists, such as raclopride, or of the selective D₁ antagonist, SCH 23390, also result in a cataleptic syndrome (Morelli and Di Chiara, 1985; Papa et al., 1993). However, the D₂ antagonist, sulpiride when systemically administered does not induce catalepsy in rats or parkinsonism in humans (Jenner and Marsden, 1984; Honda et al., 1977; Wambebe, 1987) but intrastriatal injection of this drug induces catalepsy in rats (Ossowska et al., 1990).

The antipsychotic action of neuroleptics may be due to their effect on mesolimbic and/or mesocortical dopaminergic neurones while the extrapyramidal motor effects may be due to an action on nigrostriatal dopaminergic neurones (Snyder et al., 1974).

1.4 Glutamate-dopamine interactions in the basal ganglia

The basal ganglia are made up to the caudate nucleus, the putamen (or the combined caudate and putamen, also called the corpus striatum, in the rat), the substantia nigra (SN), the medial or internal and lateral or external globus pallidus (GPi and GPe; the entopeduncular nucleus (EPN) is the rat equivalent of the GPi) and the subthalamic nucleus (STN). Other regions closely associated with the basal ganglia are the thalamus and the cortex. These regions are thought to be responsible for the initiation and coordination of motor behaviour, maintenance of posture, muscle tone, regulation of voluntary smooth muscles and cognitive functions (Beizer, 1995).

The striatum is the main input site of the basal ganglia and receives excitatory inputs from all regions of the sensory and motor cortices (Albin et al. 1989; Starr, 1995a). Within the striatum, there is a heterogeneous innervation with non-limbic cortical regions providing the major source of innervation in the rostral striatum while the amygdaloid nucleus sends projections to the caudal striatum (Albin et al., 1989). The neurotransmitter used by these projections is glutamate, which acts mainly at AMPA and NMDA receptors. These receptors are heterogeneously localised and function interdependently (Albin et al., 1992; Tallaksen-Greene et al., 1992). The other major excitatory neurotransmitter in the striatum is acetylcholine (ACh), which is mainly found
Figure 1.2 The pathways of the basal ganglia: both the indirect and direct pathways. The alterations in these pathways in parkinsonism are illustrated. In the normal basal ganglia, dopaminergic stimulation induces movement via the activation of the striatonigral pathway and inhibition of the striatopallidal pathway. However, in parkinsonism, as this dopaminergic influence is lost, glutamate neurones become hyperactive, leading to akinesia. Glutamate antagonists, possibly by acting at the corticostriatal or subthalamic pathways, may normalise the activity of these neurones.

GPe, external or lateral segment of the globus pallidus; STN, subthalamic nucleus; SN, substantia nigra; EPN, entopeduncular nucleus; GPi, internal or medial segment of the globus pallidus. The size of the arrows are indicative of activity in the neurones.

Reproduced from Starr, 1995a.
in interneurones (DiFiglia, 1987).

The striatum consists of two discrete populations of medium spiny neurones, the axons of which form the two parallel but oppositional output pathways, although other pathways to a myriad of other brain regions are not taken into account for the purposes of simplicity. Studies using dopamine, $D_1$ and $D_2$, receptor agonists and NMDA receptor antagonists have shown different responses depending on the dopamine receptor acted upon which also provide evidence for the existence of two different pathways, i.e. the two neurone projection or the direct pathway and three neurone projection or the indirect pathway. The direct pathway is thought to be under excitatory nigrostriatal dopaminergic influence and goes from the striatum to the thalamus via the GPi (EPN in rodents) and the substantia nigra pars reticulata (SNr), and includes two GABA neurones. The indirect pathway on the other hand, is under the inhibitory influence of striatal dopamine and again goes from the striatum to the thalamus but involves three GABA neurones (Mink and Thach, 1991) and additional projections to the GPe and the subthalamic nucleus (Albin et al., 1989; Bergman et al., 1990; Gerfen, 1992). Lesions of the striatonigral pathway were found to selectively reduce NMDA binding which suggests that NMDA binding sites may be concentrated in striatonigral as opposed to striatopallidal neurones (Tallaksen-Greene et al., 1992). The striato-GPe pathway preferentially expresses mainly dopamine $D_2$ receptors while $D_1$ receptors are mainly found on striatonigral neurones (Gerfen et al., 1990), but this by no means is absolute as some overlap has been noticed. The striatopallidal and striatonigral pathways are both GABAergic (Penney and Young, 1986) and they co-express different peptides. While the striatonigral pathway co-expresses substance P and dynorphin, the striatopallidal pathway expresses enkephalin (Gerfen and Young, 1988; Graybiel, 1990). These output neurones are normally silent but can be stimulated by dopamine or ACh (Chevalier et al., 1985). From the scheme shown in Figure 1.2, if glutamate or ACh stimulates the direct pathway, the inhibitory tone in the nigrothalamatic neurones is reduced and the feedback loop to the motor cortex is stimulated and thus motility is allowed. However, if these transmitters act at the indirect pathway, the end-result is akinesia due to a further suppression of the nigrothalamatic neurones.
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Dopamine, released from the nigrostriatal neurones (Moore et al., 1971) holds the activity of the cholinergic and glutamatergic systems in check. As the nigrostriatal neurones synapse on the shafts of the striatal output neurones and corticostriatal neurones terminate peripherally on the heads of these spines (Freund, et al., 1984), dopamine is thought to act postsynaptically, exerting differential action at the D₁ and D₂ receptors. Stimulating D₁ receptors is thought to increase the activity of the striatonigral neurones as shown in a number of paradigms (Berretta et al., 1992; Trugman and Wooten, 1986) while the activation of D₂ receptors reduced the activity of the striatopallidal neurones (Carlsson et al., 1990), leading to the conclusion that D₁ receptors mediate activation of the motor-excitatory direct pathway and D₂ receptors suppress the motor-inhibitory indirect pathway. The best effect is seen when D₁ and D₂ receptors are stimulated together (Braun and Chase, 1986), but the mechanism behind this is unclear, indicating either that the two receptor types are co-localised or that there is an intercellular interaction between the two pathways (Starr, 1995a). There is a reciprocal association between dopamine and glutamate in the striatum.

The GABA neurones forming part of the positive loop, the direct pathway, appear to be phasically stimulated while the GABA neurones involved in the inhibitory indirect pathway appear to be tonically active. This would explain why sometimes, both glutamate agonists and antagonists, result in behavioural stimulation. A glutamate antagonist would cause stimulation by decreasing the inhibitory influence by the indirect pathway on the thalamus while a glutamate agonist would increase the stimulatory influence of the direct pathway on the thalamus (Carlsson, 1993). Another reason for the paradoxical effects seen, when both NMDA and its antagonists produce behavioural arousal and dopamine release (Clow and Jhamandas, 1989; Kashihara et al., 1990; Rao et al., 1990), is that the striatum, as mentioned previously, is a heterogeneous structure and depending on the region injected into, different results are obtained.

ACh levels are also modulated by dopamine via D₂ receptors (Stoof et al., 1992), which explains the cholinergic hyperactivity seen after dopamine depletion and the use of anticholinergics in the therapy of PD.
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The striatum is thought to be a very important site when it comes to glutamate and dopamine interactions, but nuclei downstream of the striatum, such as the STN, GP and thalamus, may also be important in this context. The STN, which is thought to send glutamatergic neurones to the SN and EPN (Hammond et al., 1978; Robledo and Feger, 1990) and receives projections from the cortex (Afsharpour, 1985), thalamus and brainstem is an important relay station for impulses from the striatum and is the final link in the striatopallidal output pathway. The importance of the STN in motor activity is further enforced by the findings that lesioning of this nucleus causes a condition called as hemiballismus, characterised by wild and involuntary ballistic movements in primates and man (Crossman et al., 1981; Hammond et al., 1978). The STN also contains some dopamine (Versteeg et al., 1976) and binding data show the presence low levels of D₁ receptors in the STN (Wamsley et al., 1989). The dopamine neurones of the STN appear to be just as sensitive to exogenous dopamine as the dopaminergic neurones of the striatum (Beauregard et al., 1991).

Dopamine is also a neurotransmitter in the GP (Lindvall and Björklund, 1979) and is released in normal conditions from dopaminergic dendrites in the SN (Geffen et al., 1976). In the SNr, dopamine may enhance the activity of the direct pathway by increasing GABA release from striatonigral neurones via actions at D₁ receptors (Starr et al., 1987).

1.5 Glutamate-dopamine interactions in the context of PD

In PD, due to the loss of nigrostriatal dopamine neurones, there is a compensatory up-regulation of dopamine receptors and/or their transduction mechanisms, an increase in the activity of the remaining neurones, which is why parkinsonian symptoms are only evident after an approximate 80% loss of dopamine.

The loss of dopamine causes an imbalance in the activities of the striatonigral and striatopallidal pathways. As dopamine tends to modulate the activity of glutamate, dopamine depletion causes a secondary hyperstimulation of the corticostriatal and also possibly the subthalamic glutamatergic pathways (Albin et al., 1989; Greenamyre, 1993; Riederer et al., 1992). Binding studies have shown that the density of NMDA receptors
is 22-46% higher in the striatum and nucleus accumbens septi of parkinsonian patients than in normal people (Weihmuller et al., 1992). However, overactivity of the glutamatergic pathways would be expected to lead to a down-regulation of its receptors. Carlsson (1993) used this finding to postulate a decreased activity of the corticostriatal pathway. The effects of dopamine depletion and the resultant increased glutamatergic tone, on glutamate receptor number are inconclusive either way.

An increased activity is also seen in the cholinergic interneurones, due to loss of inhibition by D$_2$ receptors (Stoof et al., 1992). This then leads to an increase in the activity of the indirect pathway, which then leads to muscular rigidity and akinesia. Tremor, another characteristic PD symptom, is thought to result from the interruption of the thalamocortical pathways (Filion et al., 1988), but it may also be due to the alterations in the activity of basal ganglia pathways (Bergman et al., 1990).

Increased activity in the STN, due to reduced activity of the GP, and increased activity in the outputs to the SN and EPN is also seen in PD. Surgical interventions, such as lesions and infarctions, in the STN have been shown to alleviate parkinsonism (Sellai et al., 1992). Pallidotomies have been used previously to improve parkinsonian symptoms in man. It is therefore, feasible that pharmacological interventions in these regions may be as successful in alleviating parkinsonism.

1. 6 Current therapy of PD

1.6.1 L-Dopa

L-Dopa was first licensed for use in 1970 and it was and has been extremely useful in improving the quality of life of PD patients. L-Dopa, is the drug of choice in the treatment of PD, as it crosses the blood-brain barrier, unlike dopamine, and is converted to dopamine by aromatic-L-amino acid decarboxylase (AADC), thus effectively replacing the lost dopamine. As this conversion can also occur peripherally causing nausea and orthostatic hypotension, a peripheral AADC inhibitor, such as benserazide, is usually co-administered with L-Dopa. Both L-Dopa and benserazide can be combined into one formulation called Madopar. The response to L-Dopa is even used to confirm the
diagnosis of PD.

L-Dopa has a very strong antiparkinsonian activity initially but unfortunately more than 50% of patients will develop tolerance or response fluctuations ('on/off' episodes) or dyskinesias and choreas in the first five years of L-Dopa treatment (see Hughes, 1997). The tolerance may occur because there are less dopaminergic terminals available to convert L-dopa to dopamine. In the 'on/off' phenomenon, the patients experience wild fluctuations in function, ranging from freezing into position ('off') to abnormal involuntary movements or dyskinesias ('on'). The exact cause of the on-off phenomenon is unknown, but one theory is that sensitivity to small changes in plasma L-Dopa concentrations is enhanced (Juncos, 1992). To control these fluctuations, smaller and more frequent doses of L-Dopa have been administered or a dopamine agonist or the monoamine oxidase (MAO) inhibitor, selegiline, is co-administered with a reduced dose of L-Dopa. Sustained release preparations of L-Dopa and a peripheral AADC inhibitor, namely carbidopa/L-Dopa, have recently come onto the market but the bioavailability is less than that of the immediate-release version. A combination of immediate-release and sustained release L-Dopa is beneficial in some patients (Beizer, 1995).

In addition to adverse side-effects induced by L-DOPA, carbidopa/L-Dopa treatment induces nausea, orthostatic hypotension, arrhythmias and CNS effects, such as hallucinations and confusion.

L-Dopa may also inevitably cause further degeneration of dopaminergic pathways by the production of free radicals during metabolism (Spencer et al., 1994). L-Dopa has recently been implicated in apoptotic cell death in cultured postmitotic chick sympathetic neurones and the authors suggest that L-Dopa may be cause further nigrostriatal degeneration in Parkinson's disease (Spencer et al., 1994).

1.6.2 Direct-acting dopamine agonists

The two main types of dopamine agonists used in the treatment of PD are the predominantly D₂ agonists, bromocriptine and lisuride and a mixed D₁/D₂ agonist, pergolide. Pergolide is the more potent drug, with a longer duration of action. As AADC
activity decreases as the disease progresses, direct dopamine agonists are needed to stimulate the dopaminergic system. These drugs work by stimulating supersensitive dopamine receptors in the CS and also stimulate dopamine receptors in other brain areas, which causes side-effects (Beizer, 1995).

Dopamine agonists have been added to L-Dopa therapy when the response to L-Dopa itself was inadequate. Monotherapy with dopamine agonists early on is hampered by their side-effects, especially gastrointestinal intolerance and CNS effects, confusion and hallucinations, and orthostatic hypotension (Beizer, 1995). Lisuride treatment is restricted due to the appearance of dyskinesias and psychosis. The more recent dopamine agonists, pramipexole, ropinirole and carbegoline appear to have a more favourable profile and are effective as monotherapy in early PD and as adjuncts to L-Dopa in the later stages of the disease.

1.6.3 Selegiline

Selegiline is a MAO-B inhibitor, and was used initially to inhibit the metabolism of dopamine, thus prolonging its effect. It is antiparkinsonian when administered with L-Dopa, as it increases the duration of action of each dose while allowing for a reduction in the dose of L-Dopa. This drug has been used in Europe to reduce the motor fluctuations seen in the latter stages of the disease. Selegiline, due to its mild antiparkinsonian effect, slowed the progression of disabilities and delays the need for L-Dopa. However, a recent study reported that combined selegiline and L-Dopa treatment might be associated with an increase in morbidity after 5 years but, at present this report is unsubstantiated and remains controversial (see Hughes, 1997).

Side-effects associated with selegiline, include CNS effects, insomnia, nausea and orthostatic hypotension, but these are usually mild and their incidence is rare.

1.6.4 Anticholinergics

These agents were the only treatment of PD before L-Dopa came into the picture and even now, these agents are the second-line agents in PD therapy. They may be
administered as adjuncts to L-Dopa in patients who present with pronounced tremor and are useful in the treatment of drug-induced PD. These agents have no real effect on bradykinesia and rigidity.

Anticholinergics are poorly tolerated and psychosis, tachycardia, eye pain or urinary retention can result from treatment. Also, they should be used with caution in elderly patients. Examples of anticholinergics used in the therapy of PD are benztropine, diphenhydramine and trihexyphenidyl and more recently budipine and biperiden.

1.6.5 Amantadine

Amantadine was intended as an antiviral agent but was discovered to help PD sufferers with their symptoms. Amantadine blocks the reuptake of dopamine into presynaptic neurones and may enhance dopamine release (Jackisch et al., 1992) thereby producing an antiparkinsonian effect. Amantadine is used in the treatment of mild and drug-induced PD.

Most patients develop tolerance to the effects of amantadine within a few months of beginning treatment. The side-effects induced by amantadine, are not usually severe and only occur in approximately 10% of patients, include the symptoms often associated with anticholinergics, i.e constipation, dry mouth and urinary retention, and CNS effects, including confusion, dizziness, hallucinations, insomnia and psychosis (Beizer, 1995).

Recently amantadine, and the structurally related memantine, which is used as an antiparkinsonian in Germany, have been shown to block the NMDA ion-channel site, thus it is possible that the antiparkinsonian effect of amantadine derives from its NMDA receptor antagonism (Danysz et al., 1994b).

1.7 Novel therapies of PD

The current dopamine-based therapy of Parkinson's disease leaves a lot to be desired. L-Dopa, the main drug used in Parkinson's disease treatment has, as discussed above, undesirable side-effects such as tachykinesia and patients on prolonged L-Dopa sometimes have 'on-off' episodes, when the response to L-Dopa varies from no effect to hyperkinesia
and dyskinesia (Riley and Lang, 1993). Also, as the disease progresses, further degeneration of nigral dopamine neurones occurs and the number of dopamine receptors at which dopamine or its agonists can act decreases. Thus, new strategies are being investigated as means of controlling the symptoms. Among the avenues being explored are the use of antioxidants (Fahn, 1997), as protection against oxidative stress, unselective MAO inhibitors (Laux et al., 1995), nitric oxide (NO) synthesis inhibitors, again to prevent oxidative stress, or using trophic factors, such as BDNF and GDNF, to protect the nigro-striatal dopaminergic neurones, catechol-O-methyltransferase (COMT) inhibitors (Fahn, 1997) and glutamate antagonists, to normalise the increased glutamatergic tone that follows dopamine depletion and glutamate acts like an excitotoxin (Greenamyre and O'Brien, 1991).

Non-pharmacological interventions being investigated include the surgical procedures; ventrolateral thalamotomy, thalamic stimulation and posteroverentral pallidotomy, as possible means of relieving parkinsonian symptoms and the transplantation of foetal mesencephalic tissue into the brains of PD patients (Beizer, 1995).

1.8 The antiparkinsonian effects of glutamate antagonists

1.8.1 As monotherapy

Carlsson and Carlsson (1989b) provided the first evidence of a locomotor-stimulant effect of a glutamate antagonist, MK 801, in monoamine-depleted and α-MPT treated mice. However, the actual observed locomotion was weak in nature and not fluent when compared to the activity of normal mice, and was also accompanied by ataxia, postural abnormalities, convulsions and death.

Subsequent studies on the antiparkinsonian effects of glutamate antagonists, both competitive and non-competitive antagonists, have provided conflicting results, with the majority of groups, finding that the glutamate antagonists are ineffective or even detrimental in PD models (Crossman et al., 1989; Domingo and Sheng, 1993; Goodwin et al., 1992; Luquin et al., 1993). Treatment with these drugs is often followed by motor
deficits, ranging from a collapsed posture to severe ataxia (Carlsson and Svensson, 1990a, b; Kannari and Markstein, 1991; Klockgether and Turski, 1990; Klockgether et al., 1990; Morelli et al., 1992, Starr and Starr, 1993a, b, 1994a, b). Only, one study reported that intramuscular NBQX produced near normal activity in MPTP-treated marmosets in all parameters looked at (Klockgether et al., 1991). Carlsson's group in Sweden and Turski's group in Germany, publish reports consistently on the locomotor-stimulant properties of glutamate antagonists. Therefore, the disparity may be due to different strains of animals used or the different methodologies used.

More promising results have been seen after stereotaxic administration of these antagonists direct into regions of the basal ganglia. Thus, it is conceivable that when these drugs are injected systemically, they may act at regions outside the basal ganglia, possibly the cortex, thus inducing adverse effects and also negating any beneficial effects. Stereotaxic injections of NBQX into the STN, SNr and EPN evoked a locomotor response but injections into the striatum were ineffective (Klockgether et al., 1991). Similar data has been obtained with CPP (Klockgether and Turski, 1990). AP-5, injected into the NAc, but not the striatum, was able to produce motility (Svensson et al., 1992a; Svensson and Carlsson, 1992). Kynurenic acid, a broad-spectrum glutamate antagonist, was shown to produce dramatic improvements in MPTP-treated marmosets, when injected into the GPi. No adverse effects were seen in these animals (Brotchie et al., 1991). Brotchie's group also found that intrastriatal injections of kynurenic acid or HA-966 in models of PD were able to induce locomotion (Brotchie et al., 1991; Carroll et al., 1995). The STN is also important in the pathophysiology of PD, especially in akinesia. However, the corticostratial glutamatergic pathway is also thought to be instrumental in inducing parkinsonism but the data mentioned above, bar the studies done by Brotchie's group, do not confirm this. The theory put forward to explain this, is that the cholinergic system alone is sufficient to increase the tone in striatopallidal neurones, independently of glutamate (Starr, 1995a). The co-administration of antimuscarinics and glutamate antagonists have proved to be a very effective combination in alleviating parkinsonism (see Starr, 1995a).
1.8.2 As adjuncts to dopamine agonists or L-Dopa

As it appears unlikely that glutamate antagonists will be considered as safe monotherapy, unless more region-specific antagonists are introduced, in the control of PD, the combination of these drugs and the mainstay treatment of PD has been investigated. Generally, these agents have been found to be able to synergise with D₁ agonists and L-Dopa. L-Dopa, itself produces contralateral circling in 6-OHDA hemilesioned rats (Morelli and Di Chiara, 1990). This response was enhanced by low, behaviourally ineffective doses of MK 801. However, MK 801 and NBQX, were also shown to attenuate the effects of L-Dopa on cerebral glucose utilisation but did not affect the motor response (Engber et al., 1994). A number of groups have determined a positive interaction between glutamate antagonists and L-Dopa. Also a dose of MK 801, more than 200 times less than that required to produce a behavioural effect has been reported to synergise with a threshold dose of L-Dopa (Klockgether and Turski, 1990). CPP, CGP 37849 (Klockgether and Turski, 1990; Maj et al., 1993b) and NBQX (Klockgether et al., 1991) also produced a similar synergism. In primates, the response to L-Dopa was greatly enhanced by the administration of NBQX (Klockgether et al., 1991). Also, at this dose of NBQX, no motor deficits were seen. CGP 40116 (Wüllner et al., 1992), CPP and NBQX (Löschmann et al., 1991) have induced a similar effect in MPTP-treated marmosets. The animals treated with CPP and NBQX did not show dyskinesia but it was apparent with CGP 40116.

The synergisms detailed here, if they could be replicated in PD patients, would greatly benefit their therapy, as a lower dose of L-Dopa could be administered which would prolong its therapeutic lifetime and may also slow the degenerative process (Olanow, 1993; Riederer et al., 1993).

The mechanism behind the synergism between some of the glutamate antagonists and L-Dopa is unclear but is postulated to involve D₁ receptors (Goodwin et al., 1992; Starr and Starr, 1993 a, b, 1994a, b), especially as SCH 23390, a D₁ antagonist, can attenuate it (Hyttel, 1983). D₁ agonists, such as SKF 38393 and CY 208-243 are able to potently reverse the akinesia of parkinsonism (Temlett et al., 1989). The antiakinetic
effects of these $D_1$ agonists are further enhanced by administration of the glutamate antagonists, MK 801, PCP and CPP systemically (Morelli and Di Chiara, 1990; Morelli et al., 1992) or AP-5 into the NAc (Svensson et al., 1992a). The side-effect profile is similar to seen when glutamate antagonists are administered alone, with low doses potentiating the locomotor effect of SKF 38393 while higher doses induce ataxia and postural abnormalities (Starr and Starr, 1994a), with the additional risk of seizures. Also, the movements seen after combined treatment do not resemble those of a normal animal, becoming stereotyped. In primates, CY 208-243, while being a potent antiparkinsonian agent, did not interact with NBQX (Luquin et al., 1993). Threshold doses of the $D_1$ agonists were potentiated by MK 801 and CPP but not CGP 40116 (Starr and Starr, 1993a; Svensson et al., 1992b) but MK 801 had no effect in the 6-OHDA lesioned rat (Boldry et al., 1993). As the data is contradictory, the mechanism of action underlying the positive interactions between glutamate antagonists and L-Dopa has still not been conclusively shown.

Possible interactions of the glutamate antagonists with $D_2$ agonists have also been explored. However, the data obtained show that glutamate antagonists may actually attenuate the response to $D_2$ agonists, as MK 801 was found to reduce the circling response of LY 171555 in 6-OHDA lesioned rats (Boldry et al., 1993; Morelli et al., 1992). The response to systemic LY 171555 was reduced by intraacumbens AP-5 (Svensson et al., 1992). Similar effects have been seen with MK 801, HA 966, CGP 40116 and amantadine (Goodwin et al., 1992; Starr and Starr, 1993a, b, 1994a, b). Again, these glutamatergic antagonists combined with $D_2$ agonists, exert a behavioural profile similar to that seen with the glutamate antagonists themselves. It appears that while $D_1$ responses are potentiated by some of the glutamate antagonists, the responses to $D_2$ agonists may be attenuated, unaltered or enhanced. It appears, as well that the effects seen may be due to the combination of drugs used, e.g. CPP and lisuride increase locomotion while CGP 40116 and LY 171555 decrease it. Thus, glutamate antagonists which can increase the activity of $D_2$ agonists may be of greater use for use as adjunctive therapy to L-Dopa.
Chapter One General Introduction

The mechanism of action of glutamate antagonists is unclear but it is known that stimulation of dopamine receptors results in increased cAMP levels (Kebabian and Calne, 1979), which in turn activates cAMP-dependent protein kinase, which then acts on DARPP-32 (Beretta et al., 1992), which once phosphorylated inhibits protein phosphatase-1. The function of phospho-DARPP-32 may be to prevent dephosphorylation of other substrates in nigrostriatal neurones (Hemmings and Greengard, 1986). The activation of NMDA receptors results in dephosphorylation of DARPP-32 and thus, antagonism of the effects of dopamine. NMDA receptor antagonists prevent the dephosphorylation of DARPP-32 induced by glutamate and hence, potentiate the effect of dopamine (Halpain et al., 1990; Girault et al., 1990).

1.9 Aims of this research

The finding that the depletion of dopamine causes a secondary hyperactivity in the glutamatergic pathways of the basal ganglia, which in turn leads to the symptoms, has led to the suggestion that agents, which can normalise this increased tone, may be beneficial in the treatment of PD (Albin et al., 1989; Greenamyre and O'Brien, 1991; Riederer et al., 1992).

This hypothesis has been tested in several rodent and primate models of PD, with some success. Also, the fact that some of the drugs currently used in the treatment of PD have been found to have the ability to block glutamate, goes to show that glutamate is important in the pathophysiology of PD.

In the current work, we set out to investigate the locomotor effects of various classes of glutamate antagonists initially in drug-naive mice and then to go on to determining their locomotor effects in monoamine-depleted mice, either administered alone or in combination with various dopamine agonists. Data is already available in these paradigms for some of the glutamate antagonists, but we wanted to investigate a comprehensive list of the glutamate antagonists, acting at different sites on the NMDA receptor or at AMPA receptors, and wanted to determine their beneficial vs detrimental effects. The primary site of action of the glutamate antagonists is thought to be the...
striatum but other studies have shown that these agents can produce antiparkinsonian effects from outside the striatum (Klockgether and Turski, 1990). We studied the effects, both beneficial and adverse, of a comprehensive selection of the antagonists administered focally into the striatum or the SNr of monoamine-depleted rats.

As different glutamate antagonists are reported to respond differently in different models of PD, we chose a potent glutamate antagonist, MK 801 at doses which we know from literature to produce a marked antiparkinsonian effect, to confirm its antiparkinsonian effect in cataleptic rat model of PD. Initial experiments determined the effects of systemic MK 801 and in latter experiments we administered MK 801 into various brain areas and tried to locate its site of action. We have tried to present a comprehensive analysis of the different glutamate antagonists and have tried to determine their potential ability as antiparkinsonian agents, on the basis of results obtained in this results. We have also tried to further the understanding on the mechanism of action and/or site of action of these glutamate antagonists, or at least their mechanism and/or site of action in the models employed here.
CHAPTER TWO
MATERIALS AND METHODS
Chapter Two Materials and methods

2.1 Locomotor activity in the Mouse

2.1.1 Animals

Male albino mice (TO strain, A.R. Tuck Ltd.), weighing 25-40 g, were housed in groups of 25 at 22 ± 1° C under fluorescent lighting (light period 07.00-17.00 h) and allowed free access to food and water. Experiments were carried out between 10.00 and 17.00 h and each drug-naive animal was used at most twice while each monoamine-depleted animal was used only once. All procedures were carried out under the directives of the Animals (Scientific Procedures) Act, 1986.

2.1.2 Drug-naive mice

Vehicle or drug was administered systemically and the mice were then immediately, with the exception of lamotrigine which was administered as a 30 min pre-treatment, placed individually in Perspex observation cages (29 x 26 x 21 cm high) and spontaneous locomotor activity monitored using Radiospares 8960 Microwave Doppler Module units connected to an amplifier, timer and LED display unit (shown in Fig. 2.1). The units were constructed in our laboratory to our own design and were calibrated to detect gross motor movements only. Locomotor activity counts were recorded at 10 min intervals for up to 2 h. The presence of the following behaviours was manually noted using a checklist but not quantified.

The behaviours noted were:

- Forward locomotion
  - Locomotion around the periphery of the cage
- Sniffing
- Rearing
- Grooming
- Vacuous chewing
- Purposeful chewing
- Licking
- Ataxia
Figure 2.1. Apparatus for measuring mouse locomotor activity.
Stereotyped activity
Seizure-like activity i.e. forepaw myoclonus and/or running fits

2.1.3 Induction of akinesia and administration of glutamate antagonists

Mice were rendered akinetic by injecting reserpine (5 mg/kg) intraperitoneally. The mice were kept warm overnight, at 23 ± 1° C, to counteract reserpine-induced hypothermia. Vehicle or the test drug/s was/were administered systemically 24 h later and the mice were then immediately, with the exception of lamotrigine which was administered as a 30 min pre-treatment, placed individually in the Perspex observation cages and the locomotor activity and other behaviours were assessed as before.

2.1.4 Interaction studies with a dopamine D₁ receptor agonist, SKF 38393

Intraperitoneal injections of SKF 38393 (30 mg/kg), alone or in combination with a number of glutamate antagonists were administered, and the mice placed immediately, with the exception of eliprodil and lamotrigine which were administered with SKF 38393 as 30 min pre-treatments, into observation cages. Locomotor activity was then monitored at 10 min intervals for up to 2 h. Other behaviours were manually observed using a checklist as before.

2.1.5 Interaction studies with a dopamine D₂ receptor agonist, RU 24213

Subcutaneous injections of RU 24213 (5 mg/kg), alone or in combination with various glutamate antagonists, were administered and the mice placed immediately, with the exception of eliprodil and lamotrigine which were administered with RU 24213 as 30 min pre-treatments, into observation cages. Locomotor activity was then monitored at 10 min intervals for up to 1 h. Other behaviours were manually observed using a checklist as before.
2.1.6 Interaction studies with the dopamine precursor, L-Dopa

Intraperitoneal injections of benserazide (100 mg/kg injected 30 min prior to L-Dopa) and L-Dopa (150 mg/kg) or benserazide (100 mg/kg), L-Dopa (150 mg/kg) and various glutamate antagonists were administered and the mice placed immediately, with the exception of lamotrigine, clonidine (+) HA-966 and the competitive NMDA receptor antagonists, CPP and CGP 40116, which were administered with L-Dopa as 30 min pre-treatments, into observation cages. Locomotor activity was then monitored at 10 min intervals for up to 2 h. Other behaviours were manually observed using a checklist as before.

2.1.7 Statistical analysis

Locomotor activity was expressed as cumulative 30-120 min counts. Drug and control treatments were compared by a one-way or two-way analysis of variance (ANOVA). Post hoc analysis of each dose was performed using a Dunnett's t-test or an unpaired t-test comparing test values to control values. In instances where the standard deviations of a group were significantly different, non-parametric tests were used; a Kruskal-Wallis test followed by a Dunn's Multiple Comparisons test or the Mann-Whitney test. Significance was taken as p < 0.05.

2.1.8 Drugs

Reserpine, L-3,4-dihydroxyphenyalanine (L-Dopa), benserazide, phencyclidine (PCP), ketamine, 1-aminoadamantane sulpha (amantadine) (Sigma), 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride (SKF 38393), (+)-5-methyl-10,11-dihydro-[5H]-dibenzo[a,d] cyclo-hepten-5,10-imine (dizocilpine maleate or MK 801), dextromethorphan, (+)-3-amino-1-hydroxypyrrolidin-2-one ((+) HA-966) (Research Biochemicals Inc., Natick, MA, USA), N-n-propyl-N-phenyl-ethyl-p-(3-hydroxyphenyl)ethylamine (RU 24213) (Roussel), lamotrigine (Glaxo-Wellcome), 1-amino-3,5-dimethyladamantane HCl (memantine) (Merz), eliprodil (SL82.0715) (Synthelabo Recherche), R-DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoate (CGP
40116) (Ciba-Geigy), 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) (Tocris Neuramin), 2,3-dihydroxy-6-nitro-7-sulphamoyl-benzo(f)-quinoxaline-dione (NBQX) (Novo Nordisk) and 1-t-butyl-4,4-diphenylpiperidine (budipine) (Byk-Gulden, Konstanz, Germany) were all administered in dimineralised water. The solution of reserpine was aided with a minimum quantity of glacial acetic acid (British Drug Houses), that of lamotrigine and eliprodil with dimethylsulphoxide (DMSO, Sigma) and of NBQX with a minimum amount of sodium hydroxide (NaOH; British Drug Houses) and made up to the desired volume with dimineralised water. L-Dopa was made up as a 50 mg/ml solution and three times the volume was injected to attain a dose of 150 mg/kg. All drugs were administered in a volume of 5 ml/kg. All drugs, with the exception of RU 24213 which was administered subcutaneously, were injected intraperitoneally.

2.2 Locomotor activity in the rat

2.2.1 Animals

Male albino Wistar rats (A.R.Tuck Ltd.), weighing 180-350 g, were housed in groups of 6 and kept at 22 ± 1°C, under fluorescent lighting (light period 07.00-17.00 h) and allowed free access to rat chow and water. The experiments were carried out between 10.00 and 17.00h and each animal was used at most twice. All procedures were carried out in accordance with the Animals (Scientific Procedures) U.K. Act, 1986.

2.2.2 Stereotaxic surgery and cannulae implantation

The rats were anaesthetised with halothane (2.5% v/v in O₂ for induction) and anaesthesia was maintained throughout surgery (1.5% v/v in O₂ for maintenance). The anaesthetised animals were then secured in a Kopf stereotaxic frame. An incision was made, bregma was found and used as a reference point to determine the location of the corpus striatum (CS) and the substantia nigra pars reticulata (SNr). Four burr holes were drilled into the cranium so as to expose the dura mater. Guide cannulae (10 mm, 0.8mm outer diameter) were implanted bilaterally and anchored into place using screws and dental acrylic cement (Duralay). The guide cannulae were implanted 3.0 mm or 2.7 mm
above the CS or the SNr respectively. Dummy cannulae or styli were then placed into the cannulae to prevent clogging of the guide cannulae. The wound was then sutured (Ethicon sutures) and a local anaesthetic cream (Emla) was applied to the wound.

Table 2.1 Stereotaxic coordinates obtained from the Rat Brain Atlas (Paxinos and Watson, 1986)

<table>
<thead>
<tr>
<th>Brain area</th>
<th>AV from Bregma (mm)</th>
<th>Lateral from midline (mm)</th>
<th>Depth from Dura mater (mm) (depth of guide cannula)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus Striatum</td>
<td>± 0.2</td>
<td>± 3.0</td>
<td>3.5-6.5 (2.0)</td>
</tr>
<tr>
<td>Substantia nigra pars reticulata</td>
<td>- 5.3</td>
<td>± 2.5</td>
<td>7.6 (4.9)</td>
</tr>
</tbody>
</table>

2.2.3 Induction of akinesia and drug administration

Reserpine (5 mg/kg i.p) was injected when the rats had recovered from anaesthesia. The animals were then kept warm, 23 ± 1°C, overnight to counteract reserpine-induced hypothermia. The rats were almost completely akinetic 24 h later. The styli were removed with fine forceps and replaced with injection cannulae (33 gauge, 21 mm length), connected to a 10 µl Hamilton microsyringe by fine bore polythene tubing, which were placed into the guide cannulae. The volume of drug or vehicle injected into the CS was 0.5 µl and into the SNr was 0.25 µl per side, over a period of 2 min. The injection cannulae were left in place for a further 2 min to allow for diffusion of the drug and then slowly withdrawn.

Immediately after injection the animals were individually placed into one of two
Figure 2.2. Apparatus for measuring rat locomotor activity.
circular open fields (65 cm or 83 cm in diameter) and their locomotor activity monitored using Radiospares 8960 Microwave Doppler Module units connected to an amplifier, timer and LED display at 10 min intervals for 60 min (see Fig. 2.2). The Doppler sensors were calibrated to detect gross locomotor movements only. The absence or presence of other behaviours (as mentioned in 2.1.2) was noted using a checklist but was not quantified. Ataxia was scored on the following scale: 0-no ataxia; 1-mild ataxia consisting of a 'wobbly' gait; 2-moderate ataxia consisting of muscular relaxation causing a flattened body posture and uncoordinated movement; 3-severe ataxia consisting of difficulty in initiating movement, loss of balance and righting reflex and barrel rolling.

2.2.4 Statistical analysis

Motor activity was expressed as cumulative 60 min counts. Drug treatments were compared against vehicle-treated controls by an analysis of variance (ANOVA) with repeated measures and post hoc analysis of individual doses was carried out using a Student's t-test. The ataxia scores were compared with vehicle-treated controls by the Kruskal-Wallis test followed by a Mann-Whitney test or a Dunn's Multiple Comparisons test. Significance was taken as p < 0.05.

2.2.5 Drugs

Reserpine, phencyclidine (PCP), ketamine, amantadine (Sigma), MK 801, dextromethorphan, (±) HA-966, 2-amino-phosphonopentanoic acid (AP-5) (Research Biochemicals Inc., Natick, MA, USA), 3,5-diamino-6-[2,3-dichlorphenyl]-1,2,4-triazine (lamotrigine) (Glaxo-Wellcome), memantine (Merz), eliprodil (Synthelabo Recherche), CGP 40116 (Ciba Geigy), CPP (Tocris Neuramin) and NBQX (Novo Nordisk) were all administered in dimineralised water. The solution of reserpine was aided with a minimum quantity of glacial acetic acid (British Drug Houses: final pH 5.5), that of lamotrigine and eliprodil with a minimum amount of dimethylsulphoxide (DMSO, Sigma) and of NBQX with a minimum amount of sodium hydroxide (NaOH; British Drug Houses: final pH 8.5) and made up to volume with dimineralised water. Reserpine was administered
intraperitoneally in a volume of 1 ml/kg. All other drugs were injected intracerebrally via injection cannulae.

2.3 Catalepsy in the rat

2.3.1 Animals

Male Wistar rats (A.R. Tuck Ltd.), weighing 160-350 g, were used in these experiments. The rats were housed in groups of 6 at 22 ± 1°C under fluorescent lighting (light period 07.00-17.00 h) with food and water available ad libitum. The experiments were carried out from 09.30-17.30 h and only animals injected systemically were used at most twice while all other animals were used only once. All experiments were carried out in accordance with the Animals (Scientific Procedures) U.K. Act, 1986.

2.3.2 Stereotaxic surgery and intracerebral administration of drugs

The rats were anaesthetised using halothane (Induction with 2.5% v/v O₂ and maintenance with 1.5% v/v O₂) throughout duration of the surgery. An incision was made, bregma located and used as a reference point for determining the position of the different brain areas investigated.
### Table 2.2 Stereotaxic coordinates from the Rat brain atlas (Paxinos and Watson, 1986)

<table>
<thead>
<tr>
<th>Brain area</th>
<th>AV from Bregma (mm)</th>
<th>Lateral from midline (mm)</th>
<th>Depth from Dura mater (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpus Striatum</td>
<td>+ 1.2</td>
<td>± 1.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Substantia nigra pars reticulata</td>
<td>- 5.3</td>
<td>± 2.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>+ 0.7</td>
<td>± 1.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Globus Pallidus</td>
<td>- 0.8</td>
<td>± 2.8/3.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Ventromedial thalamus</td>
<td>- 2.3</td>
<td>± 1.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Entopenduncular nucleus</td>
<td>- 2.8</td>
<td>± 2.9</td>
<td>7.4</td>
</tr>
<tr>
<td>Subthalamic nucleus</td>
<td>- 3.8</td>
<td>± 2.5</td>
<td>7.6</td>
</tr>
</tbody>
</table>

#### 2.3.3 Induction and measurement of catalepsy

Catalepsy was induced by either injecting haloperidol systemically or intracerebrally, or muscimol intracerebrally and quantified by measuring the time taken (s) for the rats to dismount a horizontal bar (maximum 360 s), elevated 8 cm above ground, and this was expressed as descent latency (DL).
2.3.4 Systemic administration of haloperidol and MK 801

Haloperidol (1 mg/kg i.p) was administered 45 min prior to MK 801 (0.2 mg/kg i.p) administration in the first instance. In the second part of this study, MK 801 (0.2 mg/kg i.p) was administered 10 min before haloperidol (1 mg/kg i.p) injection. The animals were placed individually into Perspex observation cages (29 x 26 x 21 cm high) containing a horizontal bar (elevation 8 cm) and catalepsy measurements were commenced 15 min after haloperidol administration.

2.3.5 The effect of MK 801 on haloperidol-induced catalepsy in the corpus striatum (CS)

Haloperidol (7 μg/ 0.5 μl/ side) was administered bilaterally into the ventro-rostral striatum to induce catalepsy and 60 min later MK 801 (0.2 mg/kg) was injected intraperitoneally. Catalepsy was measured 45 min after haloperidol administration.

In the second part of this study, MK 801 (10 μg/ 0.5 μl/ side) was administered focally into the ventro-rostral striatum and 45 min later, haloperidol (1 mg/kg i.p) was administered. Catalepsy was measured 45 min after haloperidol administration.

2.3.6 The effect of NBQX on the anticytaleptic activity of MK 801

Haloperidol (7 μg/ 0.5 μl/ side) was injected into the striatum to induce catalepsy measurements started 45 min later. NBQX (12.5 mg/kg i.p) was then administered 60 min after the surgery was completed. A second injection of NBQX (12.5 mg/kg i.p) was administered simultaneously with MK 801 (0.1 mg/kg i.p) 90 min after the administration of haloperidol. Control animals were administered MK 801 (0.1 mg/kg) 90 min after haloperidol administration.

2.3.7 The effect of MK 801 on haloperidol-induced catalepsy in the nucleus accumbens (NAc)

Catalepsy was induced by administering haloperidol (7 μg/ 0.5 μl/ side) into the NAc and MK 801 (0.2 mg/kg i.p) was injected 60 min later. Catalepsy measurements were
begun 45 min after haloperidol administration.

In the second part of the experiment, MK 801 (10 µg/0.5 µl) was injected bilaterally into the NAc and haloperidol (1 mg/kg i.p) was administered 45 min later. Catalepsy was measured 45 min after haloperidol administration.

2.3.8 Anticataleptic activity of MK 801 administered into the substantia nigra pars reticulata (SNr), the entopeduncular nucleus (EPN) or the subthalamic nucleus (STN)

MK 801 was administered bilaterally into the SNr (1 µg/0.5 µl/side), the EPN or the STN (5 µg/0.5 µl/side for both regions) and haloperidol (1 mg/kg i.p) administered 45 min later. Catalepsy was measured 45 min after haloperidol administration.

2.3.9 The effect of bicuculline administered into the SNr

Bicuculline (20 ng-1 µg/0.5 µl/side) was administered into various regions in the SNr and catalepsy measurements were made 30 min later.

2.3.10 The effect of MK 801 on catalepsy induced by intracerebral muscimol

Muscimol was administered bilaterally into the GP (25 ng/0.5 µl/side) or the ventromedial thalamus (VMT) (50 ng/0.5 µl/side) to induce catalepsy. MK 801 (0.2 mg/kg i.p) was then administered 60 min later. Catalepsy was measured 45 min after the administration of muscimol.

2.3.11 Statistical analysis

Data were analysed using a one-way analysis of variance (ANOVA) and post hoc analysis was done using the one-tailed student's t-test, as we imposed a manual cut-off point. Significance was taken as p < 0.05.

2.3.12 Drugs

4-[(4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl)-1-(4-fluoro-phenyl)-1-butane (Haloperidol), muscimol, bicuculline (Sigma), NBQX (Novo Nordisk) and MK
801 (Research Biochemicals Inc., Natick, USA) were administered in dimineralised water. Haloperidol was dissolved with approximately 200 µl of glacial acetic acid (British Drug Houses) and made up to volume with distilled water. NBQX was dissolved in a minimum quantity of NaOH (British Drug Houses) and made up to volume with dimineralised water.

Drugs administered systemically were injected at a volume of 1 ml/kg, while drugs injected intracerebrally were administered using a 10 µl Hamilton microsyringe at a rate of 0.2 µl/min. The total volume injected was 0.5 µl.

2.4 Histology

The rats were sacrificed at the end of each of the rat locomotor and catalepsy experiments and the brains removed and stored in 10% v/v formal saline (Formaldehyde-AnalaR and saline). After fixation for five days, a 15% sucrose (AnalaR) solution was added and the brains left in this formal saline-sucrose mixture for 24 h. The brains were then frozen onto a freezing sledge microtome and coronal (40-60 µm thick) sections sliced and placed onto gelatin-coated slides. After air drying, the brain sections were stained with Cresyl violet (Cerstain). The following protocol was used,

Dehydrate
1. 3 min in 50% ethanol (British Drug Houses)
2. 3 min in 70% ethanol
3. 3 min in 90% ethanol
4. 3 min in absolute (100%) ethanol

Rehydrate
5. 3 min in 90% ethanol
6. 3 min in 70% ethanol
7. 3 min in 50% ethanol
8. 2 min in distilled water

Stain
9. 5-10 min in Cresyl violet (0.5%) solution
Differentiate

10. 2 min in 50% ethanol
11. 2 min in 70% ethanol
12. 2 min in 90% ethanol
13. 2 min in absolute ethanol
14. 25 min in Histaclear
15. Mount using DPX (Raymond A. Lamb)

The stained slices were then examined under a binocular microscope and the injection sites determined. The injections that did not reach the target sites were omitted from the studies.
CHAPTER THREE

LOCOMOTOR EFFECTS OF SYSTEMIC GLUTAMATE ANTAGONISTS IN DRUG-NAIVE AND MONOAMINE-DEPLETED MICE
3.1 Introduction

The loss of nigrostriatal dopamine neurones in PD leads to a secondary glutamatergic hyperstimulation which leads to parkinsonian symptoms, such as tremor, akinesia and rigidity (Albin et al., 1989).

It has been proposed that using glutamate antagonists in the therapy of PD, either alone or in conjunction with the conventional dopamine-based therapy of PD may prove beneficial by decreasing the increased glutamatergic tone, particularly in corticostriatal and/or subthalamic pathways (Gerfen, 1992; Greenamyre and O'Brien, 1991; Starr, 1995a).

This hypothesis has been tested with various classes of glutamate antagonists with some success. Glutamate antagonists are antiparkinsonian in a number of animal models of PD, including haloperidol-induced catalepsy, akinesia produced by the depletion of monoamines or by unilateral lesions of the SN (Carlsson and Svensson, 1990; Morelli et al., 1992; Snell and Johnson, 1985; Schmidt and Bubser, 1989; Schmidt et al., 1991). Experiments in dopamine-intact rodents showed an enhancement of spontaneous locomotor activity and the occurrence of stereotyped behaviours (Clineschmidt et al., 1982; Danysz et al., 1994a, b; Liljequist et al., 1991; Svensson, 1973; Svensson et al., 1991). Behavioural studies in monoamine-depleted or 6-OHDA lesioned rodents and primates do appear to show that both NMDA and AMPA receptor antagonists are antiakinetic when administered with dopamine agonists, such as L-Dopa, (Kaur et al., 1994; Klockgether et al., 1991; Klockgether and Turski, 1990; Löschmann et al., 1991; Maj et al., 1993b; Morelli et al., 1992; Wüllner et al., 1992) but have a weak effect, if any, on locomotion when administered alone (Starr and Starr, 1993a, b, 1994a, b).

As the current therapy of PD is far from desirable, glutamate antagonists may be advantageous when administered as adjuncts to the mainstay dopamine replacement-based therapy. The doses of dopamine agonists may be reduced, thereby increasing the therapeutic lifetime of L-Dopa and decreasing the incidence of debilitating side-effects resulting from chronic L-Dopa treatment (Allen et al., 1980; Boldry et al., 1995; Jenner, 1995; Lustig et al., 1992; Spencer et al., 1994). Also, as L-Dopa, itself may contribute to the pathology of PD, through metabolism to neurotoxic free radicals (Jenner et al., 1992),
the reducing of its dose would be favourable for a better long-term prognosis of PD suffers.

However, NMDA antagonists can themselves induce side-effects including amnesia, psychostimulation, muscle relaxation and ataxia. Non-competitive antagonists with a low affinity for the NMDA receptor (Lipton, 1993), competitive NMDA antagonists, glycine site antagonists, polyamine site antagonists and AMPA receptor antagonists, all have been postulated to be relatively free of the side-effects which follow the treatment of high-affinity non-competitive NMDA receptor antagonists.

A number of drugs, used clinically in the symptom control of PD have recently been found to possess NMDA receptor blocking properties. The aminoadamantanes, amantadine (Danysz et al., 1994b) and memantine (Bormann, 1989; Chen et al., 1992; Kornhuber et al., 1991), and the anticholinergics, budipine and biperiden (Jackisch et al., 1994), already used in the therapy of PD, have been found to be low-affinity NMDA receptor-linked ion channel blockers.

The present study was carried out to investigate the antiparkinsonian properties of a number of glutamate antagonists initially in dmg-naive mice, then in the monoamine-depleted mice, either alone or co-administered with the dopamine precursor, L-Dopa, a D₁ receptor agonist, SKF 38393 or a D₂ receptor agonist, RU 24213.

3.2 Results
3.2.1 Control treatments
3.2.1.1 Vehicle in dmg-naive mice

Mice injected with demineralised water, used as vehicle, exhibited exploratory locomotion with rearing and sniffing behaviours for approximately the first 10 min after being placed in the observation cages. After this period, the animals began to show signs of habituation and commenced whole body grooming. The cumulative locomotor activity counts for 30 min averaged $1733.8 \pm 218.8 \ (n=24)$, for 60 min were $2630.4 \pm 392 \ (n=24)$, for 90 min were $3099.3 \pm 468.3 \ (n=24)$ and for 120 min were $3327.6 \pm 608.2 \ (n=13)$. The 30 min cumulative locomotor counts for a 20% DMSO solution, the vehicle for
lamotrigine and eliprodil, were $857.8 \pm 174.8$ (n=6) and the 90 min cumulative counts were $2486.5 \pm 387$ (n=6) (Fig. 3.1).

### 3.2.1.2 Vehicle in monoamine-depleted mice

Twenty-four hours after reserpine (5 mg/kg i.p) treatment, the mice were rendered completely akinetic and appeared sedated. After injection of vehicle (demineralised water), the mice only exhibited slight sniffing and little locomotor activity throughout the 2 h experiment. They also remained flattened in posture. The 30 min cumulative locomotor counts were $36.7 \pm 7.9$ (n=23), 60 min cumulative locomotor counts were $70.8 \pm 17.4$ (n=23), 90 min cumulative locomotor counts were $88.6 \pm 22$ (n=23) and 120 min cumulative locomotor activity count averaged $105.8 \pm 29.5$ (n=17). A 20% DMSO solution, the vehicle for eliprodil and lamotrigine, produced 30 min cumulative counts of $5.8 \pm 2.5$ (n=6) and the 90 min cumulative counts were $48.8 \pm 15.6$ (n=6). The reduced activity of the reserpine-treated mice was significantly different from the activity of the drug-naive mice (p<0.001 for all the time points) (Fig. 3.1).

### 3.2.1.3 SKF 38393 in monoamine-depleted mice

SKF 38393 (30 mg/kg) produced a good recovery of locomotor activity in the reserpine-treated mice. The locomotor activity produced resembled the normal exploratory locomotor activity shown by drug-naive mice. The 30 min cumulative locomotor counts were $523.4 \pm 138.2$ (n=26), 60 min cumulative locomotor counts were $1649.5 \pm 320.9$ (n=26), 90 min cumulative locomotor counts were $2576.7 \pm 445.7$ (n=26) and the 120 min cumulative locomotor activity counts produced by SKF 38393 were $4358.7 \pm 725.3$ (n=18) (30 min KW statistic=35.34, p<0.001; 60 min KW statistic=38.95, p=0.001; 90 min KW statistic=35.12, p<0.001; 120 min KW statistic=34.5, p<0.001 vs reserpine-treated controls) (Fig. 3.1).

### 3.2.1.4 RU 24213 in monoamine-depleted mice

RU 24213 (5 mg/kg s.c) when injected in reserpine-treated mice produced slow
forward locomotor activity, with head down sniffing and some grooming. Mice injected with RU 24213 were only active for 30-40 min. The 30 min cumulative locomotor counts averaged 501.7 ± 84.3 (n=21) and the 60 min cumulative locomotor activity counts of RU 24213-treated mice were 850.1 ± 147.2 (n=21) (30 min KW statistic=35.34, p<0.001; 60 min KW statistic=38.95, p<0.001 vs reserpine-treated controls) (Fig. 3.1).

3.2.1.5 L-Dopa in monoamine-depleted mice

L-Dopa (150 mg/kg), administered 30 min after benserazide (100 mg/kg), induced some activity after a delay of 40-60 min, during which time the mice appeared collapsed, with indications of hind-limb abduction. However, after this delay, the animals exhibited forward locomotion, a major component of which was persistent peripheral locomotion. The locomotor activity seen in L-Dopa injected mice was not fluent and did not resemble the locomotor activity displayed by control mice. The 30 min cumulative locomotor counts were 191.8 ± 47.3 (n=35), 60 min cumulative locomotor counts were 636.8 ± 132.1 (n=35), 90 min cumulative locomotor counts were 1187 ± 254.8 (n=35), the 120 min cumulative locomotor counts obtained with 150 mg/kg L-Dopa were 1526.1 ± 369.9 (n=27) (30 min KW statistic=35.34, p>0.05; 60 min KW statistic=38.95, p<0.01; 90 min KW statistic=35.12, p<0.001; 120 min KW statistic=34.5, p<0.01 vs reserpine-treated controls) (Fig. 3.1).

3.2.2 The NMDA receptor-linked ion channel blockers

3.2.2.1 Amantadine

3.2.2.1.1 In drug-naive mice

Amantadine was administered into drug-naive mice in order to determine if the current results compared favourably with research done previously in this laboratory (Starr and Starr, 1995b). Amantadine (10-40 mg/kg) was found to increase the spontaneous locomotion of drug-naive mice (F(3,24)=4.412, p=0.0187). 10 mg/kg amantadine increased the spontaneous locomotor activity significantly (p<0.05 vs drug-naive mice)
Fig. 3.1 The effects of the control treatments on locomotion. (o) vehicle-treated drug-naive mice; (■) vehicle treatment in reserpine (5 mg/kg) treated mice; (▲) SKF 38393 (30 mg/kg) treatment in reserpine-treated mice; (♦) RU 24219 (5 mg/kg) treatment in reserpine-treated mice; (*) L-Dopa (150 mg/kg, 30 min after 100 mg/kg benserazide) treatment in reserpine-treated mice. Data are means±S.E.M. of 6–35 determinations. * represents p<0.001 vs vehicle-treated naive controls, # represents p<0.01 and ## p<0.001 vs vehicle-treated reserpinised mice by the Student’s t-test.

Fig 3.2 The effects of amantadine (10–40 mg/kg) on the locomotion of drug-naive mice. Data represent means ± S.E.M of 4–13 determinations. * represents p<0.05 vs vehicle-injected naive controls by Dunnett’s t-test.
Behavioural observations

Mice injected with 10 mg/kg and 20 mg/kg amantadine displayed sniffing, rearing and grooming in addition to forward locomotion. Mice injected with 40 mg/kg amantadine appeared flattened in posture and ataxic for approximately 10 min at the start of the experiment after which they displayed similar behaviours to the 10 and 20 mg/kg injected animals but the incidence of these behaviours was less than seen with 10 and 20 mg/kg amantadine.

3.2.2.2 Memantine

3.2.2.2.1 In drug-naive mice

Mice injected with memantine (5-20 mg/kg) displayed a dose-dependent increase in locomotor activity (n=5-13, p<0.001, F(4,34)=16.765 vs drug-naive controls). 1 and 5 mg/kg memantine did not produce a significant difference in spontaneous locomotor activity but higher doses (10 and 20 mg/kg) induced a significant increase in spontaneous locomotion (p<0.01 for both vs vehicle-injected controls) (Fig. 3.3).

Behavioural observations

Mice injected with the 1-10 mg/kg memantine displayed dose-dependent sniffing, rearing and grooming in addition to forward locomotion while at 20 mg/kg the animals appeared flattened in posture for approximately 30 min after being placed in the observation cages. After this time, the animals displayed sniffing, grooming and rearing, in addition to fast forward locomotion.

3.2.2.2.2 In monoamine-depleted mice

Monoamine-depleted mice injected with memantine (5-40 mg/kg) exhibited a weak stimulation of locomotor activity (F(5,58)=4.57, p=0.0015 vs reserpine-treated controls). Memantine (5-20 mg/kg) did not produce a significant change in the akinesia shown by the mice. However, 40 mg/kg, memantine increased locomotor activity significantly (p<0.01 vs vehicle-injected reserpine-treated controls) (Fig. 3.4).
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Behavioural observations

Memantine (5-10 mg/kg) treated mice appeared sedated throughout the duration of the experiment. Mice injected with 20 mg/kg and 40 mg/kg memantine were active after a delay of approximately 60 min.

3.2.2.2.3 In conjunction with SKF 38393

Memantine decreased the response to SKF 38393 (30 mg/kg) significantly (2-way interaction $F(4,90)=7.89, p<0.001$). 5 mg/kg memantine attenuated the response to SKF 38393 ($p<0.05$ vs only SKF 38393-treated mice) (Fig. 3.4).  

Behavioural observations

The animals, administered memantine (5-10 mg/kg) and SKF 38393, were very active, exhibiting sniffing, grooming, scrabbling in corners, rearing forward locomotion was fluent. Higher doses of memantine (20 and 40 mg/kg) administered with SKF 38393, also produced some ataxia, a flattened posture, loss of muscle tone and some seizure activity which manifested as running fits, forepaw myoclonus and 'jumpiness'.

3.2.2.2.4 In conjunction with RU 24213

The combination of RU 24213 (5 mg/kg) and memantine (5-40 mg/kg) appeared to enhance the activity of the mice compared to the effect of RU 24213 (5mg/kg) administered alone but this was not statistically significant (2-way interaction $F(4,104)=2.278, p=0.067$, using 60 min cumulative locomotor counts) (Fig. 3.4).  

Behavioural observations

Memantine (5-40 mg/kg) administered simultaneously with RU 24213 (5 mg/kg) produced some head down forward locomotion, sniffing and grooming. These behaviours were also only evident for approximately 30-40 min.

3.2.2.2.5 In conjunction with L-Dopa

Memantine (5-40 mg/kg) did not alter the response to L-Dopa (150 mg/kg administered 30 min after 100 mg/kg benserazide) (2-way interaction $F(4,99)=2.009$, 82
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Fig. 3.3 The effects of memantine (5–20 mg/kg) on the locomotion of drug-naive mice. Data represent means ± S.E.M of 5–13 determinations. * represents p<0.01 vs vehicle-injected controls by Dunnett’s t-test.

Fig. 3.4 The effects of memantine, either alone (solid bars) or in conjunction SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L–Dopa (150 mg/kg) (hatched) in reserpine (5 mg/kg) treated mice. Data are means ± S.E.M. 5–27 120min cumulative counts for the reserpine study, the SKF 38393 and L–Dopa interaction studies and 60 min cumulative counts for the RU 24213 interaction study.* represents p<0.01 vs reserpine-treated controls and # represents p<0.05 SKF 38393–treated mice by Dunnett’s t-test.
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Memantine (5-40 mg/kg) injected in combination with L-Dopa (150 mg/kg) produced forward locomotion, most of which was locomotion around the periphery of the cage, sniffing, rearing after an initial delay of about 30-50 min. The forward locomotion got increasingly stereotyped and the incidence of seizure activity increased with increasing dose of memantine.

3.2.2.3 Dextromethorphan

3.2.2.3.1 In drug-naive mice

Dextromethorphan (5-40 mg/kg) increased spontaneous locomotor activity (F(6,42)=10.27, p<0.001). The mice injected with dextromethorphan exhibited two different behavioural profiles at 40 mg/kg dextromethorphan. A fraction of the mice (4/9) injected with 40 mg/kg displayed enhanced locomotor activity while the majority (5/9) showed no statistically significant effect when compared to the control mice. The 120 min cumulative counts observed were 2955.1 ± 673.4 for the non-responding mice and 21212.8 ± 6005.7 (p<0.01 vs drug-naive controls) for the responding mice injected with 40 mg/kg dextromethorphan (Fig. 3.5).

Behavioural observations

Low doses of dextromethorphan (5 and 10 mg/kg) produced sniffing, rearing, grooming behaviours in addition to forward locomotion. 20 mg/kg and a fraction (5/9) of 40 mg/kg dextromethorphan injected mice exhibited sniffing, some rearing, grooming as well as episodes of stereotyped forward locomotion. The high-scoring mice (4/9) at 40 mg/kg dextromethorphan displayed stereotyped fast forward locomotion around the periphery of the cage, sniffing, rearing and episodes of grooming. 80 mg/kg dextromethorphan injected mice exhibited sniffing, rearing, a little grooming and forward locomotion. These animals displayed some muscular relaxation in their hind regions but this did not appear to impede locomotion.
3.2.2.3.2 In monoamine-depleted mice

Mice injected with a dose range of dextromethorphan (10-40 mg/kg) displayed a biphasic response \((F(3,42)=1.799, p=0.1633)\). 10 and 20 mg/kg dextromethorphan increased the locomotor scores obtained while mice injected with 40 mg/kg dextromethorphan produced a decreased locomotor activity score but these changes were not statistically significant (Fig. 3.6).

**Behavioural observations**

Dextromethorphan (10 and 20 mg/kg) injected mice exhibited some grooming, sniffing and little forward locomotion. Dextromethorphan, 40 mg/kg, injected mice displayed some sniffing, grooming and forward locomotion but they appeared flattened in posture and there was some muscle relaxation of the hind region.

3.2.2.3.3 In conjunction with SKF 38393

Dextromethorphan (10-40 mg/kg) increased the response to SKF 38393 (30 mg/kg) \((2\text{-way interaction } F(3,77)=17.44, p<0.001)\). Dextromethorphan, 40 mg/kg, when administered with SKF 38393 (30 mg/kg) produced statistically significant hyperactivity \((p<0.01 \text{ vs SKF 38393-treated mice})\) (Fig. 3.6).

**Behavioural observations**

Dextromethorphan (10-40 mg/kg) administered with SKF 38393 produced sniffing, grooming, rearing and fast forward locomotion, which included locomotion around the periphery of the cage. The behaviours became increasingly stereotyped with increasing dose of dextromethorphan.

3.2.2.3.4 In conjunction with RU 24213

Dextromethorphan (10-40 mg/kg) had no effect on the response to RU 24213 (5 mg/kg) \((2\text{-way interaction } F(3,85)=0.827, p=0.483)\). 10 and 20 mg/kg dextromethorphan increased the response to RU 24213 but this was not statistically significant. The combination of 40 mg/kg dextromethorphan and 5 mg/kg RU 24213 produced a cumulative locomotor count that was similar to the count produced by mice injected with
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RU 24213 5 mg/kg alone (Fig. 3.6).

Behavioural observations

Dextromethorphan (10-40 mg/kg) administered with RU 24213 (5 mg/kg) produced sniffing, rearing, grooming and forward locomotion. Dextromethorphan, 40 mg/kg, and RU 24213, treated mice appeared flattened in posture.

3.2.2.3.5 In conjunction with L-Dopa

The response to L-Dopa (150 mg/kg) appeared to be non-significantly increased by dextromethorphan (5-40 mg/kg) (2-way interaction F(3,87)=0.181, p=0.909) (Fig. 3.6).

Behavioural observations

Mice treated with dextromethorphan (10-40 mg/kg) and L-Dopa (150 mg/kg) exhibited sniffing, forward locomotion, which included locomotion around the periphery of the cage, rearing and grooming after a delay of 30-50 min, during which time the animals appeared sedated. There was also some licking behaviour observed with 20 mg/kg and 40 mg/kg dextromethorphan in combination with L-Dopa.

3.2.2.4 Phencyclidine (PCP)

3.2.2.4.1 In drug-naive mice

PCP (0.5-32 mg/kg) did not have a consistent effect on spontaneous locomotor activity (F(7,48)=5.728, p<0.001). PCP, 8 and 32 mg/kg, significantly altered the activity of the drug-naive mice (p<0.01 and p<0.05 respectively vs drug-naive controls) (Fig. 3.7).

Behavioural observations

PCP (0.5-8.0 mg/kg) injected mice exhibited sniffing, rearing and grooming as well as forward locomotion. The animals appeared increasingly stereotyped with increasing dose of PCP. Mice injected with 16 mg/kg PCP were flattened in posture for approximately 60 min at the beginning of the experiment after which they displayed sniffing, a little rearing and some grooming as well as fast forward locomotion. 32 mg/kg PCP produced some sniffing and grooming but the animals were flattened in posture and
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ataxic. There was also loss of the righting reflex. Where there was locomotion it was slow and the posture of the mice was abnormal (i.e. flattened).

3.2.2.4.2 In monoamine-depleted mice

Low doses of PCP (0.5-4 mg/kg) injected mice exhibited very little recovery of locomotor activity but at high doses (8 and 16 mg/kg) of PCP there was some recovery albeit with an initial delay of 50-60min (F(6,53)=3.97, p=0.0027). The change in locomotor activity brought about by 32 mg/kg PCP was significantly different from that of the reserpine-treated control mice (p<0.01 vs vehicle-injected reserpinised controls) (Fig. 3.8).

Behavioural observations

Mice injected with low doses of PCP (0.5-4 mg/kg) remained collapsed and sedated, with few episodes of sniffing throughout the duration of the experiment. Mice injected with 8 mg/kg and 16 mg/kg PCP showed some forward locomotion with sniffing and instances of circular forward locomotor activity. Mice administered 32 mg/kg PCP were displayed some sniffing, 'dragging' forward locomotion, but they also exhibited ataxia and muscular relaxation which made them appear flattened.

3.2.2.4.3 In conjunction with SKF 38393

PCP (0.5-32 mg/kg) did not produce any consistent change in the response to SKF 38393 (30 mg/kg i.p.) in mice (2-way interaction F(7,123)=1.263, p=0.276) (Fig. 3.8).

Behavioural observations

At low doses of PCP (0.5 and 1.0 mg/kg) and SKF 38393 (30 mg/kg), the mice appeared flattened in posture initially (t=10-30 min) after which they displayed sniffing, grooming and some forward locomotion which included locomotion around the periphery of the cage. The combination of 2.0 mg/kg PCP and SKF 38393 produced less recovery of activity compared to 0.5 mg/kg and 1.0 mg/kg PCP given with SKF 38393. These animals administered 2.0 mg/kg PCP, had fewer episodes of forward locomotion and remained flattened and still for the most part of the experiment. 4.0 mg/kg PCP, administered with SKF 38393, produced some forward locomotion, sniffing and grooming.

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whereas 8.0 mg/kg PCP and SKF 38393 injected mice were active, exhibiting grooming, forward locomotion, sniffing and some rearing. 16 mg/kg PCP and SKF 38393 were flattened in posture for about 20 min at the start of the experiment, after which they showed some sniffing, forward locomotion which included short periods of circling, grooming and also some loss of balance and loss of muscle tone. Mice injected with 32 mg/kg PCP and 30 mg/kg SKF 38393 appeared ataxic and exhibited a loss of the righting reflex and hind limb abduction. There were few instances of sniffing and grooming in these mice.

3.2.2.4.4 In conjunction with RU 24213

PCP (0.5-32 mg/kg) did not produce any statistically significant change in the response to RU 24213 (5 mg/kg) (2-way interaction F(7,109)=3.05, p=0.009, using 60 min cumulative locomotor counts). Post-hoc analysis showed no one dose of PCP, administered with RU 24213, was significantly different from RU 24213-treated controls (Fig. 3.8).

Behavioural observations

Low doses of PCP (0.5 and 1.0 mg/kg) given in combination with RU 24213 (5 mg/kg) produced some sniffing, forward locomotion, grooming and some rearing. PCP (8-32 mg/kg) administered with RU 24213 resulted in some sniffing, forward locomotion, rearing and grooming. The incidence of these behaviours decreased with increasing dose of PCP and 32 mg/kg PCP in combination with RU 24213 resulted in the animals being collapsed for the duration of the experiment.

3.2.2.4.5 In conjunction with L-Dopa

PCP (0.5-32 mg/kg) had no effect on the behaviour of the L-Dopa treated mice (2-way interaction F(6,134)=0.728, p=0.648) (Fig. 3.8).

Behavioural observations

Low doses of PCP (0.5-4 mg/kg, with the exception of 2 mg/kg) administered with 150 mg/kg L-Dopa resulted in dose-dependent increases in activity. The mice showed
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**Fig. 3.5** The effects of dextromethorphan (5–80 mg/kg) on the locomotion of drug-naive mice. Mice injected with 40 mg/kg were divided into non-responders (open) and responders (solid). Data represent means ± S.E.M of 4–13 animals. * represents p<0.01 vs vehicle-injected controls by Dunnett’s t-test.

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**Fig. 3.6** The effects of dextromethorphan, either alone (solid) or with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (150 mg/kg) (hatched) in reserpine (5 mg/kg) treated mice. Data are means ± S.E.M. of 5–27 120 min cumulative counts for the reserpine study and the SKF 38393 and L-Dopa interaction studies and 60 min cumulative counts for the RU 24213 interaction study. * represents p<0.01 vs SKF 38393-treated mice by Dunnett’s t-test.

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Fig. 3.7 The effects of phencyclidine (0.5–32 mg/kg) on the locomotion of drug-naïve mice. Data represent means ± S.E.M of 5–13 determinations. * represents p<0.05, **, p<0.01 vs vehicle-injected controls by Dunnett's t-test.

Fig. 3.8 The effects of phencyclidine, either alone (solid) or in conjunction with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (hatched) in reserpine (5 mg/kg) treated mice. Data are means ± S.E.M of 5–27 120 min cumulative counts for reserpine, SKF 38393 and L-Dopa interaction studies and 60 min cumulative counts for the RU 24213 interaction study. * represents p<0.01 vs reserpine-treated controls by Dunnett's t-test.
forward locomotion, a component of which was peripheral locomotion, as well as sniffing, grooming and some rearing after a delay of approximately 40-60 min. PCP (8-32 mg/kg) administered with L-Dopa (150 mg/kg) resulted in dose-dependent disability, in the form of loss of muscle tone and muscle relaxation in the hind region making the mice appear to be 'dragging' their hind limbs when 'walking'. There were some episodes of sniffing, grooming and a little rearing in some of the animals.

3.2.2.5 Ketamine

3.2.2.5.1 In drug-naive mice

Ketamine (2.5-80 mg/kg) did not produce any significant change in the behaviour of naive mice (\(F(6,41)=1.767, p=0.1347\)) (Fig. 3.9).

Behavioural observations

Mice injected with ketamine (2.5-20 mg/kg) displayed sniffing, rearing and grooming behaviours as well as fast forward locomotion. At 40 mg/kg and 80 mg/kg ketamine, the animals appeared to have some muscular relaxation and had episodes when they were ataxic. The effect of 80 mg/kg ketamine had a delayed onset with an effect on locomotor activity seen about 30-40 min after the experiment had begun.

3.2.2.5.2 In monoamine-depleted mice

Ketamine (2.5-20 mg/kg and 80 mg/kg) produced no change in the reserpine-treated mice but 40mg/kg ketamine increased locomotor activity significantly (\(F(6,51)=2.899, p=0.0178\)) (Fig. 3.10).

Behavioural observations

Ketamine (2.5-20 mg/kg) injected animals were flattened in posture and largely inactive with the exception of a few episodes of sniffing and grooming. 40 mg/kg ketamine injected mice exhibited sniffing, grooming and forward locomotion, which was abnormal, i.e. there was some muscular relaxation in the hind region, therefore the animals appeared to be 'dragging' their hind region when 'walking'. Ketamine 80 mg/kg, injected mice were flattened in posture, inactive and exhibited hindlimb abduction.
3.2.2.5.3 In conjunction with SKF 38393

Ketamine (2.5-80 mg/kg) decreased the response to SKF 38393 (30 mg/kg), when both drugs were administered together but this decrease was not statistically significant (2-way interaction F(6,117)=1.481, p=0.192) (Fig.3.10).

Behavioural observations

Mice injected with ketamine (2.5-10 mg/kg) and SKF 38393 exhibited sniffing, grooming, some rearing and some forward locomotion. Ketamine (20-40 mg/kg) administered with SKF 38393 produced sniffing, forward locomotion which included locomotion around the periphery of the cage, rearing, grooming and seizure activity (i.e. myoclonus and running fits). There was also slight muscle relaxation especially in the hind region which resulted in 'dragging' locomotion in these mice. The muscle relaxation was more pronounced in 80 mg/kg ketamine and SKF 38393 injected mice. These animals were ataxic with some periods of sniffing, grooming and 'dragging' locomotion.

3.2.2.5.4 In conjunction with RU 24213

Ketamine (2.5-80 mg/kg) did not produce any consistent change in the response to RU 24213 (5 mg/kg) (2-way interaction F(6,108)=1.5, p=0.186) (Fig.3.10).

Behavioural observations

Ketamine (2.5-10 mg/kg) administered with RU 24213 produced sniffing, rearing, grooming and some forward locomotion. Ketamine (20-40 mg/kg) and RU 24213 injected mice exhibited forward locomotion, sniffing and grooming while 80 mg/kg ketamine and RU 24213 injected mice were flattened in posture and there was hind limb abduction.

3.2.2.5.5 In conjunction with L-Dopa

Ketamine (2.5-80 mg/kg) had no consistent effect on the effect of L-Dopa (150 mg/kg) (2-way interaction F(6,114)=0.289, p=0.941). Ketamine (2.5-20 mg/kg) appeared to decrease the response to L-Dopa (150 mg/kg) while ketamine (40-80 mg/kg) appeared to increase locomotion but these effects were not statistically significant (Fig.3.10).

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Fig 3.9 Lack of effect of ketamine (2.5–80 mg/kg) on the locomotion of drug-naive mice. Data represent means ± S.E.M of 4–13 determinations.

Fig. 3.10 The effects of ketamine either alone (solid) or in conjunction with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (150 mg/kg)(hatched) in reserpine (5 mg/kg) treated mice. Data are means±S.E.M. 5–27 120min cumulative counts for reserpine, SKF 38393 and L-Dopa interaction studies and 60 min cumulative counts for the RU 24213 interaction study. * represents p<0.05 vs vehicle-injected controls by Dunnett's t-test.
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Behavioural observations

Ketamine (2.5-10 mg/kg) and L-Dopa injected displayed some sniffing, grooming and forward locomotion with a flattened posture and episodes of stillness. Ketamine (10-40 mg/kg) and L-Dopa (150 mg/kg) injected mice exhibited some forward locomotion, sniffing, rearing, grooming as well as some ataxia, with 'dragging' locomotion and tremor. The locomotor stimulant effects were only visible after approximately 60 min of being placed in the activity cage. Ketamine (80 mg/kg) and L-Dopa (150 mg/kg) injected mice showed forward locomotion, sniffing, a flattened posture and some seizure-like activity (also seen after 10 mg/kg ketamine and L-Dopa), i.e running fits and 'jumpiness'. Again, the behavioural stimulant effects were only seen after an approximate delay of 60 min. As the dose of ketamine was increased, the incidence of ataxia also increased.

3.2.2.6 Budipine

3.2.2.6.1 In drug-naive mice

Budipine (5-40 mg/kg) produced a biphasic effect with 5-20 mg/kg increasing locomotion while 40 mg/kg decreased it (F(4,65)=2.73, p=0.0373, using 90 min cumulative locomotor counts). Post-hoc statistical analysis indicated that none of the doses of budipine was significantly different from vehicle-injected controls (Fig.3.11).

Behavioural observations

Budipine (5-40 mg/kg) injected animals exhibited sniffing, forward locomotion, especially around the periphery of the cage, grooming, rearing and some head weaving at higher doses (10-20 mg/kg). Mice treated with budipine were active for approximately 90 min. Budipine (40 mg/kg) treated mice were ataxic, flattened in posture and exhibited 'dragging' locomotion.

3.2.2.6.2 In monoamine-depleted mice

Budipine (5-40 mg/kg) induced some locomotor activity, especially at 20 mg/kg, but this increase was not statistically significant (F(4,49)=2.077, p=0.0996, using 90 min
cumulative locomotor counts) (Fig.3.12).

**Behavioural observations**

Budipine (5-40 mg/kg) administered to reserpine-treated mice induced very little mobility, some sniffing, rearing and grooming. Higher doses of budipine (20-40 mg/kg) injected mice were ataxic and had muscular relaxation in their hind region. Budipine (5-40 mg/kg) injected mice remained mostly flattened and still for the duration of the experiment.

3.2.2.6.3 In conjunction with SKF 38393

Budipine (5-40 mg/kg) did not have a statistically significant effect on the response to SKF 38393 (30 mg/kg) although 5 mg/kg budipine did appear to enhance SKF 38393-induced locomotion while 10-40 mg/kg budipine appeared to decrease it non-significantly (2-way interaction F(1,62)=0.1, p=0.753, using 90 min cumulative locomotor counts) (Fig.3.12).

**Behavioural observations**

Budipine (5-20 mg/kg) administered simultaneously with SKF 38393 produced some sniffing, rearing, forward as well as peripheral locomotion. Mice injected with budipine (40 mg/kg) administered with SKF 38393 were not as active as mice injected with the lower doses of budipine. They remained flattened in posture while exhibiting occasional sniffing. There were some instances of seizure-like activity at 10 mg/kg budipine, but this was transient.

3.2.2.6.4 In conjunction with RU 24213

Budipine (5-40 mg/kg) non-significantly decreased the response to RU 24213 (5 mg/kg)(F(4,99)=2.455, p=0.051, using 60 min cumulative locomotor counts) (Fig.3.12).

**Behavioural observations**

Animals treated with budipine (5-40 mg/kg) and RU 24213 exhibited forward locomotion, rearing, grooming and the behaviour-stimulant effect lasted for approximately 50-60 min. At higher doses (20-40 mg/kg) budipine, administered with RU 24213, caused
Fig 3.11 The effects of budipine (5-40 mg/kg) on the locomotion of drug-naive mice. Data represent means ± S.E.M of 7-24 determinations.

Fig. 3.12 The effects of budipine, either alone (solid) or with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (150 mg/kg) (hatched) in reserpine (5 mg/kg) treated mice. Data are means±S.E.M of 5-47 90 min cumulative counts for reserpine, SKF 38393 and L-Dopa interaction studies and 60 min cumulative counts for the RU 24213 interaction study.
a flattened posture and overall, the animals were much less active than at the lower doses of budipine.

3.2.2.6.5 In conjunction with L-Dopa

Budipine (5-40 mg/kg) had no effect on the response to L-Dopa (150 mg/kg) but an increase at 5 mg/kg and a decrease at 40 mg/kg were apparent but these were not statistically significant F(4,126)=0.668, p=0.616, using 90 min cumulative locomotor counts) (Fig.3.12).

Behavioural observations

Mice administered budipine (5-40 mg/kg) and L-Dopa (150 mg/kg) some forward locomotion, sniffing, rearing and grooming but at the lower doses, the animals exhibited residual reserpine-induced akinesia, indicating that budipine and L-Dopa did not produce a complete recovery. Budipine (40 mg/kg) and L-Dopa treated animals were ataxic and appeared 'collapsed' but paradoxically, some of these animals exhibited stereotyped licking and scrabbling in corners as well as evidence of seizure activity, i.e running fits.

3.2.3 The glutamate release inhibitors

3.2.3.1 Lamotrigine

3.2.3.1.1 In drug-naive mice

Lamotrigine (5-80 mg/kg) decreased spontaneous locomotor activity (F(5,42)=6.735, p<0.001, using 30 min cumulative locomotor counts), causing significant decreases at all the doses tried (Fig.3.13).

Behavioural observations

Mice injected with lower doses of lamotrigine (5-20 mg/kg) exhibited normal exploratory activity, which included sniffing, grooming and fluent forward locomotion. Higher doses of lamotrigine (40-80 mg/kg) induced hind limb abduction and muscle relaxation causing a 'flattened' posture which was worse at 80 mg/kg. Animals treated with lamotrigine (80 mg/kg) also showed a loss of the righting reflex. This dose was therefore not used in further experiments.
3.2.3.1.2 In monoamine-depleted mice

Lamotrigine (5-40 mg/kg) had no statistically significant effect on the akinesia induced by reserpine (5 mg/kg) (F(4,42)=2.05, p=0.1067, using 30 min locomotor counts) (Fig.3.14).

Behavioural observations

Lamotrigine (5-40 mg/kg) treated mice showed some behavioural stimulation, which included sniffing, grooming and some forward locomotion which was slow and not fluent.

3.2.3.1.3 In conjunction with SKF 38393

Lamotrigine (5-40 mg/kg) had no significant effect on the response to SKF 38393 (30 mg/kg) (2-way interaction F(4,83)=1.621, p=0.178, using 30 min locomotor counts) (Fig.3.14).

Behavioural observations

Animals treated with lamotrigine (5-40 mg/kg) and SKF 38393 (30 mg/kg) produced a similar array of behaviours which accompany the administration of SKF 38393. At 40 mg/kg lamotrigine, some muscle relaxation was observed.

3.2.3.1.4 In conjunction with RU 24213

Lamotrigine (10 mg/kg) enhanced the response to RU 24213 (5 mg/kg) but this was not significant (2-way interaction F(4,90)=1.633, p=0.174) (Fig.3.14).

Behavioural observations

Lamotrigine (5-40 mg/kg) and RU 24213 treated mice displayed the range of behaviours characteristic of RU 24213 treatment with the exception of 40 mg/kg lamotrigine which also induced a collapsed posture.

3.2.3.1.5 In conjunction with L-Dopa

Lamotrigine (5-20 mg/kg) had no effect on the effect of L-Dopa (150 mg/kg) but 40 mg/kg lamotrigine potentiated the locomotor response to L-Dopa (2-way interaction
Fig 3.13 The effects of lamotrigine (5-80 mg/kg) on the locomotion of drug-naïve mice. Data represent means ± S.E.M of 7-24 determinations. All doses were given as 30 min pre-treatments. * represents p<0.05, **, p<0.01 vs vehicle injected controls by Dunnett’s t-test.

Fig. 3.14 The effects of lamotrigine, either alone (solid) or with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (150 mg/kg) (hatched) in reserpine (5 mg/kg) treated mice. Data are means ± S.E.M of 5-38 animals. All drugs were administered as 30 min pre-treatments. # represents p<0.01 vs L-Dopa-treated controls by Dunnett’s t-test.
Fig 3.15 The effects of clonidine (0.5–2mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data represent means ± S.E.M of 8–38 determinations. All drugs were given as 30 min pre-treatments. * represents p<0.05 and ** p< 0.01 vs L-Dopa treated controls by Dunnett's t-test.
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F(4,114)=3.291, p=0.014) (Fig.3.14).

Behavioural observations

The locomotor response to lamotrigine (5-40 mg/kg) and L-Dopa injections was fluent, after an initial muscular relaxation which was transient. Other behaviours seen in these mice were stereotyped sniffing, rearing, grooming and occasional licking.

3.2.3.2 Clonidine

3.2.3.2.1 In conjunction with L-Dopa in monoamine-depleted mice

Previous experiments done in this laboratory have investigated the effects of clonidine in drug-naive, reserpine-treated mice, either alone or in combination with SKF 38393 and RU 24213 (Starr and Starr, 1994b). In this study, this work is continued and the interaction, if any, between clonidine and L-Dopa is determined.

The interaction between clonidine and L-Dopa was significant, with 1 and 2 mg/kg clonidine increasing the response to L-Dopa (p<0.05 and p<0.01 respectively) (Fig.3.15).

Behavioural observations

Clonidine and L-Dopa treated animals exhibited stereotyped sniffing and forward locomotion with rearing and a little jumping and some scrabbling in corners.

3.2.4 The polyamine site antagonist, eliprodil

3.2.4.1 In drug-naive mice

Eliprodil (5-80 mg/kg) had no effect on spontaneous locomotor activity (F(5,35)=0.8114, p=0.5508) (Fig.3.16).

Behavioural observations

Animals treated with eliprodil (5-40 mg/kg) exhibited sniffing, grooming, forward locomotion, some of which was peripheral in nature, and rearing. Eliprodil, 80 mg/kg, and some of the 40 mg/kg injected mice showed postural abnormalities and mild ataxia was observed after 80 mg/kg eliprodil treatment.

3.2.4.2 In monoamine-depleted mice
Eliprodil (5-80 mg/kg) had no effect on reserpine-induced akinesia (F(5,45)=1.184, p=0.334, using 90 min cumulative locomotor counts) (Fig.3.17).

Behavioural observations

Eliprodil (5-80 mg/kg) treated mice displayed some sniffing, grooming and rearing with forward locomotion. Mice administered 80 mg/kg eliprodil exhibited seizure activity, including 'jumpiness'.

3.2.4.3 In conjunction with SKF 38393

The response to SKF 38393 (30 mg/kg) was not affected by eliprodil (5-40 mg/kg) administration (2-way interaction F(4,88)=0.369, p=0.83) (Fig.3.17).

Behavioural observations

Mice treated with eliprodil (5-20 mg/kg) and SKF 38393 displayed forward locomotion, sniffing, grooming, rearing and some vacuous chewing seen at 20 mg/kg eliprodil. At 40 mg/kg eliprodil, forepaw myoclonus and a 'collapsed' posture were observed in addition to the behaviours seen at the lower doses of eliprodil.

3.2.4.4 In conjunction with RU 24213

Eliprodil (5-40 mg/kg) administered with RU 24213 (5 mg/kg) did not alter the response to RU 24213 (2-way interaction F(4,94)=1.043, p=0.39, using 60 min cumulative locomotor counts) (Fig.3.17).

Behavioural observations

The animals administered eliprodil (5-40 mg/kg) and RU 24213 exhibited some behavioural arousal which included sniffing, rearing some locomotion. At 20 and 40 mg/kg eliprodil and RU 24213, there were instances of a 'flattened' posture and 'dragging' locomotion.
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Fig. 3.16 Lack of effect of eliprodil (5–80 mg/kg) on the locomotion of drug-naive mice. Data represent means ± S.E.M of 6 determinations.

Fig. 3.17 The effects of eliprodil, either alone (solid) or with SKF 38393 (30 mg/kg) (cross-hatched), RU 24213 (5 mg/kg) (open) or L-Dopa (150 mg/kg) hatched in reserpine (5 mg/kg) treated mice. Data are means ± S.E.M of 6–47 30 min cumulative counts in the reserpine study, the SKF 38393 and RU 24213 interaction studies and 90 min cumulative counts for the L-Dopa interaction study. All drugs, with the exception of the treatments in the L-Dopa interaction study in which the drugs were administered immediately prior to behavioural observation, were given as 30 min pre-treatments.
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3.2.4.5 In conjunction with L-Dopa

Eliprodil (1-40 mg/kg) did not alter the response to L-Dopa (150 mg/kg) (2-way interaction F(5, 128)=1.106, p=0.361) (Fig.3.17).

Behavioural observations

The mice administered eliprodil (1-40 mg/kg) and L-Dopa exhibited sniffing, rearing, grooming and forward locomotion. Seizure activity was also seen in some cases after eliprodil (1 and 5 mg/kg) administration. Eliprodil, 40 mg/kg, and L-Dopa injected mice were largely inactive, only showing occasional sniffing.

3.2.5 The strychnine-insensitive glycine site antagonist, (±) HA-966

3.2.5.1 In conjunction with L-Dopa in monoamine-depleted mice

The effects of (±) HA-966 have been investigated previously in our laboratory (Starr and Starr, 1993a, b, 1994a) in normal and monoamine-depleted mice as well as in interaction studies with SKF 38393 and RU 24213. Therefore, in this study, a continuation of previous work, the interaction, if any, of (±) HA-966 (0.1-1 mg/kg) and L-Dopa (150 mg/kg) was investigated.

(±) HA-966 (0.1-1 mg/kg) enhanced the locomotor response to L-Dopa (150 mg/kg) (p<0.05 at 0.5 mg/kg HA-966 vs L-Dopa treated controls by the Mann-Whitney test) (Fig.3.18).

Behavioural observations

(±) HA-966 (0.1-1 mg/kg) and L-Dopa treated mice exhibited sniffing, rearing, grooming and forward locomotion. The mice were very active after 0.5 mg/kg HA-966 and L-Dopa administration, exhibiting forward locomotion, rearing, sniffing which was stereotyped in some instances.
Fig 3.18 The effects of HA-966 (0.1-1mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data represent means ± S.E.M of 6-38 determinations. All drugs given as 30 min pre-treatments. * represents p<0.05 vs L-Dopa treated controls by the Mann-Whitney test.
3.2.6 The competitive NMDA receptor antagonists

3.2.6.1 CGP 40116

3.2.6.1.1 In conjunction with L-Dopa in monoamine-depleted mice

CGP 40116 has been studied previously in this laboratory, in drug-naive and monoamine-depleted mice as well as in combination with SKF 38393 and RU 24213 (Starr and Starr, 1993a, b, 1994a). These experiments are a continuation of previous work and investigate the effect of CGP 40116 (0.1-1 mg/kg) on the response to L-Dopa (150 mg/kg).

CGP 40116 (0.1-1 mg/kg) had a biphasic effect, decreasing the response to L-Dopa (150 mg/kg) significantly at 0.5 mg/kg (p<0.05 vs L-Dopa treated controls) and increasing it non-significantly at 1 mg/kg (Fig.3.19).

Behavioural observations
Mice injected with 0.1 mg/kg CGP 40116 and L-Dopa were active, showing sniffing, forward locomotion, rearing, grooming while mice administered with 0.25 mg/kg CGP 40116 and L-Dopa treated mice exhibited some sniffing, locomotion and a little rearing. 0.5 mg/kg CGP 40116 and L-Dopa were largely inactive apart from the odd bout of sniffing while 1 mg/kg CGP 40116 and L-Dopa (150 mg/kg) treatment produced sniffing, some forward locomotion which was accompanied by mild ataxia and a flattened posture.

3.2.6.2 CPP

3.2.6.2.1 In conjunction with L-Dopa in monoamine-depleted mice

The effects CPP have already been investigated in this laboratory, in normal and monoamine-depleted mice and whether CPP interacts with SKF 38393 and RU 24213 has already been determined (Starr and Starr, 1993a, b, 1994a). In these experiments, the effect of CPP (0.5-1 mg/kg) on the response of L-Dopa was investigated.

CPP (0.5 mg/kg) enhanced the locomotor effect of L-Dopa (p<0.05) (Fig.3.20).

Behavioural observations
CPP (0.5 mg/kg) and L-Dopa treated animals exhibited sniffing, rearing, forward
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locomotion, and in one mouse, seizure activity and a 'frozen stance' where the animal appears to remain in a certain position for some time, thus appearing 'frozen' while mice treated with 1 mg/kg CPP and L-Dopa showed sniffing, rearing, some forward locomotion.

3.2.7 The AMPA receptor antagonist, NBQX

3.2.7.1 In conjunction with L-Dopa in monoamine-depleted mice

NBQX has been investigated previously in this laboratory, in normal and monoamine-depleted mice and its interactions with SKF 38393 and RU 24213 have also been determined (Starr and Starr, 1993a, b, 1994a). Therefore, this study is a continuation of previous work and investigated the interaction of NBQX and L-Dopa.

NBQX (0.05-1 mg/kg) decreased the response to L-Dopa (150 mg/kg) significantly (p<0.01 at 0.1 mg/kg NBQX and p<0.05 at 0.25 mg/kg NBQX vs L-Dopa treated controls by Mann-Whitney test) (Fig. 3.21).

Behavioural observations

Mice treated with NBQX, 0.05 mg/kg, and L-Dopa exhibited sniffing, rearing and forward locomotion. NBQX, 0.1 mg/kg, 0.25 or 0.5 mg/kg, and L-Dopa treated mice were mostly inactive while 1 mg/kg NBQX and NBQX and L-Dopa injected mice produced sniffing, grooming and forward locomotion which was accompanied by a 'flattened' posture and muscular relaxation in the hind region.
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Fig 3.19 The effects of CGP 40116 (0.1–1 mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data represent means ± S.E.M of 6–38 animals. All drugs were given as 30 min pre-treatments. * represents p<0.05 vs L-Dopa treated controls vs the Mann-Whitney test.

Fig 3.20 The effects of CPP (0.5–1 mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data represent means±S.E.M of 6–38 determinations. All drugs given as 30 min pre-treatments. * represents p<0.05 vs L-Dopa treated controls by Student’s t-test.
Fig 3.21 The effects of NBQX (0.05-1 mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data represent means ± S.E.M of 6-38 determinations. All drugs were given as 30 min pre-treatments. * represents p<0.05 and ** represents p<0.01 vs L-Dopa treated controls by the Mann–Whitney test.
3.2.8 Dose-response relationship of the interaction of MK 801 with L-Dopa

MK 801 (0.01-0.1 mg/kg) interacted synergistically with L-Dopa (150 mg/kg). A significant increase was observed with 0.025 mg/kg MK 801 and L-Dopa administration (p<0.01) (Fig. 3.22).

Behavioral observations

Mice injected with L-Dopa and (0.01-0.025 mg/kg) MK 801 were active, displaying forward locomotion, some of which was around the periphery of the cage. Sniffing, rearing, grooming and some scrabbling in corners and licking at 0.025 mg/kg MK 801. 0.05 and 0.1 mg/kg MK 801 and L-Dopa injected mice were additionally flattened in posture and mildly ataxic.

Fig.3.22 The effect of MK 801 (0.01-0.1 mg/kg) on the locomotor response of L-Dopa (150 mg/kg). Data are means ± S.E.M of 11-27 determinations. * represents p<0.01 vs L-Dopa treated controls by Student's t-test.
Table 3.1 A summary table of the locomotor effects of the glutamate antagonists tested, either alone or in combination with a D<sub>1</sub> agonist, SKF 38393, a D<sub>2</sub> agonist, RU 24213 or a dopamine precursor, L-Dopa.

<table>
<thead>
<tr>
<th>Glutamate antagonists</th>
<th>In drug-naive mice</th>
<th>In reserpine-treated mice</th>
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<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>+SKF 38393</td>
</tr>
<tr>
<td>Amantadine</td>
<td>+</td>
<td>NA</td>
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<tr>
<td>Memantine</td>
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<td>Dextro</td>
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<td>NA</td>
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<tr>
<td>NBQX</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MK 801</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

+ indicates an enhancement of locomotion by the glutamate antagonists when administered alone or a potentiation of the response to dopamine agonists. - indicates a reduction in locomotion when administered alone or an attenuation of the response to the dopamine agonists. NE indicates no change in the response. NA indicates experiment not done.

Dextro-Dextromethorphan, PCP-Phencyclidine
3.3 Discussion

3.3.1 The effects of the NMDA channel blockers

The NMDA channel blockers, memantine, amantadine, dextromethorphan and PCP, all had a locomotor stimulant effect in drug-naive mice, while ketamine and budipine had no effect on locomotion. Higher doses of dextromethorphan (80 mg/kg), budipine (10-40 mg/kg), amantadine (20 and 40 mg/kg) tended to reduce spontaneous locomotion but this was not statistically significant. No such trend was seen with PCP, ketamine or memantine, possibly because the doses used were not high enough. These results compare favourably with studies done previously in this laboratory (Starr and Starr, 1993a, b, 1994a) and by other groups (Danysz et al., 1994a; Jerram et al., 1996). Danysz et al. (1994a) reported that MK 801, PGP, ketamine and memantine enhanced horizontal activity while amantadine produced a slight inhibition but dextromethorphan was ineffective. The reason for the disparity in the effects of ketamine, amantadine and dextromethorphan could be due to different doses being used, usually higher than the doses with which we obtained a significant increase, with the exception of ketamine, in which case the highest dose we used (80 mg/kg) was lower than the dose used by Danysz et al. (1994a) to enhance locomotion (100 mg/kg). Ketamine (30-150 mg/kg) was found to enhance locomotion in mice by Irifune et al. (1996) but their study differed from ours in that their mice were acclimatized to the observation cages for 30 min, so in effect they started with a lower basal activity than our mice did and thus, their model was more sensitive to minor increases in activity compared to ours. In this study, budipine appeared to increase spontaneous locomotion but this was not deemed to be statistically significant. An in vitro study showed that budipine inhibited dopamine uptake while increasing dopamine release from rabbit caudate slices (Jackisch et al., 1994). However, budipine also has weak inhibitory activity at muscarinic receptors (Menge and Brand, 1982). Previous studies have reported that budipine has a low propensity for side-effects but as we show in this study, 40 mg/kg budipine did induce motor disability in the form of ataxia and 'dragging' locomotion.

High doses of MK 801, PCP, ketamine, memantine, amantadine and dextromethorphan
have been reported to induce ataxia (Danysz et al., 1994a; Jerram et al., 1996) which is in agreement with the observations made in this study. A curvilinear dose-response is characteristic of the NMDA channel blockers and may be used to check if the drug does act via the NMDA receptor ion-channel site.

In this study, dextromethorphan had a very distinct behavioural profile at 40 mg/kg at which dose two clear-cut populations were noticed. The non-responders (5/9) were unaffected by 40 mg/kg dextromethorphan but the responders (4/9) exhibited a marked increase in locomotion. The behavioural arousal seen in the responding mice is reminiscent of the effects of PCP (Iwamato, 1984) and dextrorphan, the demethylated metabolite of dextromethorphan (Szekely et al., 1991). Dextrorphan is a more potent NMDA channel blocker than dextromethorphan (Rogawski, 1993) and may induce behavioural stimulation by causing the release of dopamine from nigrostriatal axon terminals (Hondo et al., 1994). This clear-cut difference in response may be due to the individual ability to convert dextromethorphan to dextrorphan. This variability has also been seen in humans as a small percentage of the population abuse dextromethorphan, possibly due to their ability to metabolise dextromethorphan to dextrorphan (Mussachio, 1990). The demethylation of dextromethorphan has also been reported to be strain-dependent in the rat (Bochner et al., 1994). A clearer correlation between the formation of dextrorphan and locomotor enhancement has been made by Wu et al. (1995), who found that after intraperitoneal administration, the bioavailability of dextromethorphan was lower and the formation of dextrorphan was greater than after subcutaneous administration. When they looked at the behavioural responses in the rat, the authors found that while intraperitoneal dextromethorphan enhanced locomotion 60 min post-injection, the subcutaneous injection had no effect (Wu et al., 1995), leading to the conclusion that it is dextrorphan that has an effect on locomotion. We postulate that the responding mice in our study are genetically susceptible to the effects of dextromethorphan, as they may have the ability to demethylate dextromethorphan to the more active dextrorphan. This variability in response is lost at 80 mg/kg dextromethorphan, which causes some muscular relaxation. The bell-shaped dose-response
seen with dextromethorphan is characteristic of that seen with other NMDA channel blockers (Starr and Starr, 1994a).

As the glutamate antagonists are postulated to act by stimulating dopaminergic neurotransmission, these drugs produce behavioural arousal reminiscent of dopamine agonists. The dopaminergic systems in the striatum, the NAc and the cortex are involved in the control of locomotion. The NAc is thought to play a major part in the initiation and regulation of locomotor activity (Ouagazzal et al., 1994). Non-competitive antagonists of the NMDA receptor produce motor activation in rodents which appears to be partially mediated by the release of vesicular dopamine and partially via non-dopaminergic mechanisms, especially at high doses (St.-Pierre and Bédard, 1994).

Only memantine, PCP and ketamine had an antiakinetic effect in the reserpine-treated mice. Memantine and amantadine, both belonging to the aminoadamantane group with memantine being more lipophilic and thus more potent than amantadine, have been found to counteract catalepsy induced by haloperidol (Danysz et al., 1994b; Schmidt et al., 1991) and to attenuate sedation in monoamine-depleted rats (Danysz et al., 1994a). Jackisch et al. (1992) reported that memantine had a direct dopamine releasing effect on dopamine storage granules. As reserpine does not prevent synthesis, only storage, it is feasible that dopamine continues to be synthesised and it is the newly synthesised dopamine on which memantine exerts its effect. The antiakinetic effect of memantine in this study was seen after a delay of approximately 60 min, which is consistent with dopamine being synthesised then released. PCP and ketamine also reversed akinesia in reserpine-treated mice at high doses. The locomotor response to high doses of PCP was different from that seen with the other glutamate antagonists, in that there were periods of circular locomotion. However, as this was seen only at high doses, the circular locomotion, without any directional bias, could be due to the non-specificity of PCP (Murata and Kawasaki, 1993). PCP also acts at sites apart from the NMDA ion channel site, such as sigma sites (Itzhak and Alerhand, 1989; Quirion et al., 1988). These findings bear out the results obtained in the current study but it should be noted that the locomotor-reinstating properties were only seen at high doses of these agents.
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There is a large body of evidence showing that the NMDA channel blockers tend to act via an indirect effect on dopaminergic activity. Thus, as reserpine depletes a considerable proportion of vesicular dopamine, the ineffectiveness of the NMDA channel blockers in reinstating locomotion, especially at low doses, is understandable. At high doses, some mobility is seen and this may be attributed partly to an action at other neurotransmitter systems, possibly the serotonergic system or at sigma receptors (Hiramatsu et al., 1989). Unlike, Carlsson and Carlsson (1989a, b), we have never noticed a reversal of akinesia by low doses of the channel blockers, administered on their own.

Budipine had no effect on the akinesia of reserpine-treated mice. In vivo microdialysis showed that budipine had no effect on the levels of dopamine or its metabolites in the striatum (Klockgether et al., 1996). However, budipine, like MK 801 and other glutamate antagonists, was reported to be antiscataleptic in neuroleptic-induced catalepsy (Menge and Brand, 1982) although this finding on its own does not prove an effect on dopaminergic activity, as anticholinergics are also effective in this model. Budipine is thought to act predominantly via antagonism of the NMDA receptor but has additional antimuscarinic activity but is without any dopaminomimetic activity (Klockgether et al., 1996). Budipine was found to be effective as an adjunct to L-Dopa in a clinical trial with PD patients, having a marked antitremor effect (Jellinger and Bliesath, 1987). As the therapeutic effect of budipine is particularly noticed with tremor, and as our reserpine-treated mice tend not to present with tremor, it may be that our model is not the best model to observe this effect. It may be possible that the budipine does not have a locomotor stimulant effect per se and that its primary effect is on tremor (Klockgether et al., 1996). It is conceivable that tremor is the result of compromised cholinergic neurones in the striatum and thus the antimuscarinic action of budipine would then prove to be an asset in alleviating tremor. Other muscarinic antagonists have also been shown to be more effective at alleviating tremor than improving mobility. Our study with budipine appears to be the first to look at the locomotor effects of budipine, as well as interactions with dopamine agonists. We report that budipine has no significant effect on its own or in combination with dopamine agonists, although by virtue of its antitremor
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effect, it has found a place in the PD symptom-control.

Interaction studies with the dopamine D₁ receptor agonist, SKF 38393, the D₂ receptor agonist, RU 24213 and the dopamine precursor, L-Dopa, showed that only dextromethorphan interacted synergistically with SKF 38393 and that none of the NMDA channel blockers investigated interacted with RU 24213 or L-Dopa. Memantine has been investigated in a clinical trial as an adjunct to L-Dopa in 14 PD sufferers and was found to be beneficial in five patients but psychomotor disturbances were seen in two patients (Rabey et al., 1992). In the present study, synergism was not found to exist between most of the NMDA channel blockers and the dopamine agonists, possibly due to the wide variability in the individual responses of the mice, especially to reserpine treatment. Previous studies have found there to be a positive interaction between the NMDA channel blockers and L-Dopa (Klockgether and Turski, 1990; Klockgether et al., 1991). Also, it is conceivable that the doses we used were too high and we might have bypassed a synergistic interaction by administering higher doses of the channel blockers. Such an effect is seen with the interaction between MK 801 and L-Dopa (see 3.3.7 this chapter), i.e very low doses were found to potentiate the effect of L-Dopa while higher doses of MK 801 were ineffective. It has been previously postulated that the glutamate antagonists enhance the response to L-Dopa by facilitating the D₁ component of L-Dopa, as it has been observed that the same doses also potentiated SKF 38393 (Morelli and Di Chiara, 1990; Morelli et al., 1992). However, this is too simplistic a viewpoint as the data in 3.3.7 and presented in various papers (Goodwin et al., 1992; Svensson et al., 1992) show. A large number of the NMDA channel blockers do potentiate the response to SKF 38393 while having no effect on the RU 24213 response or even decreasing it (Starr and Starr, 1993a, b, 1994a). In this study, a high dose of dextromethorphan was required to potentiate the SKF 38383 response but this same dose had no effect on the L-Dopa response. In light of the data obtained in the dose-response interaction study with L-Dopa and MK 801, very low doses of dextromethorphan may be required to see such a potentiation.

The NMDA receptor-linked ion channel blockers like memantine, MK 801,
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Phencyclidine are equipotent at both the NMDAR2A and the NMDAR2B subunits, while dextromethorphan is four times more selective for the NMDAR2A subunit (Yakamura et al., 1993). These NMDAR2 subunits are found in abundance in the striatum/NAc, thus it may be possible that these agents may be acting either in the striatum or the NAc. The NAc is thought to be more important as a site for inducing the behavioural arousal, which includes enhanced locomotion, that follows treatment with NMDA channel blockers (Narayanan et al., 1996).

3.3.2 The effects of the glutamate release inhibitors, lamotrigine and clonidine

Lamotrigine, which inhibits the release of glutamate by blocking voltage-gated Na⁺ channels (Meldrum and Leach, 1994; Messenheimer, 1994), decreased spontaneous locomotor activity. Löschmann et al. (1995) reported that lamotrigine failed to increase locomotion and Leach et al. (1991) and Baxter et al. (1990) reported that in a drug discrimination study, lamotrigine did not produce PCP-like effects. High doses of lamotrigine, in this study, produced motor impairment characterised by hind limb abduction, muscle relaxation, a flattened posture and a loss of the righting reflex, all of which impeded locomotion. The appearance of motor deficits, i.e myorelaxation and sedation, at high doses is characteristic of glutamate blockers.

Lamotrigine did not reverse the akinesia of reserpine-treated mice in the present study. Löschmann et al. (1995) reported that lamotrigine was ineffective in reserpine-treated or 6-OHDA lesioned rodents. However, Jones-Humble et al. (1993) reported that lamotrigine prevented the dopamine depletion seen after MPTP treatment in C57 black mice. Enadoline, a K-opiate receptor agonist, which is thought to act via the inhibition of striatal and nigral glutamate release (Mitchell et al., 1995), has been reported to reverse the akinesia of reserpine-treated rodents and 6-OHDA and MPTP treated primates, albeit weakly (Mitchell et al., 1995).

We found that lamotrigine did not interact with SKF 38393 while 10 mg/kg lamotrigine appeared to increase the response to RU 24213 but this was not significant when compared to the counts produced by RU 24213 alone, with the statistical test
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utilised in this study. Lamotrigine (40 mg/kg) increased the response to L-Dopa synergistically and the resultant locomotion was fluent with stereotyped licking and sniffing. This interaction with L-Dopa is in line with the clinical trial showing that combined therapy of lamotrigine and L-Dopa was beneficial in PD sufferers (Zipp et al., 1993). However, a later double-blind trial found lamotrigine to be without effect (see Löschmann et al., 1995). The way in which lamotrigine interacted with the different dopamine agonists, is opposite to what is normally seen with some of the other glutamate antagonists, i.e a potentiation of D₁ response and usually a decrease in or no effect on the D₂ response (Goodwin et al., 1992; Morelli et al., 1992; Starr, 1995a; Starr and Starr, 1994a), while with lamotrigine, there is an increase, albeit non-significant, in the response to the D₂ agonist but no effect on the D₁ response. Although, suggesting that the an enhanced D₂ response leads to the potentiation in the response to L-Dopa would be a gross simplification, as the dose required to potentiate the L-Dopa response (40 mg/kg) is much higher than the dose needed to enhance the D₂ response (10 mg/kg). The study done by Löschmann et al. (1995) showed that lamotrigine did not change the locomotor effect evoked by apomorphine, a mixed D₁/D₂ agonist or by the D₂ agonist, lisuride, which at first glance appears to contradict our results but the effects of different dopamine D₂ agonists are complex, and different D₂ agonists interact differently with glutamate antagonists. Goodwin et al. (1992) reported that while MK 801 interacted synergistically with RU 24213 in reserpine-treated mice, it did not do so with lisuride. The same may be true of the lack of interaction of apomorphine with lamotrigine and the contrasting potentiation of the response to L-Dopa.

Clonidine, a partial agonist at the α₂ adrenoceptor, in this study markedly and dose-dependently potentiated the response to L-Dopa. In a previous study, clonidine produced sedation in drug-naïve mice, had no effect in reserpine-treated mice, did not interact with SKF 38393 but enhanced the effect to RU 24213 and apomorphine (Starr and Starr, 1994b). Clonidine is able to inhibit the release of glutamate presynaptically and like lamotrigine, clonidine has been found to potentiate the response to L-Dopa and to D₂ agonists but was without effect on the response to D₁ agonists (Rubinstein et al., 1989;
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Starr and Starr, 1994b). It has also been reported that subthreshold doses of MK 801, PCP and the competitive NMDA antagonist, SDZ EAA 494 (D-CPPene) all interact synergistically with clonidine in monoamine-depleted mice (Carlsson and Svensson, 1990b).

It may be possible that this profile of interactions is characteristic of agents which act presynaptically to prevent glutamate release. Thus, unlike the postsynaptically acting glutamate antagonists which facilitate D₁ responses, these presynaptically acting drugs potentiate D₂ responses.

3.3.3 The effects of the polyamine site antagonist, eliprodil

Eliprodil had an inconsistent effect on the spontaneous locomotion of drug-naive mice with 5-10 and 40-80 mg/kg increasing locomotion non-significantly. High doses of eliprodil also induced postural abnormalities and mild ataxia. There is some evidence that eliprodil acts as a dopamine uptake blocker and enhances dopamine release in striatal tissues (Woodward and Harms, 1992). However, as eliprodil had no significant effect in this study, the effect on dopamine release or uptake may not be sufficient to translate this into an effect on behaviour. Locomotion was impaired, due to mild ataxia, after the administration of a high dose of eliprodil, which has also been observed by Ginski and Witkin (1994), who noticed that mice fell off an inverted screen suggesting that motor coordination had been impaired. A disordered gait has been noticed before with high doses of eliprodil (see Starr, 1995a) and with ifenprodil, which caused muscle relaxation without inducing ataxia (Murata and Kawasaki, 1993). Eliprodil, therefore, in intact mice has no real effect on spontaneous locomotion but induces mild ataxia with muscular relaxation.

Eliprodil did not reverse the akinesia of reserpine-treated mice but in a fraction of mice there appeared to be a stimulant effect which was not statistically significant, possibly due to the large variations between the animals. Variations in response have been seen previously with dextromethorphan in naive mice (Kaur and Starr, 1995) and PCP in a differential reinforcement paradigm (Sanger, 1992). The reason for the variation
observed with eliprodil may be due to interactions with other neurotransmitter systems, possibly noradrenergic, serotonergic systems or actions at sigma receptors (Chenard et al., 1991; Hashimoto and London, 1995). Ifenprodil was found by Mitchell et al. (1995) to potently reverse the parkinsonism of 6-OHDA lesioned marmosets which contrasts with the ineffectiveness of ifenprodil in a pilot study in Parkinson’s sufferers (Montastruc et al., 1992).

In this study, we found that eliprodil did not interact synergistically with SKF 38393, RU 24213 or L-Dopa. If anything, 40 mg/kg eliprodil appeared to reduce the response of RU 24213 and L-Dopa but this could be due to the appearance of motor deficits, which impeded locomotion, and due to sedation. From the results obtained here, eliprodil does not appear to have an antiparkinsonian effect, either alone or as an adjunct to dopamine-based treatments.

3.3.4 The interaction between the glycine site antagonist, (±) HA-966, and L-Dopa

As endogenous glycine and glutamate are necessary for the function of NMDA receptors (Johnson and Ascher, 1987), it stands to reason that if either glycine antagonists or glutamate antagonists are administered, NMDA receptor function is compromised. Thus, we would expect HA-966 to act beneficially in PD models.

(±) HA-966 has been investigated previously in this laboratory by Starr and Starr (1993a, b, 1994a) in drug-naive and monoamine-depleted mice on its own or in interaction studies with SKF 38393, RU 24213 and apomorphine. In these studies, (±) HA-966 reduced spontaneous locomotion, did not have any effect on reserpine-induced akinesia but enhanced the effect of SKF 38393, while it attenuated the effect of RU 24213. HA-966 had no effect on the activity elicited by the combined treatment of SKF 38393 and RU 24213 or by apomorphine (Starr and Starr, 1993b). In the current study, the interaction between (±) HA-966 with L-Dopa was explored. When the dose-effect relationship of the interaction between (±) HA-966 and SKF 38393 was looked at, 0.4 mg/kg (±) HA-966 was found to potentiate the effect of SKF 38393 (Starr and Starr, 1994a) which is remarkably close to the dose, 0.5 mg/kg, which we find in this study to
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enhance the response to L-Dopa. It is, hence, tempting to suggest that the D₁ component of the response to L-Dopa is enhanced by HA-966. Acute injections of HA-966 have been reported to increase dopamine levels as well as levels of DOPAC and HVA in the striatum (Broxterman et al., 1979) while in reserpine-treated rats, systemic or intrastratal HA-966 reversed the parkinsonian akinesia (Carroll et al., 1995; Slusher et al., 1994). The difference between the study done by Carroll et al. (1995) and the study done previously in this laboratory, which found that systemic HA-966 was ineffective against reserpine-induced akinesia, could be due to the fact that Carroll et al. (1995) used (+) HA-966, the active isomer, whereas in the study done by Starr and Starr (1993a, b) the racemic mixture of HA-966 was used. Another difference contributing to the differing findings may be the different doses used, i.e. while Carroll et al. (1995) used 10-100 mg/kg (+) HA-966, the dose utilised by Starr and Starr (1993a, b) was 2 mg/kg (±) HA-966. Carroll et al. (1995) also found that 60 and 100 mg/kg (+) HA-966 had muscle relaxant effects. Variation in the responses to the isomers has been noticed; a stimulant effect is seen with (+) HA-966 but sedation is observed after (-) HA-966 (Fletcher and Lodge, 1988). It may also be that the (+) HA-966 isomer crosses the blood-brain barrier more effectively than the (-) HA-966 isomer (Kemp and Leeson, 1993). However, in this study we obtained a stimulant effect when (±) HA-966 was administered with L-Dopa. It may be that such a combination is more conducive to the stimulant effect overshadowing the sedative action as no sedation was observed.

No motor disability was noted in the current study which is in agreement with Carroll et al. (1995) who observed that after intrastratal or systemic administration in reserpine-treated rodents, HA-966 induced no anaesthetic-like effects. In a drug discrimination study, (±) HA-966 did not substitute for PCP, suggesting that the behavioural profiles of these two drugs are distinct (Singh et al., 1990). Thus, HA-966 may have less propensity to produce the side-effects usually seen after the ion channel blockers.

3.3.5 The interaction between the competitive NMDA antagonists and L-Dopa

Starr and Starr (1993a, b, 1994a) found previously that CGP 40116 and CPP
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inhibited locomotion in reserpine-treated mice but they potentiated the locomotor response of SKF 38393, while the response to RU 24213 was reduced or unaltered by CGP 40116 and unchanged by CPP. These drugs also had no effect on the combined treatment of SKF 38393 and RU 24213 or on the response to apomorphine. Ataxia was evident after CGP 40116 while seizures were seen after combined CPP and SKF 38393 treatment. In the current study, we found that while CGP 40116 (0.5 mg/kg) reduced the locomotor response of L-Dopa, CPP acted synergistically to enhance the response to L-Dopa.

CGP 40116 has previously been found to enhance the antiparkinsonian effect of L-Dopa in MPTP-treated marmosets (Wüllner et al., 1992) and in MPTP-treated mice, a very low dose of CGP 40116 (0.01 mg/kg) facilitated the antiakinetic effect of a subthreshold dose of L-Dopa, as well as being effective when administered alone at 0.003 and 0.03 mg/kg (Fredriksson et al., 1994). Maj et al. (1993b), however, found that CGP 37849 and CGP 39551 inhibited the locomotion produced by L-Dopa, which concurs with our finding that CGP 40116 reduced the response of L-Dopa. CGP 40116 administered into drug-naive mice, was reported to suppress locomotion, probably by not having an effect on dopamine turnover (Starr and Starr, 1994a). It is possible that in this study CGP 40116 reduced the response of L-Dopa by inducing muscular relaxation which impeded mobility, as when observed the animals were largely inactive and remained flattened. CPP has been reported to increase the circling caused by apomorphine in 6-OHDA hemilesioned rats (Wachtel et al., 1992) and potentiated the antiakinetic response of L-Dopa in monoamine-depleted rats (Klockgether and Turski, 1990; Klockgether et al., 1991). These findings are in agreement with our observation that CPP interacts synergistically with L-Dopa.

The competitive NMDA antagonists are thought to act on neurotransmitter systems other than the dopaminergic system. A study determining the effect of competitive NMDA antagonists on dopamine turnover found that D-CPPene (Svensson et al., 1991) and CGS 19755 reduced the levels of 3-MT in the striatum while CGS 19755 decreased the levels of dopamine in striatal dialysate but instead increased the levels of 5-hydroxyindole acetic acid (5-HIAA), a metabolite of 5-HT, at the time points at which locomotion was
increased (Waters et al., 1996). CPP was reported not to have any effect on dopamine turnover in rat brain areas (Rao et al., 1991). NPC 12626, a competitive NMDA antagonist, produced locomotor hyperactivity equivalent to that seen with PCP. However, the effect of PCP, but not the effect of NPC 12626, was blocked by 6-OHDA lesions of the NAc. Intravenous injections of NPC 12626 or CGS 19755 were unable to alter the activity of A10 dopamine neurones which contrasted with the effect of PCP, which produced a dose-dependent increase/decrease in firing rate (French et al., 1991). Again, this finding shows that the competitive antagonists are unable to activate mesolimbic dopaminergic systems, unlike PCP (French et al., 1991).

Under the scheme postulated by Carlsson (1993) and Svensson et al. (1992), low doses of competitive NMDA antagonists are thought to interfere preferentially with the phasic activity mediated by the direct pathway, therefore causing behavioural depression. If, however, the doses are increased there is greater possibility of interfering with the tonic activity of the indirect pathway, thus leading to behavioural stimulation. Therefore, the doses used may play an important part in the response seen.

3.3.6 The interaction between the AMPA receptor antagonist, NBQX and L-Dopa

The current study is intended to be a continuation of work done previously in this laboratory where the effects of NBQX in monoamine-depleted mice and interactions with SKF 38393 and RU 24213 were investigated (Starr and Starr, 1993a, b, 1994a). The earlier study found that NBQX failed to reverse akinesia in reserpine-treated mice, but interacted synergistically with SKF 38393 while having no effect on the response to RU 24213. NBQX also had no effect on the locomotor response induced by combined administration of SKF 38393 and RU 24213 (Starr and Starr, 1993a, b, 1994a). In this study, NBQX reduced the response to L-Dopa with a marked decrease seen at 0.1 and 0.25 mg/kg NBQX. There was some indication of motor disturbance with muscle relaxation in the hind regions of the mice and a flattened posture. Sedation has been noticed before with high doses of NBQX (Starr and Starr, 1994a). Danysz et al. (1994a) found that NBQX induced a slight inhibition in spontaneous horizontal activity while
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NBQX was reported to diminish the hyperlocomotion induced by AMPA injected into the NAc (Boldry et al., 1993). NBQX (1-10 mg/kg) was found to act synergistically with a dose of L-Dopa (50 mg/kg), known to have no effect by itself (Klockgether et al., 1991). In contrast to the observation made with MK 801 and L-Dopa, where a very low dose of MK 801 (0.025 mg/kg) potentiated the response to L-Dopa (Kaur and Starr, 1994), in this case, it may be that we did not administer a high enough dose of NBQX to see a potentiation of the response of L-Dopa. Also, the dose of L-Dopa we used was suprathreshold compared to the threshold dose used by Klockgether et al. (1991), which may be another reason for the discrepant findings. NBQX (2.5-10 mg/kg) was found to have antiparkinsonian actions in MPTP-treated monkeys (Klockgether et al., 1991; Löschmann et al., 1991) but Luquin et al. (1993) reported that NBQX was ineffective in MPTP-treated monkeys. The reason for this discrepancy could be previous chronic drug exposure in the subjects used by Luquin et al. (1993) causing changes in sensitivity or due to the doses of NBQX used. Luquin et al. (1993) used 1 mg/kg NBQX while Klockgether et al. (1991) found that low doses of NBQX worsened the parkinsonism of the monkeys with a beneficial effect only seen at high doses. NBQX, 12.5 mg/kg, induced rotations in unilaterally 6-OHDA lesioned rats (Löschmann et al., 1991). A previous study found that while NBQX interacted with SKF 38393 in reserpine-treated rodents (Starr and Starr, 1994a), it failed to have any effect on the D₁ response in MPTP-treated monkeys (Luquin et al., 1993). From the above results, we infer that simply extrapolating results from rodents to primates, may be unwise in certain cases, especially when it comes to determining interactions with dopamine agonists. The decrease we obtained in the response to L-Dopa at a low dose of NBQX can only be compared to the worsening of parkinsonism observed in MPTP-treated primates with low doses of NBQX (Klockgether et al., 1991). In the catalepsy model, NBQX was ineffective against or decreased the anticaataleptic action of MK 801 and was ineffective when combined with L-Dopa (Hauber and Andersen, 1993; Zadow and Schmidt, 1994).

NBQX seems to act further downstream of the striatum, as focal injections into the striatum were ineffective in reserpine-treated rats but a reversal of akinesia was seen when
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NBQX was administered into the GP, the STN or the SNr (Klockgether and Turski, 1990; Young et al., 1990). DNQX, an AMPA antagonist, injected into the NAc or the ventral pallidum, reduced the hyperactivity induced by amphetamine, which again goes against the view that administration of glutamate antagonists facilitate dopaminergic activity (Willins et al., 1993). It is thus conceivable that the antiakinetic effects induced by NBQX from the level of the STN/GP/SN may be negated by its deleterious effects from further upstream, i.e. the NAc and/or the striatum and thus the site into which NBQX is administered will determine the response seen.

3.3.7 The dose-relationship of the interaction between MK 801 and L-Dopa

MK 801 has previously been found to enhance spontaneous locomotion and to have no effect or a very weak effect on reserpine-induced akinesia. MK 801 has been reported to act synergistically to potentiate the locomotor response of SKF 38393 while having no effect on the response to RU 24213 (Starr and Starr, 1993a, b). MK 801 injected systemically into rodents at low doses (0.05-0.3 mg/kg) produced stimulation of coordinated locomotion (Maj et al., 1991; Ouagazzal et al., 1994; Starr and Starr, 1994a; Willins et al., 1993). In studies carried out by Morelli’s group (Morelli and Di Chiara, 1990; Morelli et al., 1992), the dose of MK 801 which potentiated the effect of SKF 38393, also increased the response to L-Dopa and apomorphine in 6-OHDA lesioned rats.

It has previously been postulated that the glutamate antagonists act to facilitate the antiparkinsonian effect of L-Dopa by enhancing the D_1-dependent component of the L-Dopa response (Boldry et al., 1993; Goodwin et al., 1992; Starr and Starr, 1993a, b; Svensson et al., 1992). However, the doses at which MK 801 potentiated the effect of SKF 38393, had a detrimental effect on the locomotor response evoked by apomorphine (Starr and Starr, 1993b), or by the combined treatment of SKF 38393 and RU 24213 (Starr and Starr, 1993a). Some of the NMDA or AMPA antagonists have been reported to potentiate the response to L-Dopa (Klockgether et al., 1991; Löschmann et al., 1991; Wüllner et al., 1992) but not that of the D_1 agonists (Domingo and Sheng, 1993; Luquin et al., 1993). Thus, the potentiation of the antiparkinsonian action of L-Dopa by the
glutamate antagonists cannot be simply explained as a potentiation of the D_2 response. This was also noticed by Svensson et al. (1992) who found that MK 801 facilitated the SKF 38393 response but had no effect on the apomorphine response. In this study, we found that MK 801 potentiated the response to a suprathereshold dose of L-Dopa at doses that were much lower than those used to facilitate the response to SKF 38393. Other groups have also found that glutamate antagonists tend to have a biphasic effect on the locomotion, with low doses facilitating it and high doses attenuating it (Goodwin et al., 1992; Klockgether et al., 1991; Morelli et al., 1992; Wachtel et al., 1992). Also as we saw in this study, administering very low doses of MK 801 permits fluent locomotion with motor disability only appearing at doses upward of 0.05 mg/kg. Previous studies using higher doses of MK 801 have found these doses induce motor disturbances which seemed to accompany any motor enhancement (Carlsson and Svensson, 1990b; Kannari and Markstein, 1991) but as we show careful dosing may be required to separate out the motor enhancing and the myorelaxant effects of the glutamate antagonists. 

Glutamate receptor antagonists tend not to have any effect on the response to the D_2 agonist, RU 24213 or may even decrease it (Starr and Starr, 1993a, b, 1994a, b) but as the antiakinetic effect of L-Dopa is thought to predominantly derive from its agonism of D_2 receptors, with D_1 receptors playing a minor role (Clark and White, 1987), and agonists of the D_2 receptor, such as bromocriptine, have been found to be antiparkinsonian, this finding is therefore puzzling. MK 801 is thought to produce locomotor enhancement by facilitating dopaminergic transmission by acting via presynaptic (Whitton et al., 1992) and/or postsynaptic (Gandolfi and Dall'Olio, 1993) mechanisms. In this study, we postulate MK 801 may be enhancing the response to L-Dopa, by possibly increasing the activity of AADC, the enzyme responsible for the bioconversion of L-Dopa to dopamine, thus increasing the synthesis of dopamine and possibly also release. There is some evidence to show that MK 801 does indeed increase the activity of the enzyme AADC (Hadjiconstantinou et al., 1995). Also, MK 801 could potentiate the postsynaptic effect of L-Dopa, which could be acting as a neurotransmitter (Komori et al., 1993). MK 801 could act in any of these or other ways to enhance the
Chapter Three Systemic administration of glutamate antagonists

response to L-Dopa but the separation of the beneficial and detrimental effects of MK 801 by reducing the doses administered shows that the glutamate antagonists cannot be dismissed as potential antiparkinsonian agents.

3.4 Conclusions

We found in this study that a number of the NMDA channel blockers enhanced spontaneous locomotion while lamotrigine reduced it. The animals treated with 40 mg/kg dextromethorphan could be differentiated into non-responders or responders. We postulated that this difference arose from the individual ability to convert dextromethorphan to its more active and PCP-like metabolite, dextrorphan. In the reserpine-treated mice, we found only high doses of some of the NMDA channel blockers were able to reinstate locomotion but this was not at all fluent. Thus, it does appear that the use of these agents as monotherapy in PD is unlikely and they might be more useful as adjuncts to the conventional dopamine-based therapy of PD. When the interactions with the dopamine D₁ or D₂ receptor agonists or with L-Dopa were investigated, only dextromethorphan out of all the channel blockers was able to potentiate the activity of SKF 38393 but it had no effect on the response to L-Dopa. However, both the glutamate release blockers, lamotrigine and clonidine, were able to potentiate the response to L-Dopa, possibly by facilitating the D₂ component of the L-Dopa response. The glycine site antagonist, (±) HA-966, and the competitive NMDA antagonist, CPP, were also able to potentiate L-Dopa but CGP 40116 and the AMPA antagonist, NBQX, reduced the response to L-Dopa.

As these series of experiments show, the responses seen vary with the glutamate antagonists used. Also, these drugs may prove beneficial when used as adjuncts to the dopamine-based therapy of PD, as they would allow a lowering of the dose of L-Dopa, thus, possibly sparing the surviving dopamine neurones from damage caused by the metabolites of dopamine. However, the drugs to be used must be chosen with great care as we see in this study, some glutamate antagonists decrease the response to L-Dopa. Also, as we see in the interaction study between L-Dopa and MK 801, the doses used
must be chosen carefully. The doses of MK 801 required to potentiate the response of L-Dopa were very low, much lower than the doses needed to potentiate the effect of SKF 38393. Thus, the hypothesis that the glutamate antagonists potentiate L-Dopa by enhancing the responding to its D₁ component does not appear to hold true. It is conceivable that these glutamate antagonists may be increasing the conversion of L-Dopa to dopamine and also increasing its release. There is some evidence that MK 801 may indeed be increasing dopamine synthesis by increasing AADC activity (Hadjiconstantinou et al., 1995).

We find that the response of the glutamate antagonists follows a bell-shaped curve, with low doses potentiating the locomotor response and high doses producing motor disabilities such as ataxia and muscle relaxation. These adverse motor side-effects, resulting from the administration of high doses of NMDA receptor antagonists in particular, may arise from the action of these drugs outside the basal ganglia, possibly at sites within the cortex (Brotchie et al., 1991).
CHAPTER FOUR
THE EFFECTS OF GLUTAMATE ANTAGONISTS ADMINISTERED INTO THE STRIATUM OR THE SUBSTANTIA NIGRA OF MONOAMINE-DEPLETED RATS
Chapter Four Glutamate antagonists in the Basal Ganglia

4.1 Introduction

The corticostriatal and/or the subthalamic glutamatergic pathways (Miller and De Long, 1987; Mitchell et al., 1989) are considered to be hyperactive in conditions of dopamine deficiency and therefore, glutamate antagonists may prove beneficial in these conditions by normalising the increased glutamatergic tone (Greenamyre and O'Brien, 1991; Riederer et al., 1992; Rogawski, 1993; Starr, 1995a).

This hypothesis has been tested in models of dopamine deficiency with some success. NMDA and AMPA receptor antagonists administered systemically into monoamine-depleted animals have been able to reinstate at least a modicum of locomotor activity (Carlsson and Carlsson, 1989a; Carlsson and Svensson, 1990b; Clineschmidt et al., 1982; Klockgether and Turski, 1990; Klockgether et al., 1991; Schmidt et al., 1992; Starr, 1993a, b, 1994a, b; Svensson et al. 1991).

The locus/loci of the glutamate receptors which mediate the stimulant and/or detrimental effects produced by glutamate antagonists has/have not been conclusively proven. The general consensus is that the glutamate antagonists induce locomotor stimulant effects from the striatum (Carlsson and Carlsson, 1989a; Schmidt and Bubser, 1989; Schmidt et al., 1990; Yoshida et al. 1991, 1994) and/or the nucleus accumbens (NAc) (Narayanan et al., 1996; Ouagazzal et al., 1994).

Other groups have reported that the stimulant effect of the glutamate antagonists may not be confined to the striatum as direct injections into the substantia nigra (SN), the pallidum, entopeduncular nucleus (EPN) or the cortex have also been found to induce locomotor stimulation (Brothie et al., 1991; Carroll et al., 1995; Klockgether and Turski, 1990; Klockgether et al., 1991; Mitchell et al., 1995; Pierce and Rebec, 1993; Slusher et al., 1994; St-Pierre and Bédard, 1994).

Therefore, it appears that locomotor stimulation induced by glutamate antagonists can arise from striatal as well as extra-striatal sites. The activity produced by the NMDA antagonists is dose-dependent and at high doses side-effects such as psychostimulation, flattened body posture, sedation, memory impairment and ataxia arise
(Crossman et al., 1989). After high doses of intrapallidal kynurenic acid, a broad-spectrum glutamate antagonist, in marmosets (Brotchie et al., 1991) or intrastriatal CPP in rats (Carroll et al., 1995), an anaesthetic-like effect was seen. After systemic MK 801, sedation was observed in parkinsonian macaques (Crossman et al., 1989). The motor side-effects, such as sedation or ataxia, resulting from the use of high doses of glutamate antagonists have been hypothesised to arise from an alteration in the transmission of the thalamocortical pathway (Löschmann et al., 1991).

The above studies have demonstrated differences between the sensitivities of the different brain regions to the effects of the different classes of glutamate receptor antagonists investigated. These differences may suggest a regional heterogeneity of glutamate receptors with respect to their molecular structure and/or their function.

In this study, we investigated the locus of locomotor stimulant action of various glutamate antagonists within the basal ganglia and tried to determine if motor deficits arose from the same site. We targeted two main regions within the basal ganglia, the corpus striatum (CS) and the substantia nigra pars reticulata (SNr), of rats rendered akinetic with reserpine.

4.2 Results

4.2.1 Injections into the CS

4.2.1.1 Vehicle injections

The animals which received control injections were active in the initial 5-10 min post-injection interval displaying some locomotion, grooming and sniffing but this can be attributed to the injection itself and to the stress which accompanies the handling and restraining of the animals. The cumulative 60 min locomotor count obtained by intrastriatal vehicle-injected animals was 372±143. Statistical analysis of the time courses showed that the motor activity observed in the 10 min period immediately after intrastriatal vehicle administration was significant when compared to all the other time points (F(8,62)=6.34, p<0.001).
4.2.1.2 Motor restorative properties of glutamate antagonists

Only the competitive NMDA receptor antagonists, CPP (1.25-10 µg) and CGP 40116 (1.25-20 µg) reinstated dose-dependent motor activity in the reserpine-treated rats. There was a latency of 30 min before the motor restorative effect of CPP (1.25 µg) was observed and the response lasted for approximately 60 min (F(8,44)=2.45, p=0.034) while motor activity was reinstated by CGP 40116 (1.25 µg) after approximately 40 min and the animals were still active at 90 min (F(11,47)=2.511, p=0.0202) (Fig. 4.1A).

Animals administered low doses of CPP and CGP 40116 exhibited fluent locomotion resembling that of normal animals but at higher doses the motor activity of the animals was less fluent and motor deficits, ranging from muscular relaxation leading to a flattening of posture to severe ataxia and barrel rolling, were evident. The dose-dependency of the locomotor stimulant effect and the occurrence of ataxia produced by CPP are shown in Fig. 4.2.

MK 801 (0.5-10 µg), phencyclidine (PCP) (10-20 µg), AP-5 (2.5-5 µg), (±) HA-966 (0.5-28 µg), lamotrigine (3.75-7.5 µg) and NBQX (1-20 µg) had no effect on the reserpine-induced akinesia when administered into the CS (Fig. 4.3).

The glutamate antagonists, with the exception of lamotrigine, induced episodic sniffing and grooming as well as vacuous chewing which lasted longer than the behaviours observed after vehicle administration. Behaviours such as head weaving, forepaw treading and vacuous chewing occurred in some cases without being accompanied by locomotion. Compounds which reinstated locomotor activity markedly also induced motor deficits such as ataxia at the same doses. A full complement of behaviours observed after intracerebral glutamate antagonists is given in Table 4.1.

Histological investigation showed that the effective injections of CPP were distributed throughout the dorsoventral gradient of the striatum (see Fig. 4.4).
Fig. 4.1 Time-courses for locomotor activity produced by bilateral injections of competitive NMDA receptor antagonists into the corpus striatum (CS)(A) or substantia nigra pars reticulata (SNr)(B) of reserpine (5 mg/kg) treated rats. Vehicle, 0.5μl in the CS, 0.25μl in SNr(open circle), CPP 1.25μg in CS, 0.5μg (solid circle), CGP 40116 1.25μg in CS, 1μg in SNr (solid square). Data are means ± S.E.M. of 4–12 determinations.
Chapter Four Glutamate antagonists in the Basal Ganglia

Fig 4.2 Dose-dependence of the locomotion (solid bars) and ataxia (cross-hatched) induced by CPP injected into the CS of reserpine-treated rats. Data are means±S.E.M. of at least 6 determinations. * represents p<0.05, ** p<0.01 and *** p<0.001 vs controls by Student's t-test (locomotion) or Mann-Whitney test (ataxia).

Fig 4.3 The effects on locomotion (solid) and ataxia (cross-hatched) of bilateral injections of glutamate antagonists in reserpine-treated rats. Veh, vehicle; PCP, phencyclidine; HA, HA966; CGP, CGP 40116; LTG, lamotrigine. Data are means±S.E.M. of at least 6 animals. * represents p<0.05 and *** p<0.001 vs controls by Student's t-test (locomotion) or Dunn's Multiple Comparisons test (ataxia).
4.2.2 Injections into the SNr

4.2.2.1 Vehicle injections

Vehicle injections into the SNr elicited transient activity in the 10 min post-injection period with the animals averaging cumulative 60 min locomotor counts of 73±21. Similar results were obtained after DMSO (the vehicle for eliprodil) injections into the SNr. Statistical analysis of the time courses showed that the motor activity seen in the 10 min period immediately after intranigral vehicle injection was significantly different from the activity observed at all the other time points (F(8,44)=3.58, p=0.0045).

4.2.2.2 Motor restorative properties of glutamate antagonists

Glutamate antagonists applied directly into the SNr produced different responses when compared to injections into the CS. Lower doses of CPP (0.5-1 μg) and CGP 40116 (1 μg) were required to reinstate activity in the akinetic rats from the SNr compared to the CS. NBQX (5 μg) also induced marked locomotion but lower doses (0.5-1 μg) were without effect. AP-5 (2.5-5 μg), MK 801 (0.5-5 μg), PCP (10-20 μg), (±) HA-966 (0.5-1 μg), lamotrigine (10 μg) but not eliprodil (5-7.14 μg) produced stimulation which was statistically significant. The dose of CGP 40116 which counteracted reserpine-induced akinesia also caused ataxia. Data are shown in Fig. 4.5. The locomotion elicited by CPP (F(8,98)=1.957, p=0.063) and CGP 40116 (F(8,53)=1.989, p=0.073) from the SNr was rapid in onset appearing within 1-2 min of injection but also had a shorter duration (approximately 20-30 min)(see Fig. 4.1B).

The behaviours noted after intranigral glutamate antagonists, with the exception of lamotrigine, consisted of vacuous chewing, sniffing and grooming which lasted longer than the behaviours induced by intranigral vehicle administration. Intranigral (±) HA 966 also induced forepaw treading and head weaving without inducing appreciable locomotion. Again as for intrastriatal injections, the doses of CPP and CGP 40116 which induced marked locomotor stimulation also produced motor side-effects such as a flattened or collapsed posture and ataxia. The behavioural responses seen after intranigral glutamate
glutamate antagonists are shown in Table 4.1. The injection sites of CPP in the SNr are illustrated in Fig 4.4.

Fig. 4.4 Sites of injection of CPP into the CS (A) or the SNr (B) of reserpine-treated rats. Closed circles represent bilateral drug administration while open circles represent unilateral injections (in instances where one cannula was blocked).
Figure 4.5 The effects of intranigral injections of glutamate antagonists on locomotion (solid) and ataxia (cross-hatched) in reserpine-treated rats. PCP, phencyclidine; CGP, CGP40116; LTG, lamotrigine; ELIP, eliprodil. Vehicle motor scores were 73±21 and no ataxia was seen. Data are means±S.E.M. of at least 6 animals. * represents p<0.05, *** p<0.001 vs controls by Student’s t-test (locomotion) or Dunn’s Multiple Comparisons test (ataxia).

Table 4.1 Behaviours observed after administration of vehicle or various glutamate antagonists into either the CS or the SNr of reserpine-treated rats.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Vehicle</th>
<th>MK 801</th>
<th>PCP</th>
<th>HA966</th>
<th>CPP</th>
<th>CGP</th>
<th>AP-5</th>
<th>Eliprodil</th>
<th>Ltg</th>
<th>NBQX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>A/A</td>
<td>A/P</td>
<td>A/P</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>A/P</td>
<td>NA/A</td>
<td>A/A</td>
<td>A/P</td>
</tr>
<tr>
<td>Rearing</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>A/A</td>
<td>NA/A</td>
<td>A/A</td>
<td>A/P</td>
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<tr>
<td>Sniffing</td>
<td><em>/</em></td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
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<td>P/P</td>
<td>NA/*</td>
<td><em>/</em></td>
<td>P/P</td>
</tr>
<tr>
<td>Grooming</td>
<td><em>/</em></td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>NA/*</td>
<td><em>/</em></td>
<td>P/P</td>
</tr>
<tr>
<td>V chewing</td>
<td>A/A</td>
<td>A/P</td>
<td>A/A</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>NA/A</td>
<td>A/A</td>
<td>A/P</td>
</tr>
<tr>
<td>Head weave</td>
<td>A/A</td>
<td>A/P</td>
<td>A/A</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>NA/A</td>
<td>A/A</td>
<td>A/P</td>
</tr>
<tr>
<td>Forepaw tread</td>
<td>A/A</td>
<td>P/P</td>
<td>A/P</td>
<td>A/P</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>A/P</td>
<td>NA/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Flattened posture</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>A/P</td>
<td>NA/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Ataxia</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>A/A</td>
<td>P/P</td>
<td>P/P</td>
<td>P/P</td>
<td>A/P</td>
<td>NA/A</td>
<td>A/A</td>
</tr>
</tbody>
</table>

The results shown represent the absence (A), presence (P) of behaviours for the CS/SNr respectively. * means the behaviour was transient and occurred immediately after injection only. NA, not available because not tested. V chewing, vacuous chewing.
4.3 Discussion

In this study, we confirm the view that in conditions of dopamine deficiency, there is secondary glutamate hyperstimulation both in striatal and nigral pathways, as focal administration of some glutamate antagonists into the CS or the SNr can alleviate parkinsonism in the reserpine-treated rodent (Klockgether and Turski, 1990; Riederer et al., 1992; Schmidt et al., 1992; Starr, 1995a).

The CS receives glutamatergic input from the cortex and the thalamus and sends projections to the SN and the globus pallidus (GP) (Albin et al., 1989; Greenamyre, 1993; Schmidt et al., 1992). The SNr receives glutamatergic input from the cortex, (Albin et al., 1989; Carter and Fibiger, 1978), the subthalamic nucleus (STN) (Albin et al., 1989) and the pedunculopontine nucleus (Tokuno et al., 1988) while it sends projections to the thalamus (Albin et al., 1989; Greenamyre, 1993; Starr, 1995a).

The glutamate receptors found in the CS and SN consist of NMDA (Albin et al., 1992; Monaghan and Cotman; 1985) and non-NMDA, i.e AMPA, kainate and metabotropic receptors. There is a greater density of AMPA receptors in the SN than NMDA receptors while NMDA receptors are abundant in the CS (Albin et al., 1992).

In our study, as in previous studies, we find that although a number of NMDA receptor antagonists and an AMPA receptor antagonist, can reinstate mobility in monoamine-deficient animals, the extent to which they can do this depends on the drug used and the brain area the drug was injected into. Thus, differences in the sensitivity of NMDA receptors to different antagonists has been postulated. The difference could be due to regional heterogeneity of the receptors with respect to molecular structure and/or function (Standaert et al., 1994).

4.3.1 Glutamate antagonists administered into the CS

In the CS, only the competitive antagonists, CPP and CGP 40116, reinstated motor activity in the akinetic rats in this study. All the other glutamate antagonists administered had no effect on locomotion in the reserpine-treated rats but they did produce some
Previous studies have shown a marked locomotor-enhancing effect of the glutamate antagonists, both competitive and non-competitive NMDA receptor antagonists as well as AMPA receptor antagonists, administered into the CS of dopamine-intact animals (Alkhatib et al., 1995; Pierce and Rebec, 1993; Schmidt, 1986) and a marked synergistic effect when these drugs were administered in conjunction with dopamine agonists (Carlsson and Svensson, 1990a; Klockgether and Turski, 1990; Starr and Starr, 1993a, b, 1994a, 1995a).

Glutamate antagonists administered more ventrally, into the NAc, have been reported to have a greater effect on spontaneous locomotor activity than striatal administration. Focal injections of NMDA antagonists into the NAc were reported to induce locomotor activation (Carlsson, 1993; Narayanan et al., 1996) while injections into the anterodorsal striatum resulted in stereotyped behaviours (Schmidt and Bury, 1988).

In contrast to injections into the dopamine-intact CS, injections of glutamate antagonists into the CS of dopamine-deficient animals have been reported to induce weak locomotor stimulation, if any. Kynurenic acid when administered into the striatum of reserpine-treated rats or MPTP-treated marmosets was ineffective (Brotchie et al., 1991). Klockgether and Turski (1990) found that CPP, a selective competitive antagonist which binds to striatal postsynaptic NMDA receptors, when injected into the striatum of reserpine-treated rats had no effect on locomotion whereas Carroll et al. (1995) and the current study found that intrastriatal CPP was strongly antiakinetic. When injection coordinates were compared, we discovered that Klockgether and Turski (1990) administered CPP into a site approximately 1.0 mm anterior to where we injected CPP. Also, Klockgether and Turski (1990) administered α-methylparatyrosine (α-MPT), an inhibitor of dopamine synthesis, in addition to reserpine. Although we did not detect any rostrocaudal differences in the sensitivity to CPP and CGP 40116, other groups have reported such a difference in other models of PD. Yoshida et al. (1991, 1994) showed that AP-5 potently reversed haloperidol-induced catalepsy when injected only into the rostral
Our finding that AP-5 was ineffective in our study agrees with the observations made by Svensson and Carlsson (1992) and Carroll et al. (1995). The reason why AP-5 in our study was ineffective may be due to injection placement as Yoshida et al. (1991, 1994) administered AP-5 into a site approximately 1.5 mm anterior to the site where we injected AP-5. The striato-GPe pathway (the indirect pathway) originates from the anterior striatum while pathways to the GPi (EPN) and SNr (the direct pathway) originate from more posterior regions of the striatum (Albin et al., 1989). As it is the indirect pathway which is hypothesised to be hyperstimulated in parkinsonian syndromes while the direct pathway is underactive, it stands to reason that a more rostral placement of glutamate receptor antagonists would produce desirable results as compared to a caudal administration (Schiffman et al., 1993).

Intrastriatally administered non-competitive antagonists, PCP and MK 801, were ineffective in our model of PD. St-Pierre and Bédard (1994) found that although an injection of MK 801 into the SNr of 6-OHDA lesioned animals produced a circling response, it had no effect when injected into the striatum. Bresink et al. (1995) have demonstrated two binding sites for \[^{3}H\] MK 801 in the striatum and their displacement studies showed a lower affinity for unlabelled (+) MK 801 in the striatum, a possible reason for the ineffectiveness of MK 801 in counteracting reserpine-induced akinesia in this study. Studies have shown that MK 801 and PCP, at doses which produce hyperlocomotion: (1) increase dopamine and DOPAC levels in the medial frontal cortex, anterior cingulate cortex and in the NAc , without corresponding changes or even decreases in the striatum (Imperato et al., 1990; Wedzony et al., 1994); (2) increase dopamine outflow from the prefrontal cortex (PFC) (Wedzony et al., 1994); (3) evoke dopamine-dependent locomotion and stereotyped sniffing (Liljequist et al., 1991; Maj et al., 1991; Svensson et al., 1991; Wedzony et al., 1994); (4) enhance dopaminergic neurotransmission and metabolism in the striatum and NAc (French and Ceci, 1990; Löschler and Honack, 1992; Maj et al., 1991; Rao et al., 1990; Wedzony et al., 1993); (5) enhance the release of dopamine (Imperato et al., 1990; Wedzony et al., 1993); (6)
increase the firing rate of both the A9 and A10 dopaminergic neurones (French et al., 1991) and increase burst firing in the VTA A10 dopaminergic neurones (French et al., 1991; Murase et al., 1993); (7) increase the firing rate in the SN, regularise the pattern of firing and increase burst firing (Murase et al., 1993). On the contrary, the competitive NMDA antagonists appear not to have any effect on dopaminergic activity, either neurochemically or behaviourally (Rao et al., 1991). CPP applied directly to A9 dopaminergic neurones reduced the rate of firing, indicating that tonic glutamatergic input to these neurones, mediated by NMDA receptors, is required for normal basal activity (Overton and Clark, 1992). The competitive NMDA antagonists, NPC 12626, CGS 19755 and CPP had no effect on the firing rate and burst firing of VTA A10 dopamine neurones (French et al., 1991). CGP 37849, an analogue of AP-5, did not have any effect on dopamine outflow from the prefrontal cortex (PFC) (Wedzony et al., 1994). D-CPPene produced a decrease in the levels of 3-MT, a metabolite of dopamine and also a measure of dopamine release, and had no effect on DOPAC or HVA levels (Svensson et al., 1991). The NMDA channel blockers increase the firing rate of dopaminergic neurones in the ventral tegmental area (VTA) and enhance burst firing (French and Ceci, 1990; French et al., 1991), which denotes an increase in dopamine release, but competitive NMDA antagonists are ineffective (French et al., 1991). These findings appear to indicate that dopamine is a prerequisite for the behavioural stimulant effect of the non-competitive antagonists whereas the competitive antagonists do not appear to require dopamine for them to have an effect on behaviour (Kretschmer et al., 1994; Svensson et al., 1991). These data can be used to explain our results in the CS, i.e only the competitive antagonists, CGP 40116 and CPP, were antiakinetic while the NMDA channel blockers were ineffective as 24 hour reserpine treatment depletes approximately 95% of the vesicular dopamine (Anden and Johnels, 1978; Bertler, 1961; Starr et al., 1987). It, therefore, stands to reason that the competitive antagonists would be effective while the non-competitive antagonists would not in the monoamine-depleted rodent.

Lamotrigine, a glutamate release inhibitor which works by blocking voltage-gated
Na\(^+\) channels (Meldrum and Leach, 1994; Messenheimer, 1994) and also has the ability to block high voltage-activated Ca\(^{2+}\) currents in rat corticostriatal axon terminals (Stefani et al., 1996), when administered systemically into reserpine-treated mice, in an earlier study carried out in this laboratory, did not reinitiate locomotion (see Chapter Three). Löschmann et al. (1995) also showed that lamotrigine failed to reverse the parkinsonism of reserpine-treated rats or of nigral 6-OHDA lesioned rats. A neurochemical study found that lamotrigine did not have an effect on the dopamine levels of MPTP-treated mice (Jones-Humble et al., 1993) and lamotrigine has also been found to be a weak inhibitor of the release of dopamine (Leach et al., 1991). All the above findings may be used to confirm our data that intrastratial lamotrigine did not affect the parkinsonian akinesia induced by reserpine in this study.

NBQX, a selective AMPA receptor antagonist (Parsons et al., 1994), was found unable to reinstatement locomotion when administered into the laterodorsal striatum but was effective when injected into the STN, GPi, and SNr of reserpine-treated rats and when administered intramuscularly into the MPTP-treated monkey (Klockgether et al., 1991). Our results in the CS correspond to the observation made by Klockgether et al. (1991). The reason for this may be that the density of AMPA receptors in the CS is low compared to the STN and target areas of the STN, such as the SN (Albin et al., 1992). Luquin et al. (1993) reported that intravenous NBQX did not improve MPTP-induced motor deficits in monkeys. A microdialysis study showed that systemic NBQX or CNQX decreased dopamine levels in the striatum (Moghaddam and Bolinao, 1994; Sakai et al., 1997). Systemic administration of NBQX also produced a slight decrease in spontaneous locomotion (Daniels et al., 1994a). Our results subscribe to the data obtained previously.

(±) HA-966, an antagonist at the strychnine-insensitive glycine site (Fletcher and Lodge, 1988; Singh et al., 1990; Vartanian and Taylor, 1991), was ineffective in our study against parkinsonian akinesia but the (R) HA-966 or (+) HA-966 isomer, injected into the striatum of bilaterally 6-OHDA lesioned marmosets and both reserpine-treated (Slusher et al., 1994) and 6-OHDA-lesioned rats, was antiakinetic (Carroll et al., 1995; Mitchell
et al., 1995). Carroll et al. (1995) and Mitchell et al. (1995) found (+) HA-966 increased locomotion in reserpine-treated rats, when systemically administered, without inducing anaesthetic-like side effects. Kretschmer et al. (1994) reported that (+) HA-966 had no effect on locomotion in monoamine-depleted rats while a later study reported that 7-chlorokynurenate, also a glycine site antagonist, injected into the anterodorsal striatum or NAc, had no effect on spontaneous locomotion (Kretschmer and Schmidt, 1996). R or (+) HA-966 is the isomer which antagonises the effects of glutamate, aspartate and NMDA, thereby reinstating locomotion in models of PD while the S or (-) isomer is thought to produce sedation (Fletcher and Lodge, 1988). As we used the racemic mixture of HA-966, the locomotor stimulant effect of the (+) isomer may have been cancelled out by the sedation induced by the (-) isomer.

The differences seen between the effects of different classes of NMDA antagonists and also the difference in the response to competitive NMDA antagonists, i.e CPP and CGP 40116 were antiakinetic while AP-5 was not, may be due to differing sensitivities to receptors made up of different subunits. There is a high density of NMDA receptors in the basal ganglia and the most abundant variant consists of NMDAR2B subunits while the NMDAR2A subunits are only present at low levels (Buller et al., 1994; Standaert et al., 1994). The NMDA receptors, made up of NMDAR2B subunits, found in the medial striatum, have a low affinity for antagonists but a high affinity for agonists (Buller et al., 1994). The channel blockers like MK 801 and PCP are equipotent at both the NMDAR2A and the NMDAR2B subunits but may act preferentially at receptors made up of the NMDAR1/NMDAR2B subunits. A study expressing mouse NMDA receptor subunits in Xenopus oocytes found that the mouse counterparts of the NMDAR1/NMDAR2A and the NMDAR1/NMDAR2B channels are more sensitive to (+) MK 801. The competitive antagonists, such as CPP, preferentially act at NMDAR1/NMDAR2A receptors. The glycine site antagonists, L-689,560 and 7-chlorokynurenate, were reported to be more active at the NMDAR2A subunit (Buller et al., 1994).

In this study, AP-5 was ineffective at counteracting akinesia while CPP and CGP
40116 were very potent at reversing akinesia. A reason for this discrepancy may be that AP-5 has low affinity for the NMDAR2A subtype, which is found in the striatum (Standaert et al., 1994) and possibly for which CPP and CGP 40116 may have greater affinity. It has also been reported that AP-5 has the ability to inhibit[^H]glycine binding, which CPP does not have. Although, how this would make AP-5 ineffective is not clear but as HA-966 was also found to be ineffective, it is clear the role played by glycine in the modulation of the effects of NMDA receptor antagonists is more complex than initially thought (Danysz et al., 1989).

Local infusion of AP-5 (Schmidt and Bury, 1988), SDZ EAA 494 (D-CPPene), a competitive NMDA antagonist (Svensson et al., 1991) or MK 801 (Imperato, 1990) into the dorsal striatum increased sniffing in rats and induced locomotion while infusion of NMDA had the opposite effect (Schmidt and Bury, 1988). Antagonising NMDA receptors or enhancing the dopaminergic system in the striatum stimulates stereotyped behaviour, such as sniffing and head movements (Imperato et al., 1990; Pierce and Rebec, 1993; Scatton et al., 1982; Schmidt, 1988) whereas inhibition of the glutamatergic activity or activation of dopaminergic activity in the NAc stimulates locomotion (Imperato et al., 1990; Ouagazzal et al., 1994). The injection of 7-chlorokynurenate into the anterodorsal striatum induced stereotypical snout contacts but had no effect on locomotion (Kretschmer and Schmidt, 1996). These findings correspond to the observations made by us in this study, i.e (±) HA-966 did not reverse reserpine-induced akinesia but it did induce behaviours, such as sniffing, grooming and vacuous or undirected chewing. In the current study, the non-competitive NMDA antagonists induced stereotyped behaviours such as sniffing, grooming, vacuous chewing and forepaw treading without inducing locomotion. These results concur with previous studies which report the administration of non-competitive NMDA antagonists into the striatum produces stereotyped behaviour rather than locomotion (Schmidt and Bury, 1988). The competitive NMDA antagonists induced locomotion, stereotyped activity as well as motor deficits such as a collapsed posture and ataxia.
Chapter Four Glutamate antagonists in the Basal Ganglia

The competitive NMDA antagonists are postulated to be relatively free of undesirable side-effects which plague the use of non-competitive NMDA antagonists. This has been shown after systemic administration of the competitive NMDA antagonists, which unlike the noncompetitive NMDA antagonists, only induce amnesia when administered in doses higher than the doses normally required to alleviate parkinsonian and did not induce ataxia or stereotypies (Maj et al., 1993). However, as we show in this study and as shown by some previous studies (Brotchie et al., 1995; Carroll et al., 1995), local administration of the competitive NMDA antagonists into the CS or SNr can induce ataxia and stereotyped behaviour. Also, D-CPPene treatment was found to induce ataxia without any locomotor stimulation at low doses while at high doses, locomotion was observed with stereotyped behaviours (Svensson et al., 1991). Intrastriatal CPP has been found to induce anaesthetic-like side-effects in rodents (Carroll et al., 1995) and intracerebroventricular CPP has been shown to induce hyperactivity and ataxia (see Svensson et al., 1991) which corresponds to our finding that the doses of CPP which induced locomotion also resulted in ataxia.

4.3.2 Glutamate antagonists administered into the SNr

Overactivity of the STN leads to hyperstimulation of the glutamatergic pathways to the medial globus pallidus (GPI) and to the SNr which in turn lead to the characteristic symptoms of PD (Albin et al., 1989; Klockgether and Turski, 1989; Mitchell et al., 1989). Thus, the administration of glutamate receptor antagonists in these regions may alleviate parkinsonian symptoms.

Glutamate antagonists have been shown to be able to reverse parkinsonism from brain regions outside the striatum. Brotchie et al. (1991) administered kynurenic acid into the EPN/medial globus pallidus of reserpine-treated rats and MPTP-treated primates and found that it reversed parkinsonism dose-dependently. CPP or NBQX administered into the SNr, EPN or the STN increased locomotor activity in reserpine-treated rats (Klockgether et al., 1990; Klockgether and Turski, 1991).
Almost all the glutamate antagonists we investigated induced locomotion from the SNr, albeit to varying degrees. The non-competitive antagonists, MK 801 and PCP; the glycine site antagonist, (±) HA 966; the glutamate release inhibitor, lamotrigine; all produced weak locomotor stimulation while the most marked antiakinetic effect was seen after the administration of the competitive NMDA antagonists, CPP, CGP 40116 and AP-5; and the AMPA receptor antagonist, NBQX.

Receptor localisation studies have shown that AMPA and NMDA receptors are present in the SN but there is a greater density of AMPA receptors, especially in the terminal areas of pathways from the STN (Albin et al., 1992; Nakanishi, 1992; Standaert et al., 1994), therefore it appears both these receptor subtypes are involved in the pathophysiological process that leads to the suppression of locomotion. The majority of the SNr neurones share a similar phenotype characterised by NMDAR1C and NMDAR2D subunits (Standaert et al., 1994).

The most marked locomotor-stimulant effects were induced by the competitive NMDA antagonist, CGP 40116, which to our knowledge has not been tested intracerebrally in this paradigm. CGP 40116 has recently been described as not affecting the release of dopamine or its metabolites in intact rats and even inhibiting the release of dopamine and its metabolites in reserpinised and α-MPT treated rats, when infused into the SNr (Biggs et al., 1996) which again seems to suggest that the stimulant effect seen in this study is not fully dopamine-mediated. Conversely, CGP 40116 increased dopamine levels in the SN when administered systemically at doses which produce hyperactivity but unlike the study carried out by Biggs et al. (1996), this study was done in dopamine-intact rats (Wedzony and Czyrak, 1996). CGP 40116, CPP and even AP-5, which was ineffective in the striatum, all induced locomotion from the SN in our study. CPP administered into the STN, the GPi or the SNr reduced rigidity in monoamine-depleted rats (Klockgether and Turski, 1990) which parallels the data obtained in this study. The reason for the effectiveness of AP-5 in the SNr, as opposed to its ineffectiveness in the CS, may be due to the presence of NMDA receptors made up of the appropriate...
NMDAR2 subunits.

The channel blockers, MK 801 and PCP, induced behavioural effects as well as some locomotion in the reserpine-treated rats. MK 801 administered into the SNr of 6-OHDA-lesioned rats elicited circling (St.-Pierre and Bédard, 1994). MK 801 administered systemically has been found to increase dopamine, DOPAC and HVA levels in the SN (Maj et al., 1991; Rao et al., 1990). This could explain why we saw a reversal of akinesia and some behavioural arousal with the channel blockers administered into the SNr. Also, Elverfors and Nissbrandt (1991) reported that while, there was pronounced depletion of dopamine in the striatum induced by reserpine, the decrease in dopamine levels in the SN was not as great. The decreases of dopamine metabolites in the SN were also not as pronounced as in the striatum. The authors concluded that there was a pool of dopamine present in the SN which was insensitive to reserpine, probably due to different storage mechanisms, in smooth endoplasmic reticulum in addition to classical storage granulas (Elverfors and Nissbrandt, 1991). This finding may explain the effectiveness of the NMDA channel blockers in the SN, as opposed to their ineffectiveness in the striatum. As the NMDA channel blockers are postulated to act via enhancing dopaminergic neurotransmission, the depletion of dopamine produced by reserpine, would prevent these drugs producing behavioural arousal but as these drugs do produce behavioural arousal in the reserpine-treated rats, it appears that there is some endogeneous dopamine present which is resistant to the depleting effects of reserpine. However, it is also plausible that these agents may have an action downstream of the striatum decreasing the increased activity in the SN and the GP, thereby disinhibiting the thalamocortical pathway, leading to the reversal of parkinsonism (St.-Pierre and Bédard, 1994).

Eliprodil (SL82.0715), a polyamine site antagonist (Carter et al., 1988, 1990), failed to reverse reserpine-induced akinesia when administered intranigrally in this study. Perrault et al. (1989) reported that eliprodil, administered systemically or into the NAc, failed to induce locomotor stimulation while Voltz et al. (1994) found that systemic ifenprodil, another polyamine antagonist, did not antagonise the effects of intrastriatal
Eliprodil had no effect on locomotor activity when systemically administered into reserpine-treated mice (Brooks et al., 1996). A pilot study investigating the effect of ifenprodil in PD patients found it to be ineffective (Montastruc et al., 1992). Conversely, ifenprodil, administered into the striatum of bilaterally 6-OHDA lesioned marmosets, restored apparently normal mobility (Mitchell et al., 1995). We were unable to increase the dose of eliprodil investigated due to poor solubility, so we cannot reach any definite conclusions as to the possible use of eliprodil in PD therapy. The polyamine site antagonists act specifically at NMDAR2B subunit, but the subunits present in the SN are NMDAR2C and NMDAR2D, thus possibly explaining the ineffectiveness of eliprodil in the SNr (Avenet et al., 1997). Ifenprodil, tested against NMDA and kainate-induced excitotoxicity was protective against NMDA neurotoxicity but left behind a subpopulation of NMDA-susceptible neurones, suggesting a heterogeneity in the NMDA receptor population (Zeevalk and Nicklas, 1990).

The most marked ataxia was observed after administration of the competitive NMDA antagonists, especially CGP 40116. The dose-window between the beneficial antiakinetic effects of these drugs and the ataxia and motor deficits appears to be very narrow and in some cases the doses of the glutamate antagonists which potently reversed akinesia also produced undesirable motor side-effects. An attenuation of dopamine receptor function in the SNr has been found to cause an increase in muscle tone (Double and Crocker, 1995). The ataxia observed after the MK 801, PCP, HA-966, lamotrigine, eliprodil and NBQX was mild, consisting of flattened posture and an occasionally unsteady gait with some postural bias at times, but then the locomotor stimulation produced by these drugs was weak.

4.4 Conclusions

Focal administration of glutamate antagonists into the striatum of reserpine-treated rats showed that locomotion was induced only by a subgroup of competitive NMDA receptor antagonists while locomotion was induced by the administration of the competitive and
non-competitive NMDA antagonists as well as the AMPA receptor antagonist, NBQX, into the SNr. For the action of the NMDA channel blockers to manifest, the suppression of the excitatory drive in pathways to the SNr appears to be of greater importance than the suppression of the excitatory drive in projections to the striatum. In some instances, behavioural responses were observed without being accompanied by locomotion. Finally, ataxia arose from injections of the glutamate antagonists into the CS and the SNr and appears to accompany the locomotion induced by these drugs, which indicates careful titration will be necessary if glutamate antagonists are to be used in PD therapy. This finding supplements studies postulating that interruption of the thalamocortical pathways leads to motor deficits.
CHAPTER FIVE
THE ATTENUATION OF NEUROLEPTIC-INDUCED CATALEPSY BY MK 801 IS MEDIATED STRIATALLY AND EXTRASTRIATALLY
5.1 Introduction

The parkinsonian syndrome, characterised by akinesia, rigidity and a prolonged reaction time, resulting from the use of typical neuroleptics forms the basis of using neuroleptic-induced catalepsy, defined as a state of immobility in rodents, as a model of drug-induced PD (Elliott et al., 1990; Sanberg, 1980).

The general consensus is that neuroleptic-induced catalepsy emanates from the striatum (Dunstan et al., 1980; Honma and Fukushima, 1978; Yoshida et al., 1991, 1994) or the nucleus accumbens (NAc) (Ellenbroek et al., 1988; Ossowska et al., 1990). The cataleptic effect in the striatum seems to be localised to the rostral striatum (Ossowska et al., 1990; Yoshida et al., 1991, 1994).

Glutamate receptor antagonists have been investigated in the neuroleptic-induced catalepsy with some success. A number of glutamate antagonists administered systemically have been found effective in reducing or even preventing the catalepsy induced by systemically administered dopamine D_1 and/or D_2 receptor antagonists (Danysz et al., 1994b; Elliott et al., 1990; Klockgether and Turski, 1990; Maj et al., 1993a; Moore et al., 1993; Papa et al., 1993; Schmidt and Bubser, 1989; Schmidt et al., 1991; Verma and Kulkarni, 1992). Systemic NMDA potentiated haloperidol-induced catalepsy (Mehta and Ticku, 1990) while systemic MK 801 has been reported to prevent catalepsy produced by injections of neuroleptic drugs into the striatum (Elliott et al., 1990). AP-5, a competitive NMDA receptor antagonist, when injected into the dorsorostral striatum, reduced systemic haloperidol-induced catalepsy (Yoshida et al., 1991, 1994) while systemic haloperidol antagonised the behavioural arousal induced by AP-5 administered into the dorsorostral striatum (Schmidt and Bury, 1988).

GABA is one of the major neurotransmitters in the basal ganglia and alterations in GABAergic transmission can result in movement disorders. GABA mimetics, such as muscimol, have been found to be able to reinstate the catalepsy induced by haloperidol after frontal cortex lesions (K.G. Lloyd, unpublished results). GABA_A receptor agonists administered into the striatal-pallidal complex (Vrijmoed-deVries et al., 1987), ventromedial thalamus (VMT) (Di Chiara et al., 1979; Starr and Summerhayes, 1983) or

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the globus pallidus (GP) (Ossowska et al., 1993) have been reported to induce catalepsy. The GABA$_A$ receptor antagonists, bicuculline or picrotoxin, injected into the SN have also been reported to induce catalepsy (Di Chiara and Gessa, 1978; Olianas et al., 1978).

Klockgether and Turski (1990) reported that CPP, a competitive NMDA receptor antagonist, administered into the subthalamic nucleus (STN), entopeduncular nucleus (EPN) or the substantia nigra pars reticulata (SNr) reversed the catalepsy induced by reserpine. As we found earlier (see Chapter Four), MK 801 injected into the SNr was effective in alleviating the akinesia induced by reserpine while intrastriatal MK 801 was not. Therefore, we reasoned that MK 801 could also act at any or all of the glutamatergic sites within the brain. Preliminary experiments where the cataleptogenic agent, haloperidol, muscimol or bicuculline, was administered intracerebrally into brain structures and MK 801 injected intraperitoneally were undertaken. MK 801 was then administered into various brain areas and its effect on the cataleptogenic response of haloperidol was determined.

5.2 Results

5.2.1 Systemic administration

5.2.1.1 The effect of MK 801 administered after haloperidol pretreatment

Haloperidol (1 mg/kg i.p) induced marked and consistent catalepsy within 30 min of injection which lasted for up to 2 h, with the animals remaining on the horizontal bar for up to 360 s (our cutoff period).

MK 801 (0.2 mg/kg i.p) administered 45 min after haloperidol (1 mg/kg i.p) administration decreased the catalepsy induced by haloperidol ($F(17,116)=1.94$, $p=0.0227$) (Fig. 5.1). The animals displayed occasional sniffing, some vacuous chewing, muscle relaxation, a collapsed posture and ataxia. Vehicle injected controls only displayed episodic vacuous chewing and some grooming and remained cataleptic for the duration of the experiment.
5.2.1.2 The effect of MK 801 administered prior to haloperidol

MK 801 (0.2 mg/kg i.p) administered 10 min before haloperidol (1 mg/kg i.p) administration prevented haloperidol-induced catalepsy (F(17,107)=27.26, p<0.001). The animals showed obvious signs of MK 801 induced behavioural stimulation, which included forward locomotor activity, sniffing, grooming, stereotyped head movements as well as some muscle relaxation causing a collapsed posture and ataxia (Fig. 5.2).

5.2.2 Effects in the CS

5.2.2.1 The effect of systemic MK 801 on intrastriatal haloperidol-induced catalepsy

Haloperidol (7 µg/ 0.5 µl/ side), administered bilaterally into the ventroorostral striatum, induced catalepsy within 45 min of injection. The catalepsy induced was marked and lasted for up to 2 h.

MK 801 (0.2 mg/kg i.p), injected 60 min after intrastriatal haloperidol (7 µg/ 0.5 µl/ side), significantly reversed haloperidol-induced catalepsy (F(17,152)=21.257, p<0.001) (Fig. 5.3). Behavioural stimulation associated with 0.2 mg/kg MK 801 was seen, with behaviours like sniffing, grooming, locomotion and some ataxia evident.

5.2.2.2 The effect of systemic NBQX on the anticafeleptic activity of systemic MK 801

Catalepsy was induced by administering haloperidol (7 µg/ 0.5 µl/ side) into the ventroorostral striatum. NBQX (12.5 mg/kg i.p) was administered 60 min later and the catalepsy was unchanged. A second dose of NBQX (12.5 mg/kg i.p) was administered simultaneously with MK 801 (0.2 mg/k i.p) 30 min later. NBQX was found to reduce the anticafeleptic effect of MK 801 for 45 min after which time the stimulant effect of MK 801 was evident (F(17,152)=12.344, p<0.001) (Fig.5.4).
Chapter Five MK 801 in neuroleptic-induced catalepsy

Fig. 5.1 The effect of MK 801 (0.2 mg/kg i.p) on the catalepsy induced by haloperidol (1 mg/kg i.p) administered 45 min before MK 801 (▲) compared to haloperidol-treated controls (■). The arrow represents the time at which MK 801 was administered. Data are means ± S.E.M. of 6–7 determinations. * represents p < 0.05 using one-tailed Student’s t-test.

Fig. 5.2 The effect of MK 801 (0.2 mg/kg i.p) on the catalepsy induced by haloperidol (1 mg/kg i.p) administered 10 min after MK 801 (▲) compared to haloperidol-treated controls (■). Data are means ± S.E.M. of 6 determinations. *** represents p < 0.001, ** represents p < 0.01 using one-tailed t-test.
Fig. 5.3 The effect of systemic MK 801 (0.2 mg/kg) on the catalepsy induced by intrastriatal haloperidol (7 µg/0.5 µl/side) compared to haloperidol treated controls (■). The arrow represents the time at which MK 801 was administered. Data are means ± S.E.M of 6 determinations. ** represents p < 0.01 and *** represents p < 0.001 using the one-tailed t-test.

Fig. 5.4 The effect of NBQX (12.5 mg/kg i.p) on the anticataleptic activity of MK 801 (0.2 mg/kg i.p). Catalepsy was induced with intrastriatal haloperidol (7 µg/side) administered 90 min before the first injection of NBQX (12.5 mg/kg) while the second NBQX (12.5 mg/kg) injection was administered with MK 801 (0.2 mg/kg i.p) 30 min later. Control animals received MK 801 90 min after haloperidol (■). The arrows represent the drug injections. Data are means ± SEM of 6–11 determinations. * represents p < 0.05, ** p < 0.01 using a one-tailed t-test.
5.2.2.3 The anticaatleptic effect of intrastriatal MK 801

MK 801 (10 µg/0.5 µl/ side), administered into the ventrorostral striatum produced behavioural stimulation, which included forward locomotion, sniffing as well as some ataxia. As the animals were allowed 30 min to recover from the anaesthesia, the ataxia observed was due to the action of MK 801 and not residual anaesthesia.

MK 801 delayed the onset of the catalepsy induced by haloperidol (1 mg/kg i.p; administered 45min after MK 801) for 90 min (F(17,125)=9.978, p<0.001) (Fig. 5.5). The animals appeared active, displaying sniffing, grooming, some circling, locomotion, muscle relaxation and ataxia. Animals injected with saline in the striatum were only active in the initial 15 min interval post-injection period showing occasional vacuous chewing, sniffing, grooming, some locomotion and a collapsed posture.

5.2.3 Effects in the NAc

5.2.3.1 The effect of systemic MK 801 on intraaccumbens haloperidol-induced catalepsy

Haloperidol (7 µg/ 0.5 µl/ side) injected bilaterally into the NAc was found to induce marked and consistent catalepsy which lasted for up to 2 h.

MK 801 (0.2 mg/kg i.p) injected 60 min after haloperidol administration, attenuated haloperidol-induced catalepsy markedly (F(17,143)=16.931, p<0.001) (Fig 5.6). The animals were very active, displaying exploratory activity with some ataxia also evident.

5.2.3.2 The anticaatleptic effect of intraaccumbens MK 801

MK 801 (10 µg/0.5 µl/ side) injected into the NAc produced behavioural stimulation which included forward locomotion and ataxia. Haloperidol (1 mg/kg i.p) administered 45 min after MK 801, failed to produce catalepsy for 90 min (F(17,107)=19.006, p<0.001) (Fig. 5.7). These animals were active, displaying locomotion, grooming, sniffing, turning and ataxia. Vehicle administered into the NAc produced initial post-injection forward locomotion and grooming.
Fig. 5.5 The effect of intrastriatal MK 801 (10μg/0.5μl/side) on catalepsy produced by systemic haloperidol (1 mg/kg)(▲) compared to intrastriatal vehicle and systemic haloperidol treated controls (■). Data are means ± S.E.M of 7 determinations. * represents p < 0.05, ** p < 0.01 and *** p < 0.001 using the one-tailed t-test.

Fig. 5.6 The effect of systemic MK 801 (0.2 mg/kg) on catalepsy induced by intraaccumbens haloperidol (7μg/0.5μl/side)(▲) compared to haloperidol treated controls (■). The arrow represents the time at which MK 801 was administered. Data are means ± S.E.M of 7-9 determinations. *** represent p < 0.001 using one-tailed Student's t-test.
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Fig. 5.7 The effect of intraaccumbens MK 801 (10µg/0.5µl/side) on catalepsy induced by systemic haloperidol (1 mg/kg) (●) compared to intraccumbens vehicle and systemic haloperidol-treated controls (■). Data are means ± S.E.M of 6 determinations. ** represents p < 0.001, *** represents p < 0.001 using one-tailed Student's t-test.

Fig. 5.8 The effect of MK 801 administered into the SNr (1 µg/0.5µl/side)(open bars), the EPN (5 µg/0.5µl/side)(solid) or the STN (5 µg/0.5µl/side)(hatched) on catalepsy induced by systemic haloperidol (1 mg/kg) compared to intracerebral vehicle and haloperidol treated controls (dashed line). Data are means ± S.E.M. of 6 determinations. * represents p < 0.05 and *** represent p < 0.001 using the one-tailed student's t-test.
5.2.4 The anticataleptic activity of MK 801 injected into the SNr, the EPN or the STN

MK 801 (1-10 μg/0.5 μl/ side) injected into the SNr, EPN or the STN, 45 min prior to haloperidol (1 mg/kg i.p) administration, attenuated catalepsy. MK 801 (1 μg/0.5 μl/ side) administered focally into the SNr delayed the onset of the catalepsy induced by haloperidol (1 mg/kg i.p) for 60 min (F(16,101)=7.154, p<0.001). MK 801 administered into the EPN or the STN (5 μg/ 0.5 μl/ side) also attenuated systemic haloperidol-induced catalepsy for 30 min and 60 min respectively (F(17,107)=12.562, p<0.001 for the EPN and F(17,107)=21.199, p<0.001 for the STN) respectively (Fig.5.8).

MK 801 injected into the SNr displayed grooming, locomotion, sniffing and ataxia. Vehicle injected into the SNr produced initial 15 min activity which included sniffing. MK 801 injected into the EPN produced locomotion, sniffing, muscle relaxation, a flattened posture, head bobbing, a loss of balance whereas saline injected into the EPN produced initial 15 min activity including locomotion, sniffing and grooming. MK 801 injected into the STN produced initial 15 min activity including locomotion, sniffing, some rearing, a collapsed posture and ataxia, whereas saline produced initial 15 min activity, including some locomotion.

5.2.5 The cataleptogenic activity of bicuculline injected into the SNr

A range of doses (20 ng-1 μg/ 0.5 μl/ side) of bicuculline was tried and the drug was injected into a number of different sites within the SNr but we found that bicuculline did not induce catalepsy. On the contrary, the animals were very active, especially at 1 μg, with the animals exhibiting tight circling or epileptic seizures. Efforts to investigate the effects of MK 801 on catalepsy derived from the SNr were therefore discontinued.
Fig 5.9 The effect of systemic MK 801 (0.2 mg/kg) on the catalepsy induced by intrapallidal muscimol (25ng/0.5μl/side) (▲) compared to muscimol treated controls (□). Data are means ± S.E.M of 6-7 determinations.

Fig 5.10 The effect of systemic MK 801 (0.2 mg/kg) on the catalepsy induced by intrathalamic muscimol (50ng/0.5μl/side) (▲) compared to muscimol treated controls (□). Data are means ± S.E.M of 6-8 determinations.
5.2.6 The effect of systemic MK801 on catalepsy induced by muscimol administered into the globus pallidus (GP) or the ventromedial thalamus (VMT)

Muscimol, a GABA$_A$ receptor agonist, injected bilaterally into the GP (25 ng/0.5 µl/ side) (Fig. 5.9) or the VMT (50 ng/0.5 µl) (Fig. 5.10) induced marked catalepsy on which MK 801 (0.2 mg/kg i.p) had no effect (F(17,116)=7.137, p<0.0001 for GP and F(17,125)=4.524, p<0.001 for the VMT, when all time points were pooled but comparison of individual time points vs those of controls did not yield any significance). After MK 801 administration the animals displayed some behavioural arousal which included locomotion, sniffing, grooming and a little circling but this was insufficient to counteract the catalepsy induced by muscimol.
5.3 Discussion

In the present study, haloperidol administered systemically, intrastriatally or into the NAc, elicited catalepsy as did muscimol administered into the GP or the VMT. MK 801 administered systemically counteracted the catalepsy evoked by systemic, intrastriatal or intraacumbens haloperidol. We also found that MK 801 administered focally into the rostral ventromedial striatum, the shell region of the NAc, the SNr, the EPN or the STN was effective in alleviating systemic haloperidol-induced catalepsy albeit to a variable degree. However, systemic MK 801 had no effect on the catalepsy produced by intrapallidal or intrathalamic muscimol. Our results showing MK 801 injected systemically or into various nuclei of the basal ganglia induced behavioural stimulation, with sniffing, forward locomotion and episodic rearing evident, correspond to studies carried out by other groups (Hauber and Schmidt, 1990; Imperato et al., 1990; Willins et al., 1993).

5.3.1 The mechanisms involved in catalepsy evoked by neuroleptic treatment

Neuroleptic treatment causes blockade of dopamine receptors (Calabresi et al., 1992), especially the D$_2$ receptors located on glutamatergic axon terminals (Maura et al., 1988), which then enhances the tonic release of glutamate. The glutamatergic corticostriatal afferents (Van Der Kooy and Carter, 1981), which are the major source of excitatory input to striatal GABAergic neurones (Gerfen, 1992), then become hyperactive and via an action at NMDA receptors, cause an increase in GABAergic output (Young and Bradford, 1993) to the lateral (or external) globus pallidus (GPe) which in turn results in increased activity in the subthalamic glutamatergic output (Moore et al., 1993). The increase in GABAergic activity can be mimicked by administering muscimol, a GABA$_A$ receptor agonist, directly into the striatum (Turski et al., 1984), GP (Ossowska et al., 1993; Scheel-Kruger et al., 1981) or the VMT (Starr and Summerhayes, 1983) or by administering bicuculline, a GABA$_A$ receptor antagonist, into the SN (DiChiara and Gessa, 1978; Olianias et al., 1978). The catalepsy evoked by muscimol can be localised to a discrete area of the thalamus, the ventroanterior-ventromedial nucleus, which receives projections from the striatum via the EPN (Carter and Fibiger, 1978) and the SN (Clavier
et al., 1976) and from the cerebellum (Rinvik, 1975). The blockade of striatal dopamine receptors would result in GABAergic stimulation in the thalamus, possibly via the activation of the GABAergic nigrothalamic pathway and of the inhibitory EPN-thalamic pathway (Di Chiara and Gessa, 1978). This explains why intrathalamic muscimol mimics intrastratal haloperidol, in inducing catalepsy.

Neuroleptic-induced catalepsy is thought to arise from the antagonism of dopamine receptors in the striatum (Dunstan et al., 1981), especially in the rostral ventromedial striatum (Meyer et al., 1993; Ossowska et al., 1990; Sanberg et al., 1992; Yoshida et al., 1991, 1994). This discrete region in the striatum appears to be a hotspot for eliciting catalepsy as injections of neuroleptics into the rostroventral or the dorsal striatum were either ineffective or produced weak catalepsy (Ossowska et al., 1990; Yoshida et al., 1991). We administered haloperidol into the rostral ventromedial striatum and found that the catalepsy produced was marked and long-lasting which corresponds to previous studies (Ossowska et al., 1990; Yoshida et al., 1991, 1994).

The role of the NAc in neuroleptic-induced catalepsy is uncertain, with conflicting data obtained by different groups. However, our results concur with reports that haloperidol administered into the NAc induces catalepsy (Honma and Fukushima, 1978; Meyer et al., 1993; Ossowska et al., 1990) but other groups have found no effect (Yoshida et al., 1994). The NAc receives inputs from the cortex, the amygdala and the hippocampus and forms part of the limbic system. The NAc is hypothesised to be involved in the antipsychotic effect of neuroleptic drugs (Robertson and Fibiger, 1996; Wan et al., 1995).

Worms et al. (1985) and Jaskiw et al. (1993) found that lesions of the frontal or parietal cortical areas decreased haloperidol-induced catalepsy. As striatal dopamine release is enhanced by glutamate released from the corticostriatal pathway (Giorgueff et al., 1977; Roberts and Anderson, 1979), the authors reasoned that neuroleptic-induced catalepsy may be mediated by a blockade of dopamine receptors located on corticostriatal pathways and cortical ablation would then suppress neuroleptic-induced glutamate release and thereby decrease the stimulation of striatal output pathways by glutamate (Nieoullon
and Dusticier, 1983). Frontal cortex lesions also decrease nigral but not striatal GABA synthesis (Scatton et al., 1982) and increase [$^3$H] GABA binding in the pallidum and the SNr (Kupersmith and Lieberman, 1980). Lesions of the caudate putamen (Costall and Naylor, 1973; Honma and Fukushima, 1978) and NAc have been reported to markedly reduce haloperidol-induced catalepsy (Al-Khatib et al., 1989; Koffer et al., 1978) and an increase in the tone of the gastrocnemius muscle and triceps muscle was detected when haloperidol was injected into the rostral striatum and the NAc respectively (Ellenbroek et al., 1988). Meyer et al. (1993) found that haloperidol injected into the NAc, GP as well as the caudate putamen increased the dorsal immobility response (elicited by grasping a rat by the dorsal skin at the nape of the neck and lifting the rat off its feet causing the rat to become immobile for a period of time until it emits escape-like behaviours) but injections into the substantia nigra pars compacta (SNc) and the cortex had no effect.

5.3.2 The effects of systemic MK 801 on catalepsy induced by systemic haloperidol

Studies performed by other groups show that systemic MK 801 can attenuate the catalepsy induced by systemically administered neuroleptic drugs (Elliott et al., 1990; Mehta and Ticku, 1990; Moore et al., 1993; Schmidt and Bubser, 1989) which corresponds to the results we obtained after systemic administration. However, we found that MK 801 administered prior to the cataleptogenic agent, haloperidol, was more effective than when administered after the cataleptogenic agent. This did not hold true for later experiments where the one drug was intracerebrally administered with the other systemically administered. Haloperidol has been reported to able to bind to the ion-channel site of NMDA receptors (Ilyin et al., 1996; Lynch and Gallagher, 1996) and by so doing may compete with MK 801 but as haloperidol has a greater affinity for dopamine receptors than for NMDA receptors, the catalepsy prevails. Alternatively, systemic haloperidol may reduce the availability of systemic MK 801 in the brain. There is some evidence that neuroleptic drugs such as haloperidol, when administered systemically, prevent dopamine agonists (MK 801 acts indirectly like a dopamine agonist) from accumulating in brain areas (Vetulani et al., 1978; Westerink et al., 1984) but more
recent studies showing that haloperidol has no effect on blood-brain barrier permeability or even increases it in aged rats (Saija et al., 1992), have contradicted earlier studies. As this disparity was not noticed when haloperidol is administered intracerebrally while MK 801 is systemically administered, it seems logical to assume that haloperidol is having an effect either peripherally or in a region of the brain, distinct from the rostroventral striatum or the NAc, which then undermines the anticataleptic effect of MK 801.

However, the converse would be expected as haloperidol, acting at the level of the striatum would lead to a disinhibition of glutamatergic neurones (Yoshida et al., 1991), and MK 801, a use-dependent NMDA ion-channel blocker, would then be expected to be even more effective at alleviating catalepsy.

In studies where the anticataleptic effects of MK 801 or other glutamatergic antagonists were investigated, the glutamatergic antagonists were administered prior to or simultaneously with the cataleptogenic agent (Elliott et al., 1990; Klockgether and Turski, 1990; Mehta and Ticku, 1990; Meyer et al., 1993; Moore et al., 1993; Papa et al., 1993; Schmidt and Bubser, 1989; Schmidt et al., 1991; Verma and Kulkarni, 1992), with the exception of the study carried out by Maj et al. (1993a). Maj et al. (1993a) administered MK 801 30 min after haloperidol treatment in mice and found a reduction in catalepsy at a dose higher (0.4 mg/kg) than the dose that we used. Additionally, it is important to note that while our study was carried out using rats, Maj et al. (1993a) used mice.

5.3.3 Effects in the ventro-rostral striatum

In this study, haloperidol was administered directly into the rostral striatum to induce catalepsy which was attenuated by systemic MK 801. Also, MK 801 injected into the rostral striatum counteracted the catalepsy induced by systemic haloperidol.

The striatum is heterogeneous in terms of the activity of the neuroleptic drugs and glutamate receptor antagonists. The rostral striatum appears to be an important locus for the anticataleptic effect of MK 801, as it is for the cataleptogenic action of haloperidol. NMDA injected into the rostral striatum has been reported to restore haloperidol-induced catalepsy in frontally decorticated rats (Yoshida et al., 1991) while AP-5, a competitive
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NMDA receptor antagonist, injected into the rostral striatum reduced haloperidol-induced catalepsy (Yoshida et al., 1994) which corresponds closely with the results we obtained after the intrastriatal administration of MK 801. Conversely, when Klockgether and Turski (1993) administered NMDA into the rostral striatum parkinsonism was the result. The highest levels of L-aromatic amino acid decarboxylase (AADC), the enzyme responsible for the biochemical transformation of L-Dopa to dopamine, have been reported to occur in the rostral region of the striatum as compared to other areas in the striatum (Fonnum et al., 1978). The rostral region of the dorsal striatum is innervated by afferents originating in the medial substantia nigra pars compacta (SNc) while the caudal striatum receives afferents from the lateral SNc (Fonnum et al., 1978; Havemann et al., 1983). The indirect pathway originates from the anterior (rostral) striatum while pathways to the GPi (EPN) and SNr (the direct pathway) originate from more posterior regions of the striatum (Beckstead and Cruz, 1986). As the indirect pathway is thought to be hyperstimulated in instances of dopamine deficiency, it is feasible that glutamate antagonists administered focally into regions innervated by the indirect pathway would alleviate the symptoms produced by dopamine deficiency.

MK 801 administered into the striatum produced some behavioural arousal, which included sniffing and grooming which is in agreement with data obtained previously in this laboratory (Kaur and Starr, 1997) and by other groups (Pierce and Rebec, 1993; Schmidt et al., 1990).

There is also a high density of NMDA receptors in the rat caudate putamen, which might explain the marked anticataleptic effect of intrastriatal MK 801 (Monaghan and Cotman, 1982). The prevalent NMDAR2 subunits found in the striatum are the NMDAR2A and NMDAR2B subunits. MK 801 binds to these subunits with high affinity (Yakamura et al., 1993), which may explain its potent anticataleptic activity in the striatum.

5.3.4 The effect of NBQX on the anticataleptic activity of MK 801

In this study, NBQX reduced the anticataleptic effect of MK 801 for 45 min but by
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itself, NBQX had no effect on haloperidol-induced catalepsy. NBQX has been reported to have a short duration of action, approximately 30 to 40 min (Klockgether et al., 1990).

Papa et al. (1993) found that unlike MK 801, NBQX increased the catalepsy produced by raclopride while having no effect on the catalepsy produced by SCH 23390. The authors explained that the increase in catalepsy elicited by NBQX may have occurred via an action on AMPA receptors situated in the NAc. AMPA administered into the NAc has been reported to stimulate locomotion (Shreve and Uretsky, 1988) which can be antagonised by NBQX (Boldry et al., 1993). Therefore, any beneficial effect of NBQX at the level of the STN, EPN or SNr, may be counteracted by actions in the NAc. Also, by inference, NBQX may be able to cause an increase in the catalepsy produced by dopaminergic antagonists. However, our experimental set-up, i.e the assigning of a cutoff point, prevented us from observing such an effect. Alternatively, due to the differential effect of NBQX on catalepsy produced by D₁ or D₂ receptor antagonists, NBQX might act in the GP, blocking the stimulatory input from the STN and therefore increasing the inhibition of the pallidal cells, which receive an inhibitory input due to the disinhibition of the striatopallidal pathway by a D₂ receptor antagonist.

MK 801 is thought to act at the level of the striatum to attenuate catalepsy possibly by potentiating dopaminergic activity but MK 801 could also act at the STN to reduce catalepsy. NBQX may block the indirect dopaminergic stimulation of MK 801 by acting as suggested by Papa et al., (1993) in the GP to increase the inhibition of the pallidal cells therefore increasing the activity of the STN. Hauber and Andersen (1993) found that GYKI 52466, an AMPA receptor antagonist, did not affect haloperidol-induced catalepsy when administered on its own but antagonised the anticataleptic activity of MK 801. This corresponds to our finding with NBQX. Other groups have also found that systemic NBQX administered on its own has no effect on catalepsy or akinesia (Klockgether et al., 1991; Papa et al., 1993; Zadow and Schmidt, 1994). Hauber and Andersen (1993) suggest that GYKI 52466 may be acting at postsynaptic AMPA receptors situated on striatal neurones which form part on the indirect pathway. Such a blockade would result in reduced depolarisation, which is necessary for the voltage-sensitive activation of the
NMDA receptor linked ion channels and the binding of MK 801 (Roberts et al., 1995). Zadow and Schmidt (1994) postulated that NBQX, indirectly inhibits striatal GABAergic neurones thereby inhibiting thalamic activity, and therefore, could potentiate catalepsy.

5.3.5 Effects in the NAc

An important component of the afferents to the NAc are glutamatergic and they originate from the amygdaloid nucleus, the hippocampus and the frontal cortex (Fuller et al., 1987; Kelley and Domesick, 1982). The NAc also sends projections to the SN (see McGeer et al., 1984).

MK 801 injected into the NAc reduced systemic haloperidol-induced catalepsy while systemic MK 801 attenuated the catalepsy induced by intraaccumbens haloperidol. Systemic MK 801 was found to elicit behavioural activation which could be blocked with dopamine antagonists administered focally into the NAc which suggests that the effect of MK 801 is dependent on the activation of dopamine receptors in the NAc (Narayanan et al., 1996). The administration of systemic MK 801 increased the firing rate in dopaminergic neurones in the VTA, which is then expected to increase dopaminergic neurotransmission in the NAc, thereby antagonising the effect of haloperidol and leading to an alleviation of catalepsy (Narayanan et al., 1996). Additionally, AMPA receptor activation in the NAc is thought to be necessary for the expression of MK 801-induced locomotor stimulation (Donzanti and Uretsky, 1984; Raffa et al., 1989; Willins et al., 1993). Dopamine D₁ or D₂ receptor antagonists, administered into the NAc reduced the increase in motor activity seen after systemic MK 801 administration (Ouagazzal et al., 1994). Akaike et al. (1983) reported that haloperidol antagonised dopamine-induced inhibition of glutamate responses which resulted in increased glutamatergic activity in the NAc and this could be blocked by MK 801, thereby negating the effect of haloperidol. All the above findings show that the effect of MK 801 in the NAc is dopamine-dependent as it can be antagonised by dopamine antagonists or it can antagonise the effects of the dopamine antagonists, which is in agreement with the data obtained in this study.

There appears to be a distinction between the core and shell regions of the NAc,
with locomotor activity being unchanged or decreased by infusion of AP-5 into the core region but increased by infusion into the shell region (Maldonado-Irizarry and Kelley, 1994; Pulvirenti et al., 1994). The area where we administered haloperidol to induce catalepsy and MK 801 to alleviate systemic haloperidol-induced catalepsy corresponds to the shell region of the NAc. The shell receives projections from the basolateral amygdala, the ventral pallidum, the A8, A9, A10 regions, the bed nucleus of stria terminalis, preoptic area, lateral hypothalamus and the medial amygdala (Zahm and Brog, 1992). Projections from the shell region, unlike those from the core region which form the basal ganglia circuit, form the limbic circuit. It is assumed that AP-5 increases locomotor activity by blocking hippocampal glutamatergic input into the shell of the accumbens (Mogenson and Nielsen, 1984) but it is possible that the infusion of AP-5 may affect glutamate input from structures such as the thalamus or the cortex.

Raffa et al. (1989) reported that the behavioural activation produced by intraaccumbens MK 801 was not antagonised by haloperidol which suggests that MK 801 may also act at non-dopaminergic neurotransmitter systems. Carlsson and Carlsson (1989a) also showed that the increase in locomotor activity produced by systemic MK 801 was not affected by haloperidol. Therefore, it is possible that effect of MK 801 may not be dependent solely on the dopaminergic system to produce behavioural stimulation but instead may act on other catecholaminergic systems such as the adrenergic system (Clineschmidt et al., 1982). It also appears that the dose of MK 801 required to produce an anticataleptic effect is much lower than that required to produce locomotor stimulation. Hence, the anticataleptic effect of MK 801 may be dopamine-dependent while the locomotor stimulant effect may be only partially dopamine-dependent.

5.3.6 Effects in the SNr, EPN or the STN

MK 801 applied directly into the SNr, EPN or the STN alleviated the catalepsy produced by systemic haloperidol. The animals were active albeit to different degrees.

The STN and its afferent projections are implicated in the pathophysiology of movement disorders such as PD (DeLong, 1990). There is a glutamatergic output from
STN to the EPN and the SNr (Albin et al., 1989) and stimulation of the STN, as occurs in parkinsonism, enhances the activity of the EPN and SNr (Nakanishi et al., 1991; Robledo and Feger, 1990). Electrophysiological experiments have shown increased activity in the EPN after MPTP treatment (Filion and Tremblay, 1991; Voloshin et al., 1993) and hyperactivity in the input from the STN to the EPN (Bergman et al., 1990). Brotchie et al. (1991) administered kynurenic acid into the internal globus pallidus (Gpi)/EPN of MPTP-treated marmosets or reserpine-treated rats and noticed a reversal of parkinsonism. Lesions of the STN in MPTP-treated macaques were found to attenuate the parkinsonian symptoms (Bergman et al., 1990).

Pharmacological interruption of glutamatergic pathways in the SN or the Gpi (EPN in the rat) induced locomotor stimulation (Brotchie et al., 1991; Carroll et al., 1995). Therefore, administering a glutamate antagonist such as MK 801 into the SNr, the EPN or the STN opposes the hyperstimulation of the subthalamo-EPN and subthalamonigral pathways, thereby promoting motor recovery and counteracting catalepsy.

There is a low density of NMDA receptors in the SN and the STN of normal rats (Albin et al., 1989, 1992; Monaghan and Cotman, 1982) which could be the reason for the short-lived effect of MK 801 administered into these nuclei. Klockgether and Turski (1993) found that in the SNr, Gpi, EPN and the STN, activation of AMPA/kainate and metabotropic receptors produced parkinsonian rigidity, whereas the activation of NMDA receptors had no effect, suggesting that in these areas, AMPA and metabotropic receptors are of greater importance than NMDA receptors, in the context of dopamine depletion. Also, the predominant NMDA receptor subunits found in these nuclei are of the NMDAR2C and NMDAR2D variety (Standaert et al., 1994) but MK 801 has been found to have greater potency at NMDA receptors made up of NMDAR1/NMDAR2A or NMDA2B subunits (Beaton et al., 1992). Thus, as the NMDA receptors required by MK 801 to have a more potent effect may be in short supply in these brain nuclei, this might also help explain the short duration of action of MK 801 in these brain areas.
5.3.7 The lack of effect of MK 801 on muscimol-induced catalepsy

In the present study we found that MK 801 had no effect on the catalepsy produced by muscimol administered into the GP or the VMT. These observations correspond to the finding made by Mehta and Ticku (1990), that MK 801 had no effect on baclofen-induced, GABA$_B$ receptor-mediated, catalepsy. Systemic muscimol enhanced catalepsy induced by systemic SCH 23390 or raclopride (Ögren and Fuxe, 1988) while muscimol applied iontophoretically was shown in an electrophysiological study to exert an inhibitory effect on activity of nigral dopamine neurones (Grace and Bunney, 1985). SL 76 002, a GABA mimetic, has also been reported to inhibit dopamine neurones in the extrapyramidal system resulting in decreased dopamine levels and the potentiation of neuroleptic-induced catalepsy (Bartholini, 1980). The behavioural stimulation induced by systemic muscimol was found to be independent of dopamine activity as haloperidol did not antagonise it which implies that muscimol acts downstream from striatal dopamine receptors, probably on the the striato-entopeduncular or pallido-subthalamc GABAergic pathways (Scheel-Kruger and Magelund, 1981). Di Chiara et al. (1979) reported that high doses of apomorphine did not reverse intrathalamic muscimol-induced catalepsy. Also, systemic muscimol has been found to inhibit striatal dopamine release, based on 3-methoxytyramine levels in a microdialysis study (Wood and Altar, 1988). As MK 801 is postulated to act by indirect dopamine stimulation via the release and/or synthesis of dopamine, it is feasible that via its inhibitory action on dopamine release in the striatum and nigra, muscimol prevents the anticataleptic action of MK 801. Another explanation for the failure of MK 801 to alleviate the catalepsy induced by muscimol could be due to the potentiation of Mg$^{2+}$ blockade of the NMDA receptor by muscimol. Chaudieu et al. (1994) showed that muscimol inhibited NMDA-evoked [$H]$ dopamine release in mesencephalic cell cultures and this effect was specific to NMDA receptors, having no effect on kainate or quisqualate-evoked release. The authors rationalised that muscimol acting at GABA$_A$ receptors was enhancing the Mg$^{2+}$ blockade of NMDA receptors. They based this on the fact that the ability of muscimol to affect NMDA-evoked release was highly dependent on the presence of Mg$^{2+}$. As MK 801 is a use-dependent antagonist of
NMDA receptors, the ion channel needs to be open for MK 801 to have an effect. However, if muscimol enhances the Mg²⁺ blockade of the NMDA receptor ion channel, this prevents MK 801 from binding to its site of action and therefore not producing the desired effect of reducing catalepsy.

The thalamic nucleus receives glutamatergic input from the cerebellum and cortex as well as GABAergic fibres from the EPN (Albin et al., 1989). Alterations in the transmission in the thalamus occur in the pathophysiology of PD (Klockgether et al., 1985). Klockgether et al. (1986) found that application of AP-7, an NMDA receptor antagonist, into the VMT induced catalepsy while administering NMDA increased locomotor activity. Brotchie et al. (1991) found that injections of kynurenic acid made into the GP or ventral thalamus of reserpine-treated rats were devoid of antiparkinsonian activity but injection into the EPN was antiakinetic. These findings, along with other reports (Loschmann et al., 1991) suggest that glutamate antagonists administered directly into the thalamus, instead of alleviating catalepsy may indeed potentiate it. This can be explained anatomically, as in dopamine-deficient conditions the activity of the glutamatergic thalamocortical projection is reduced and administering MK 801 would further reduce locomotor activity. We did not see a potentiation of catalepsy as our experiments were set up to detect a decrease in catalepsy, as we used a cutoff point. Receptor localisation studies have shown a low density of NMDA receptors in the rat GP (Albin et al., 1992), which mean less NMDA receptors for MK 801 to act at and thus, a diminished effect of MK 801.

An alternative hypothesis suggesting that decreased activity in the external segment of the GP (GPe) is not necessary for increased activity in the STN has been put forward recently (Chesselet and Delfs, 1996; Levy et al., 1997). The model used previously suggested that a decreased activity in the pallidum was necessary for hyperstimulation of the STN (Albin et al., 1989) but recent electrophysiological and biochemical studies show that GABAergic activity in the GPe is not decreased in parkinsonian subjects. The STN receives an input from the cortex which may instead be implicated in hyperactivity of the STN. Our finding that systemic MK 801 was unable to
modulate intrapallidal muscimol-induced catalepsy, which should mimic striatal dopamine receptor blockade according to the currently accepted model, may provide further evidence to support this model (Levy et al., 1997).

5.3.8 The failure of intranigral bicuculline to induce catalepsy

We administered bicuculline into various regions of the SNr and also tried increasing the dose of bicuculline gradually from 20ng to 1μg, but were unable to reproduce the results obtained by Olianas et al. (1978), Scheel-Kruger et al. (1981) or Di Chiara et al. (1978). Conversely, the higher doses of bicuculline seemed to have a paradoxical behaviour-stimulant effect. The premise that a GABA_A receptor antagonist would induce catalepsy when administered into the SNr is valid seeing that muscimol, administered into the SNr inhibited intrastriatal sulpiride-induced catalepsy (Ossowska et al., 1993). Muscimol administered focally into the SNr has also been reported to produce hyperlocomotion (Olianas et al., 1978; Scheel-Kruger et al., 1977) while bicuculline administered into the caudal SNr produced catalepsy and tonic activity in the EMG, which was prevented by muscimol (Havemann et al., 1983). The stereotaxic coordinates used for the administration of muscimol by Havemann et al. (1983), who observed muscimol-induced hyperactivity, corresponded to the caudal SNr while we administered our drugs in an area that appeared to be in the middle of the rostral-caudal gradient. Our study does not bear out the results found by Olianas et al. (1978) and Di Chiara et al. (1978). When the coordinates we used to target the SNr were compared with those used by Olianas’ and Di Chiara’s groups, we discovered that their injections stopped short of the SNr and the drug was instead administered into the zona incerta. Also, the bicuculline salt used by Havemann et al. (1983) was the methiodide salt whereas we used bicuculline methobromide. Bicuculline methiodide has been found to be more stable than bicuculline itself (Olsen et al., 1975). Therefore, it is possible that our inability to induce catalepsy with intranigral bicuculline could be due to any one of the factors mentioned above.

The administration of the GABA_A receptor antagonists bicuculline or picrotoxin into the nigra has been reported to increase dopamine release in the caudate nucleus
(Bartholini et al., 1975). Recent studies have also shown that bicuculline at high concentrations acts as a use-dependent non-competitive NMDA receptor antagonist (Odile-Krebs et al., 1994; Wright and Nowak, 1992). These findings may explain why we saw a paradoxical increase in activity after high doses of intranigral bicuculline.

5.4 Conclusion

In the present study, MK 801 was found effective at reducing haloperidol-induced catalepsy at all the nuclei investigated, which seems to suggest that its anticataleptic activity is not restricted to the striatum only but extends to the nuclei which are downstream of the striatum. NBQX was found to reduce the anticataleptic effect of MK 801, which concurs with data obtained by other groups. The reason why MK 801 failed to counteract muscimol-induced catalepsy could be due to muscimol actively preventing dopamine release, by preventing MK 801 binding to its ion-channel site, or the GP not having as major a role in STN hyperstimulation as previously thought.
CHAPTER SIX
GENERAL DISCUSSION
6.1 The behavioural effects of systemically administered glutamate antagonists

The present research has employed a behavioural paradigm to assess the effects of various classes of glutamate antagonists, administered systemically, on locomotion in mice. The behavioural effects of these agents were determined initially in drug-naive mice to ascertain an appropriate dose-effect relationship. Once this was done, the antiparkinsonian effects of these drugs were investigated in the reserpine-treated (monoamine-depleted) mouse model of PD. As earlier studies have shown that glutamate antagonists can synergise with dopamine agonists to alleviate parkinsonian symptoms (Goodwin et al., 1992; Klockgether and Turski, 1990; Maj et al., 1993a, b; Starr and Starr, 1993a, b, 1994a, b), we then co-administered glutamate antagonists with a dopamine D₁ agonist, a D₂ agonist or the dopamine precursor, L-Dopa, to determine what interactions, if any, occurred between these agents.

We can confirm that some glutamate antagonists, particularly low doses of MK 801, lamotrigine, clonidine, (±) HA-966 and CPP, while having a minimal effect on locomotion of their own, can interact positively with L-Dopa to reverse the akinesia of monoamine-depleted mice, while having little effect on the response to the D₁ agonist, SKF 38393, or the D₂ agonist, RU 24213. If the synergism between the glutamate antagonists and L-Dopa can be transposed into the human condition, this might allow for a reduction in the dose of L-Dopa, therefore prolonging the therapeutic lifetime of L-Dopa and possibly reducing the incidence of dyskinesias resulting from chronic L-Dopa treatment (Jenner, 1995; Spencer et al., 1994). A reduction in the dose of L-Dopa may reduce the neurotoxic damage caused by L-Dopa itself (Naudin et al., 1995; Spencer et al., 1994). Also, there has been some evidence, showing that the glutamate antagonists may actively prevent L-Dopa induced dyskinesias (Allen et al., 1980; Boldry et al., 1995; Lustig et al., 1992). Therefore, all in all, the co-administration of glutamate antagonists would immensely benefit sufferers of PD. In the few clinical trials that have investigated the effect of the glutamate antagonists, dextromethorphan (Bonucelli et al., 1992), amantadine (Kornhuber et al., 1989), memantine (Rabey et al., 1992), budipine (Jellinger and Bliesath, 1987) and lamotrigine (Zipp et al., 1993), as add-on therapy to L-Dopa in
PD patients, there appears to be an overall benefit although this is by no means conclusive with contradicting data also published (Montastruc et al., 1994). The reasons for this mixed success could be that the age-groups used appear to differ, with the beneficial results seen in a lower age-group (Bonucelli et al., 1992; Montastruc et al., 1994). It appears from an earlier study that amantadine may also be effective, when administered alone, in producing motor improvement in PD patients (Parkes et al., 1970), but amantadine has also been found to have dopaminomimetic effects, although these are only apparent at high doses (Jackisch et al., 1992; Scatton et al., 1970).

Some of the compounds, already used in PD symptom control, have been found to be able to antagonise the effects of glutamate and which tends to suggest that glutamate is a key player in the pathophysiology of PD (Olney et al., 1987). Budipine, biperiden, both anti-muscarinic agents (Jackisch et al., 1994), amantadine and memantine (Danysz et al., 1994b; Kornhuber et al., 1991), are examples of NMDA ion-channel blockers, which have found a place in the treatment of PD.

The locomotion induced by the NMDA ion-channel blockers is often accompanied by psychostimulation, memory impairment and motor deficits such as ataxia and muscle relaxation, whereas the glycine site antagonists, polyamine site antagonists, and the AMPA receptor antagonists, have previously been found to reinstate mobility without the induction of motor side-effects. However, as we find in this study, this does not hold true as at doses of these agents which reverse akinesia, there is also some form of motor impairment. This motor impairment is thought to arise from the action of these drugs in regions of the brain apart from the basal ganglia, such as the cortex (Brotchie et al., 1991). Therefore, careful dosing is required to limit the induction of these side-effects, as it has been observed in this study that very low doses of MK 801 can positively interact with L-Dopa without inducing apparent motor side-effects, whereas the locomotion produced by higher doses is accompanied by muscular relaxation and ataxia, which can impede mobility.

Some of the glutamate antagonists, such as MK 801, PCP and ketamine, are not considered to be 'clean' drugs, as they can also act at sites other than the NMDA
ion channel, such as sigma receptors (Sharp, 1997), and can affect acetylcholine (Hasegawa et al., 1996) or 5-HT release (Lösch et al., 1991, 1993; Lösch and Hönack, 1992; Whitton et al., 1992). However, it has been indicated that the effects on acetylcholine (Hasegawa et al., 1996) and 5-HT release (Maj et al., 1996) may be secondary to effects on the dopaminergic system. Sharp (1997) reported that the stereotypies and abnormal behaviour, produced by PCP may be due to its action at σ sites. Even though, binding studies have shown that these drugs bind potently to NMDA channel sites (Albin et al., 1992; Bresink et al., 1995; Chen et al., 1992), the effects at other sites may preclude these drugs from use as potential antiparkinsonian agents. Therefore, the search is on for more specific glutamate antagonists and even subunit-specific agents which would target NMDA or AMPA receptors made up of particular subunits. This would also reduce the effects of these drugs at sites such as the cerebral cortex, thereby reducing the incidence of side-effects. The polyamine site antagonists, the glycine site antagonists, the competitive NMDA antagonists and AMPA receptor antagonists, may all be of some use towards reaching this goal. Low affinity NMDA ion channel blockers, such as amantadine, memantine and dextromethorphan, may also be contenders by virtue of their fast kinetics (Chen et al., 1992) which means that, as these drugs spend less time trapped in the ion channel site (Blanpied and Jonhson, 1994), the probability of them inducing detrimental effects is also lowered (Lipton, 1993).

The locomotor activity induced by the NMDA channel blockers has been shown to result from an indirect activation of the dopamine system (Clineschmidt et al., 1982; Maj et al., 1991; Morelli and Di Chiara, 1990). MK 801 increases dopamine turnover (Hiramatsu et al., 1989; Lösch et al., 1991, 1993; Rao et al., 1990) and increases its release (Imperato et al., 1990; Wedzony et al., 1993). The doses of MK 801 which enhanced the response to L-Dopa were much lower than those required to potentiate the effect of SKF 38393 (Kaur and Starr, 1994; Starr and Starr, 1993a, b, 1994a). Previously, it has been found that the glutamate antagonists, particularly the NMDA ion channel blockers, potentiate the response to a D₁ receptor agonist, SKF 38393, while having no effect or even attenuating the response to the D₂ receptor agonist, RU
24213 (Goodwin et al., 1992; Starr and Starr, 1993, 1994a, b) and enhancing the response to L-Dopa (Kaur and Starr, 1994; Klockgether and Turski, 1990). From the previous data, it was assumed that the glutamate antagonists potentiated the response to L-Dopa by possibly enhancing the \( D_1 \) component of L-Dopa. However, the data obtained in the current study indicates that this is too simplistic a viewpoint, as doses of the non-competitive glutamate antagonists which potentiate SKF 38393 had no effect on the L-Dopa response and vice versa, as seen in this study. This disparity in the dose-response has also been noticed previously (Goodwin et al., 1992). The glutamate release blockers, lamotrigine and clonidine, also seem to increase the response to L-Dopa by possibly enhancing the \( D_2 \) response (Starr and Starr, 1994b). In this study, there did appear to be an enhancement of the RU 24213 response by lamotrigine although this was not significant, but in previous studies, clonidine has been shown to increase the antiakinetic effect of RU 24213, L-Dopa and apomorphine but having no effect on the \( D_1 \) response (Starr and Starr, 1994b). The mechanism of action of the glutamate release blockers differs from that of the NMDA receptor antagonists, in that they appear to potentiate L-Dopa by increasing the \( D_2 \) component of L-Dopa (Rubinstein et al., 1989; Starr and Starr, 1994b).

6.2 Stereotaxic injections of the glutamate antagonists into the CS or the SNr of monoamine-depleted rats

After determining that systemic administration of glutamate antagonists can reverse akinesia in monoamine-depleted mice, we next investigated the site of action of these drugs in the basal ganglia. The glutamate antagonists were directly administered into the CS or the SNr of monoamine-depleted rats via indwelling guide cannulae. The CS and the SNr were chosen as these regions are important in the pathophysiology of PD.

We found that only the competitive NMDA receptor antagonists, CPP and CGP 40116, were able to reinstate locomotion in the reserpine-treated rats while the non-competitive and the competitive NMDA receptor antagonists, including AP-5 which was ineffective in the striatum, as well as (±) HA-966 reversed akinesia in the reserpine-
treated rats. Also, we found that often the doses of the agents which induced locomotion also produced motor disability in the form of ataxia and muscle relaxation which made the animals appear collapsed. The motor deficits were produced by injections into the CS and also into the SNr so there is no one area from which these side-effects arise. The reason for the differing responses of the different glutamate antagonists from the CS or the SNr may be due to the presence of heterogeneous NMDA receptors, i.e. made up of different NMDAR2 subunits (Buller et al., 1994; Standaert et al., 1994). Using NMDAR2 subunit-specific antagonists to target the brain regions involved in the symptoms of PD may reduce the incidence of side-effects caused by the glutamate antagonists.

The mechanism of action of the competitive and non-competitive NMDA antagonists is different. The NMDA ion channel blockers are thought to act via an enhancement of the dopaminergic system, possibly by increasing the synthesis of dopamine, and dopamine seems to mediate the action of these drugs whereas the competitive NMDA antagonists appear to act upon neurotransmitter systems other than dopamine, possibly 5-HT (Kretschmer et al., 1994; Svensson et al., 1991). Also, the non-competitive NMDA antagonists may act at the 'tonic' indirect (striatopallidal) pathway as they are use-dependent, the more active the pathway, the more efficacious the drug, while low doses of the competitive NMDA antagonists are thought to act at the 'phasic' direct (striatonigral) pathway, with high doses interfering with the indirect pathway (Carlsson, 1993; Svensson et al., 1992).

6.3 The effect of MK 801 on neuroleptic-induced catalepsy

In the previous set of experiments, it was found that MK 801 was effective in alleviating the akinesia of monoamine-depleted rats only when administered into the SNr, and not when administered into the striatum. However, it has been observed that the doses of glutamate antagonists required to counteract catalepsy are much lower than those required to induce motility (Danysz et al., 1994b). In the current study, we investigated the site of the ant cataleptic action of MK 801 and tried to determine if the locus of this action was in the striatum, as suggested by other groups (Dunstan et al., 1981; Honma and
Initially catalepsy was induced by systemic haloperidol and the effect of MK 801 determined. MK 801 was antica
taleptic both when administered before and after haloperidol administration but we found that the antica
taleptic effect of MK 801 was much greater when it was administered 10 min before haloperidol administration, possibly due the competition produced by the NMDA receptor antagonistic properties of haloperidol itself (Ilyin et al., 1996; Lynch and Gallagher, 1996). Catalepsy induced by direct injections of haloperidol into the CS or the NAc was attenuated by systemic MK 801 while systemic haloperidol-induced catalepsy was counteracted by intrastriatal or intraccumbens MK 801. MK 801 applied directly to the SNr, the EPN or the STN was also effective in alleviating the catalepsy induced by systemic MK 801. From these results, we determined that the locus of antica
taleptic action of MK 801 was not restricted to the striatum and MK 801 could also act downstream of the striatum, at the level of the SNr/STN/EPN.

The action of MK 801 was then investigated against the catalepsy induced by intrapallidal or intrathalamic muscimol. The administration of muscimol into the GP or the VMT mimics the blockade of dopamine receptors in the striatum. We found that MK 801 was ineffective against muscimol-induced catalepsy. These data corroborates evidence for a hypothesis put forward recently by Levy et al. (1997) and Chesselet and Delfs (1996), suggesting that the hyperstimulation of the STN does not result from a reduced activity in the globus pallidus but may instead be due to the corticosubthalamic pathway. Also, we tried to induce catalepsy by administering bicuculline into the SNr but were unable to do so, instead obtaining a paradoxical increase in the activity of the rats at high doses of bicuculline, which may be caused by the antagonism of the NMDA receptor-linked ion channel by bicuculline at high doses.

Therefore, from the results obtained here, MK 801 appears to act downstream of the CS and upstream of the thalamus. MK 801 would be expected to further reduce motility by acting at the level of the thalamus (see Starr, 1995a). The reason for the disparity in
the effectiveness of MK 801 in this set of experiments and in the locomotion study is probably due to the doses administered, as lower doses appear to alleviate catalepsy than those required to induce motility.

6.4 Concluding remarks

The data obtained from the current research augment the findings of previous studies, that glutamate antagonists, especially the NMDA antagonists, can relieve the akinesia of PD. We conclude that the glutamate antagonists, at present, are not a viable option for use as monotherapy of PD but may be more suitable for use in the polytherapy of PD. As seen in the animal models of PD and in the few clinical trials that have been carried out in PD sufferers, these drugs are beneficial in the symptom-control of PD, when combined with L-Dopa. However, it appears that different models of PD yield different results, with respect to the action of the glutamate antagonists, thus it is unwise to draw conclusions from data obtained from just one model of PD. However, our results do compare favourably with data obtained in other models of PD. The disparity within models may due to the doses administered, as the doses of the glutamate antagonist required to alleviate catalepsy are lower than the doses needed to reinstate locomotion. Also, there are variations in the effectiveness of the glutamate antagonists, between the rodent and primate models.

Therefore in conclusion, glutamate antagonists may find a place in the symptom-control of PD, when co-administered with dopaminergic agents like L-Dopa, provided the doses used are carefully selected to preclude the induction of motor side-effects. The glutamate antagonists may also help to curb the dyskinesias usually seen after chronic L-Dopa administration (Allen et al., 1980; Boldry et al., 1995; Lustig et al., 1992). However, more research is needed into the long-term effects of these drugs, as it appears as chronic use of these drugs may cause an upregulation of postsynaptic dopamine receptors, particularly dopamine D2 receptors, which would also contribute to the therapeutic usefulness of these drugs (Gianutsos et al., 1985; Wedzony and Czyrak, 1996).
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